Olive mill composted residues as new resource for bio-control of plant pathogens

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Olive mill wastes (two and three-phase olive husks, olive mill wastewater) might cause unfavourable impact on plants, soil structure, microbial population and activity, aquatic ecosystems and even on air media because of the phenolic, fatty acid, mineral salts content and high COD and BOD5. In the last decade, many pilot and industrial scale composting trials on agricultural residues (including solid and liquid olive wastes) proved the reliability of this microbial biotechnology to allow the transformation of these by-products into organic fertilizers (cured composted residues) with no phytotoxicity, and able to improve soil fertility and plant production (Alfano *et al.*, 2008). More, olive mill composted residues showed positive properties which suppress soilborne plant pathogens (Lima *et al.*, 2008). In fact, cured composts consistently reduced the growth *in vitro* of *Verticillium dahliae* and other important fungal pathogens. The inhibitory activity decreased or disappeared when the cured composts was autoclaved before its use. The inhibitory activity seem to involve the beneficial residual microbial population selected spontaneously during the composting process. In other trials performed on young olive plants grown on soil artificially contaminated by *Verticillium dahliae* microsclerotia (MC), when the antagonistic fungus *Trichoderma viride* was enriched with 15% (w/w) of OMW cured compost, a significant reduction in the density of *Verticillium dahliae* MS in the soil, was observed. In conclusion the application of good quality cured composts with suppressive characteristics for the control of fungal pathogens of some important vegetal crops in organic and integrated agriculture systems seem to be a very promising strategy.

Keywords: olive mill wastes, compost, biocontrol, soilborne plant pathogens.

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Medicago truncatula improves salt tolerance when nodulated by an indole-3-acetic acid over-producing Sinorhizobium meliloti strain

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Among the abiotic stresses, salinity, even caused by irrigation, is one of the most important environmental constraints limiting the growth and productivity of the major crop species including legumes, and will soon become even more severe (Vinocur and Altman, 2005). In our study, the abiotic stress resistance of wild type *Sinorhizobium meliloti* 1021 was compared with that of RD64, a derivative of 1021 strain harbouring an additional pathway for the synthesis of indole-3-acetic acid (IAA), expressed both in free-living bacteria and bacteroids (Imperlini *et al*., 2009). The IAA-overproducing RD64 strain accumulated higher level of trehalose as endogenous osmolyte and showed an increased tolerance to several stress conditions (55 °C, 4 °C, UV-irradiation, 0.5 M NaCl and pH 3). *Medicago truncatula* plants nodulated by RD64 (*Mt*-RD64) showed a phytohormones re-modulation with a higher IAA content in nodules and roots and a decreased IAA level in shoots as compared to the plants nodulated by the wild type strain 1021 (*Mt*-1021). The response of nodulated *Medicago truncatula* plants to salt stress, when 0.3 M NaCl was applied, was analyzed. For *Mt*-RD64 plants higher internal proline contents, almost unchanged hydrogen peroxide levels and enhanced activity of antioxidant enzymes (superoxide dismutase, total peroxidase, glutathione reductase and ascorbate peroxidase) were found as compared to *Mt*-1021 plants. These results were positively correlated with reduced symptoms of senescence, lower expression of the ethylene signalling genes, lower reduction of shoot dry and better nitrogen-fixing capacity observed for these plants. Upon re-watering, after 0.3 M NaCl treatment, *Mt*-1021 plants almost die whereas *Mt*-RD64 plants showed visual recovery signs. Finally, the shoot dry weight of *Mt*-RD64 plants treated with 0.15 M NaCl was not statistically different from that of *Mt*-1021 plants grown under non-stressed conditions.

Keywords: antioxidant enzymes, hydrogen peroxide, legumes, proline; salinity.

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How to improve intestinal health in poultry and to control pathogens widespread

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The gut microbiota of young birds is considered to be relatively unstable and it is constantly submitted to a wide range of factors that can negatively influence the delicate microbial balance, such as sub-clinical infections posed by pathogenic challenge.
Thus, the ability to maintain a normal or optimal gut microbiota composition becomes one of the key factors in determining the health status of the animals linked with the production performance of birds in commercial poultry operations.
Furthermore, there is no doubt that poultry is a major source of *Campylobacter* spp. and there is scope for cross-contamination of other foods.
Concerns about development of antimicrobial resistance and about transference of antibiotic resistance genes from animal to human microbiota, led to withdraw approval for antibiotics as growth promoters in the European Union. On the other hand, the use of probiotics to promote health and nutrition has attracted a great deal of attention and claims have been made in this context with regard to daily weight, improvement in feed conversion and resistance to disease.
Previously, we selected two probiotics strains, *Lactobacillus plantarum* (PCS 20) and a *Bifidobacterium longum* (PCB 133), for their *in vitro* antimicrobial activity against *Campylobacter jejuni* subsp. *jejuni*; our current interest in these two microorganisms is to obtain information on their ability to survive and persist in the poultry gut when orally administered by gavages and their influence in the gut microbiota.
We conducted two trials using in each one 32 broiler chickens (control group/probiotic group). The administration period of the two probiotics strains was 15 days consecutive with a daily dose of 106-107 /g of feed; fresh faeces from individual chickens were collected on days 0, 15 and 20 (wash out period) and immediately transferred to the laboratory for DNA extraction (QIAamp DNA Stool Mini Kit-Qiagen).
Detection and enumeration were performed with culture independent techniques based on polymerase chain reaction; qPCR and Sybr Green chemistry were used for quantification of *L. plantarum*, while *B. longum* was analysed by direct semi-quantitative PCR according to a previously described protocol.
Chickens treated with *L. plantarum* did not show any presence of the target microorganism in all the faecal samples; *B. longum* was detected in most of the samples analysed with a population assessed around 105-106 CFU/g of faeces.
The results underlined the potential ability of the strain *B. longum* o survive in the chicken gut and probably influence positively the microbiota balance; qPCR is in progress to quantify the chicken gut microbiota with relevance to *Campylobacter* spp. and *C. jejuni*.
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Keywords: gut microbiota, probiotics, antimicrobial activity.

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A potential new role of nirK gene in Rhizobium sullae

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Rhizobium sullae, a nitrogen fixing symbiotic bacterium, induces nodule formation on *Hedysarum coronarium* (Casella *et al.*, 1984; Squartini *et al.*, 2002). *Rhizobium sullae* strain HCNT1 is known to possess a truncated denitrification chain, harbouring only a gene (*nirK*) that encodes for a dissimilatory nitrite reductase (Toffanin *et al.*, 1996). Since this strain does not obtain any obvious benefits from it, the conservation of this gene is currently unexplained. *Rhizobium sullae* HCNT1 was recently found able to grow on high concentrations of selenite and to reduce this oxyanion to the less toxic elemental selenium. Since its *nirK*-minus mutant grows weakly in the presence of even low selenite and it is not able to grow at higher concentrations, a defence mechanism for detoxification of selenite was proposed to explain one of the potential roles of *nirK* (Basaglia *et al.*, 2007).
Nitrite reductase activity of *R. sullae* was then evaluated in different environmental conditions, in the presence of nitrite, selenite or both. In this approach it was possible to clarify if nitrite reductase and selenite reductase are, in this strain, the same protein that works in different way, according to the substrate and to the atmospheric conditions, or they are two distinct proteins. Firstly, it was observed that selenite reduction activity occurred either under aerobic or anaerobic atmosphere, while the reduction of nitrite to NO can be attained only after a microaerobic preincubation, and that the presence of nitrite in the cultural medium together with selenite did not influence selenite reduction to elemental red selenium. On the contrary, the addition of selenite to cultures containing nitrite inhibits the production of nitrogen oxides. The presence of selenite has no effect on the expression level of *nirK*; mRNA analysis confirmed that *nirK* is always expressed, as the related transcript is present either during aerobic or under microaerophilic conditions. Moreover, the use in the culture of a specific chelator for a copper-containing nitrite reductase such as NirK, together with selenite, inhibited also the reduction of this oxyanion to elemental red form. This latest result indicates that the putative selenite reductase in *R. sullae* contains copper, like nitrite reductase.
The above evidences seem to suggest that nitrite and selenite reductase could be the same protein; the enzyme studied could be a selenite reductase, rather than a nitrite reductase, which reduce selenite under aerobic condition and that becomes able to reduce also nitrite but only after a treatment under microaerobic condition. Therefore, protein purification was essential to better understand its properties. A recombinant-proteins approach (His-tag) allowed to purify a subunit of about 40 kDa encoded by *nirK* gene, that is a subunit which form the overall architecture of the homotrimeric enzyme (120 kDa). Preliminary *in vitro* experiments with the purified protein, for testing its ability to reduce nitrite and selenite separately, were undertaken. Although NirK showed the ability to reduce nitrite, medium-long term experiments are required to obtain the optimal conditions under which the protein could also reduce selenite.

Keywords: denitrification, *nirK*, nitrite reductase, selenite reductase, *Rhizobium sullae.*

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Effect of Azospirillum brasilense Sp245 on grapevine vegetative propagation

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Vegetative propagation of grapevine is nowadays performed by means of bench-grafting. The rooting formation of grafted cuttings is often a critical phase, due to the low rooting ability of some rootstocks. In order to improve the performance of grapevine propagation we tested the effect of *Azospirillum brasilense*, a diazotroph capable of colonizing the rhizosphere, on *Vitis vinifera* cv. Sangiovese grafted on various rootstock cuttings. Bacterial cells of *Azospirillum brasilense* Sp245 were inoculated at different stages of the vines production in a conventional nursery (cv. Sangiovese grafted on 420A, 161-49, 157-11, SO4, 140Ru, 775P, 1103P, 101-14, 3309 rootstocks), and in an organic nursery (cv. Sangiovese grafted on 1103P) located in Pisa area (Italy). As concerns the experiments in the conventional nursery two treatments have been tested: cuttings inoculated just before the bench-grafting (A); bench-grafted cuttings inoculated just before field planting after a period of 15 days at 25 °C, high relative humidity and in the dark (B). In the organic nursery the grafted cuttings have been inoculated during the 15 days treatment at 15 days at 25 °C, high humidity rate and in the dark. The following parameters were considered: rooting percentage; number of adventitious roots; total biomass of rooted vines. In addition the effect of the bacterial inoculation on the callus diameter formation at the end of the 15 days at 25 °C in treatment A has been evaluated. In the conventional nursery we found that the inoculum statistically significantly increased the callus diameter of grafted-cuttings produced during the treatment in the dark at 25 °C. The rooting percentage of inoculated grafted cuttings was significantly higher in the case of 161-49, 140Ru and 775P rootstocks, while the biomass of the grafted rooted cuttings was significantly higher for SO4 and 101-14. In the organic nursery the rooting percentage, the number of roots and the biomass per vine appeared significantly higher for grafted-cuttings inoculated during the 25 °C treatments. In conclusion, *Azospirillum brasilense* improved adventitious root formation and biomass formation of grapevine grafted-cuttings in function of rootstock type and stage of inoculation. These results confirm for grapevine the positive effect showed by *A. brasilense* on the root growth of crop plants.

Keywords: grapevine propagation, *Azospirillum brasilense* Sp245, rooting.

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Oenological yeasts as source of extracellular hydrolytic enzymes for future applications in bioethanol production

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Bioethanol is an attractive, sustainable and alternative energy source to conventional fuel. Current ethanol production processes using crops (sugar cane and corn) as a starting substrate are well-established. However, a cheaper feedstock such as lignocellulosic biomass could make bioethanol more competitive with fossil fuel, although its complex structure makes this material more resistant to biological degradation. Recent efforts have focused on the one-step microbial conversion of plant biomass into biofuel since simultaneous microbial hydrolysis and fermentation of lignocellulosic material could represent a strategy to allow cost-effective production of ethanol. Wild-type microorganisms having both the properties to utilise biomass polysaccharides and to produce ethanol have not been described. In this respect, yeasts isolated from oenological environments for their fermentative abilities and possessing efficient hydrolytic enzymes could be very promising.

The aim of this study was to investigate on the extracellular enzymatic activity profile of yeast strains previously selected on the basis of their optimal fermentative performance, in order to start the development of a strain suitable for the one-step bioconversion of biomass into ethanol. One hundred and eighty non-*Saccharomyces* strains and two hundred and twenty *Saccharomyces cerevisiae* strains isolated from grape marcs were screened for their activity on cellulose, hemicellulose, pectin, protein and starch. Few strains showed activity on cellulose, pectin, protein and starch, while no xylanolytic strains were observed. Two non-*Saccharomyces* strains were found effective for the production of cellulase and starch-degrading enzymes. Thirteen strains of *S. cerevisiae*, potentially able to use starch as the sole carbon source, were selected (Favaro *et al.*, 2008). Their weak growth on starch minimal agar plates was unexpected since the common dogma (Pretorius, 1997) is that wild-type *S. cerevisiae* cannot grow on media where starch is the only carbon source. In addition, extensive biochemical, physiological and genetic knowledge on their potentially amylolytic enzyme(s) was performed to look into this possible new starch-hydrolytic mechanism. The pattern of starch degradation halo of the selected *S. cerevisiae* strains was very similar to that exhibited by *Saccharomyces diastaticus*, which is clearly related to *S. cerevisiae*, except for ethanol performance and extracellular glucoamylase production. Starch utilisation in *S. diastaticus* depends on the expression of three unlinked genes, *sta1*, *sta2* and *sta3*. The search of the above genes or genes with the same function is in progress in the selected strains of *S. cerevisiae* mentioned above. These observations provided the basis for a further research on the gene-regulation and enzymatic efficiency of these DNA-sequences in order to enhance and improve their starch degrading activity through molecular approaches.

Since at least few strains showed good hydrolytic activities on complex polysaccharides (cellulose and starch), main components of low-cost plant biomass, this study encourages the selection of oenological microorganisms possessing interesting enzymatic profiles for future applications in bioethanol production.

This approach could allow the development of one-step bioconversion process, relying on wild-type oenological yeasts with desired properties for both lignocellulose hydrolysis and fermentation. These natural yeasts could also be the base of genetic modification for the construction of more efficient recombinant strains.

Keywords: bioethanol, one-step microbial conversion, oenological yeasts, extracellular enzymatic activities.

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AniA, a regulatory protein involved in polymer accumulation in Ensifer meliloti

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Bacterial biosynthesis of different polymers depends upon the genetic traits of the strain in use and by the culture conditions adopted. Although the separate production of exopolysaccharides (EPS) or poly-3-hydroxybutyrate [P(3HB)] is well documented, much less attention has been paid to the relationships between the synthesis of these two major compounds produced by bacteria. The biosynthetic pathways of these polymers are not directly linked, but they can be produced at the same time in some microorganisms. In rhizobia the general conditions governing the biosynthetic pathways (the nature and availability of carbon and nitrogen sources, the oxygenation, the energetic and redox states of cells, the environmental constraints, etc.) seem to be the same. Since the extent of EPS and P(3HB) production implies a significant energetic cost, a sensitive regulatory mechanism is required. A control of the synthesis and degradation of P(3HB) in bacteroids is important to maintain an effective symbiosis. Therefore, a well-regulated P(3HB) cycle results as a key factor for an optimal use of the available energy and for a balanced distribution of the carbon resources. Regulation of carbon flux into P(3HB) production occurs at multiple levels, and the comprehension of this regulation is essential for understanding the physiological functions of P(3HB) and possibly for applying this knowledge to industrial production of polyesters. AniA, a putative regulatory protein previously described (Povolo and Casella, 2000), and identified in the polyhydroxyalkanoates locus in *Ensifer meliloti* (Tombolini *et al.*, 1995), was found to be involved in carbon/energy regulation under normal growth conditions. The occurrence of AniA orthologs (described in some cases as PhaR) and organization of the respective genes were described in detail in many different bacteria (Pötter *et al*., 2005).
The present work gives a better inside of the role of the carbon flux regulator (*aniA*) in *E. meliloti* 41. Previous studies on other bacterial species indicated that the impaired synthesis of one polymer causes other reserve materials to be turned over (Breuer and Babel, 1999). A strain carrying a *lacZ* transcriptional fusion inside the *aniA* gene was constructed from *E. meliloti* 41 and from the mutant strain *E. meliloti* 41003 unable to accumulate polyhydroxyalkanoates (Povolo and Casella, 2008). *Ensifer meliloti* 41003 accumulates also less exopolysaccharides as compared to the wild-type strain 41 (Povolo and Casella, 2008). The transcription of *aniA-lacZ* fusions was studied in the wild-type and in the *phaC*-mutant backgrounds under different conditions. We also showed that an EPS negative mutant of *E. meliloti* 2011 (strain H3a) could accumulate more P(3HB) than the wild-type strain 2011.
All together these results indicate a clear correlation between P(3HB) and EPS biosynthesis. On the other hand, an *aniA-Km* mutation was transferred to different *E. meliloti* strains carrying *exp-lacZ* and *exo-lacZ* fusions. Phenotypic analysis of these double mutants showed a change from rough colonies of the single mutants to mucoid colonies in the double mutants (strains *exoY-lacZ/aniA-*, *exoL-lacZ/aniA-* and *exoP-lacZ/aniA-*) indicating an effect of *aniA* on EPS production.

Keywords: poly-3-hydroxybutyrate, exopolysaccharides, *Ensifer meliloti*, *aniA.*

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Co-localizing symbiont and endophytic bacteria in legumes by tagging with different fluorescent proteins

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Bacterial endophytes can promote plant growth and yield, suppress pathogens, solubilize nutrients or contribute to nitrogen uptake in plants. The use of fluorescent reporters is nowadays a key tool for studying microbe-plant interactions. In a previous work we assessed the presence of different endophytes in addition to rhizobia inside root nodules of wild legume plants and showed that rhizobia share nodules with a variety of different co-infecting taxa (Muresu *et al.*, 2008). In the present report we explore the plant-endophyte relationships attempting the co-localization of endophytes (*Pseudomonas* sp., *Enterobacter agglomerans*) and rhizobia (*Rhizobium leguminosarum* bv. *trifolii*) by the introduction of different fluorescent proteins as bacterial markers for the different kinds of bacteria. This would help their localization throughout the plant and in particular the distinction of rhizobia from other endophytes co-infecting root nodules. Green fluorescent protein (GFP) and red fluorescent protein (RFP) markers were integrated into the bacterial chromosome. For the marked constructs, a suicide plasmid, carrying the gfp gene in a transposable element was mobilized into the endophytic species. To obtain rfp-tagged bacteria, a replacement of *gfp* with *rfp* was made starting from the pRL765gfp plasmid. The use of dual fluorescence markers will allow to co-localize different bacterial taxa within plant tissues and will enable to plan different innovative applications in the field of symbiosis, biocontrol and other endophytic plant-microbe interactions.

Keywords: endophytes,confocal fluorescence microscopy.

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In vitro/in vivo tests and DGGE analysis to evaluate biocontrol activity of Azospirillum brasilense Sp245 against Rhizoctonia solani

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Plants under acclimatation are very vulnerable to phytopathogens. The rooted seedlings at the time of transplantation in peat become possible hosts of fungal agents such as *Rhizoctonia solani*. This species is the major fungus responsible for damping-off and root rot diseases (Matta, 1996) and is observed in over two hundred different plant species belonging to more than sixty phanerogam families. In this perspective the use of systemic fungicides to protect plants against these pathogens becomes widely necessary.
Rhizobacteria of the genus *Azospirillum* have been extensively used as inoculum for crop phytostimulation thanks to the positive interaction between these bacteria and plants at root level (Dobbeleare *et al*., 2001; Russo *et al*., 2005). *Azospirillum brasilense* Sp245 is a motile, free-living, Gram-negative bacterium that occur in the soil, capable of nitrogen fixation and phytohormone production, particularly indole acetic acid (IAA). In addition, an auxin-like molecule with antimicrobial properties was isolated (Somers *et al*., 2005) from the culture supernatant of *Azospirillum brasilense*. This molecule, identified as phenylacetic acid (PAA) seems to be involved in the biocontrol activity of the bacterium; thus, suggesting its use not only for phytostimulation but also for plant protection.
In this study the biocontrol effect of *Azospirillum brasilense* Sp245 against *Rhizoctonia solani* was evaluated by *in vitro* and *in vivo* inhibition tests. Moreover, Denaturing Gradient Gel Electrophoresis (DGGE) was used to detect presence/absence of *Rhizoctonia solani* in infected soil samples when treated with a formulation based on *Azospirillum. brasilense* Sp245. Both inhibition tests and DGGE profiles of rhizosphere microbial community showed a biocontrol activity of the bacterium against *Rhizoctonia solani*, suggesting that *Azospirillum brasilense* Sp245 could be helpfully used in place of chemicals in case of moderate *Rhizoctonia solani* plant infections.

Keywords: biocontrol activity, plant growth promoting rhizobacteria, *Azospirillum*, *Rhizoctonia solani*, Denaturing Gradient Gel Electrophoresis (DGGE).

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Sulphur dioxide affects viability, culturability and resuscitation of Brettanomyces/Dekkera bruxellensis

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The environmental and chemical conditions at the end of alcoholic fermentation (high ethanol concentrations, low content of fermentable sugars and low pH), allow the growth of only a few yeasts and bacteria. If uncontrolled, the metabolic activities of these micro-organisms can irreversibly affect the chemical composition of wine. Indeed the growth of *Brettanomyces/Dekkera bruxellensis* in wines may cause organoleptic modifications due to the production of volatile phenols and off-flavour and animal odours, resembling smells of a farm, horse sweat, medicine and animal leather (Chatonnet *et al*., 1995). Moreover Brettanomyces is able to produce biogenic amines, nitrogen compounds that are noxious to human health (Caruso *et al*., 2002; Vigentini *et al*., 2008; Agnolucci *et al*., 2009). During the last decade *Brettanomyces* has been extensively studied in order to gain knowledge and to establish adequate control measures. Sulphur dioxide and dimethyl dicarbonate are the only two allowed preservatives in the oenological practices to inhibit the growth of the spoilage micro-organisms. The effects on *Brettanomyces* populations in wines are not clearly understood. Traditionally the isolation and characterization of these yeasts from wine are carried out by using selective/differential microbiological media allowing to characterize the micro-organisms which can grow, multiply and give rise to colonies (the viable and culturable population). Thus, the information about viable but non-culturable microbial population (VNC) is lost. The aim of this research was to investigate the effect of sulphur dioxide on *Brettanomyces* by assessing its culturability (microbiological analysis by plating on selective media) and viability (vital staining with trypan blue and microscopic counts in a Thomas chamber). In this work, one representative of each haplotype previously characterised by Agnolucci *et al*. (2009), has been analyzed for its culturability by using a solid synthetic like-wine medium added with different concentrations of potassium metabisulphite. The results obtained show a different response of the haplotypes. The short-term effect of sulphur dioxide on the culturability and viability of the seven haplotypes has been analyzed for 24 hours since the addition of different concentrations of sulphur dioxide in a liquid synthetic like-wine medium (Vigentini *et al*., 2008). The results obtained show that the sulphur dioxide can affect the viability and the culturability of all *Brettanomyces* haplotypes and that the response to this chemical stressor is dose-dependent. A sulphur dioxide concentration that allows the transition from a culturable to nonculturable state, has been identified for the different haplotypes. For the haplotypes 1L, 3T, 20T, 12T, B2 and BF4 sulphur dioxide more than 0.2 mg l^{-1} allows the transition VC to VNC state, while for the haplotype BD7 this concentration is more than 0.4 mg l^{-1}. For one haplotipe, in a long-term storage experiment, culturability, viability and production of volatile phenols and biogenic amines have been analyzed, in the presence of a concentration of sulphur dioxide that can induce the VNC state with respect to a lethal dose and a control. It was possible to identify the experimental conditions in which viable non-culturable are induced to revert into culturable cells, a phenomenon described as resuscitation in the literature.

Keywords: *Brettanomyces/Dekkera bruxellensis*, sulphur dioxide, resuscitation, volatile phenols.

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Debaryomyces hansenii polymorphism analysis and food traceability: a multiplex PCR SSRs-related methodology

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Debaryomyces hansenii is a homotallic yeast with an essentially haplontic life cycle which can tolerate salinity levels up to 24%. Cryo- and osmo-tolerance account for its important role in several agro-food processes. *Debaryomyces hansenii* is the most common species of yeast found in all types of cheese and in brine thanks to its ability to grow in the presence of salt at low temperature and to metabolise lactic and citric acids. *Debaryomyces hansenii* also provides proteolytic and lipolytic activities during cheese ripening. *Debaryomyces hansenii* is one of the most frequent yeast species to be associated with chilled food and, although normally considered as non-pathogenic, one case of bone infection associated with this yeast was reported; moreover several clinical samples were identified as *D. hansenii* (or as its anamorph *Candida famata*) in superficial infections (Lépingle *et al*., 2000). The 26S rDNA sequences did not show any variation, confirming the extreme homogeneity of this species as predicted considering the available sequences deposited in GenBank. (Del Bove *et al*., 2009). In order to study the biodiversity of *D. hansenii* strains isolated from traditional cheese factories, a high discriminatory SSRs-related molecular tool has been designed. Microsatellites proved to be versatile molecular markers, particularly for population analysis, with high levels of inter- and intra-specific polymorphism (Zane *et al*., 2001). The complete genomic dataset of type strain *Debaryomyces hansenii* CBS767 available revealed that the genome seems to have the highest coding capacity among yeasts, accounting for 79.2% of the whole genome with a putative number of 6906 detected CDS. *Debaryomyces hansenii* is also the yeast with the most redundant genome with an overall redundancy of 49.2% (Dujon *et al*., 2004). In contrast to the triplets SSRs, di-and tetranucleotides SSRs are much less frequent in coding regions than in non-coding regions. On genomic yeast, triplet SSRs appear to be equally distributed on translated e non-translated regions (Li *et al*., 2002). The possibility to explore the whole genomic sequence gave us the opportunity to design and optimize a powerful 5-loci multiplex PCR based on tri-nucleotides patterns placed among the whole genome and on different chromosome positions. The results, presented and discussed, showed that this technique can provide enough variability to characterize and study the members of this species.

Keywords: *Debaryomyces hansenii*, Multiplex PCR, SSRs analysis, food traceability.

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Potential exploitation of lysozyme in the winemaking of Sicilian wines from "organic grapes"

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Lysozyme is a natural enzyme with muramidase activity which can work against a range of lactic acid bacteria (LAB), including *Oenococcus*, *Pediococcus* and *Lactobacillus* spp. that can affect wine stability (Cunningham *et al*., 1991). For such reason, over the last decade, there has been a growing interest in lysozyme as a supplement to sulphur dioxide for bacterial inhibition (Sonni *et al*., 2009). Since, lysozyme applications proved to be enough specific to require technical knowledge and expertise to be efficient, an experimental winemaking was performed to evaluate the efficiency of this enzyme application to produce a Sicilian wine from "organic grapes". Specifically, two experimental conditions were considered by using SO2 or lysozyme, alternatively, according to a defined industrial winemaking scheme, with periodically samplings in order to: i) control organic acid content, ii) control unwanted LAB and iii) prevent malolactic fermentation. Musts to be employed derived by grapes of "Catarratto", an ancient white variety, whose wine presented susceptibility to oxidation phenomena. The evaluation during time of lactic microflora revealed an appreciable microbial reduction following to lysozyme addition, even if this drop took a longer time (about 48 h) if compared to the production with SO2 (about 2 h). In addition, in wine produced with lysozyme the content of acetic acid was half of the value determined on the wine added with SO2 after 30 days by the end of alcoholic fermentation. Seventy-three LAB cultures isolated by different phases of both thesis were identified and characterized through molecular approach. Identification revealed the prevalence of *Lactobacillus plantarum* (about 60%) followed by the species *Lactobacillus mali*, *Lactobacillus hilgardii*, *Lactobacillus casei/paracasei/zeae* and *Leuconostoc mesenteroides* subsp. *mesenteroides*. By evidences emerged from minimal inhibition concentration (MIC) to lysozyme, lactobacilli resistance appeared to be strain's related; however, strains could be grouped into three classes according to their resistance behaviour. Representative strains of these three classes, (*Lactobacillus plantarum* B30, *Leuconostoc mesenteroides* subsp. *mesenteroides* B1 and *Lactobacillus mali* B4p) were employed in a winemaking trial by using synthetic must. In detail, each strain was inoculated in must added of lysozyme (20 mg ml^{-1}) singly or in combination with a commercial strain of *Saccharomyces cerevisiae*. Inoculated musts were used as controls. Lactic microflora was monitored for twenty days of fermentation, confirming evidences emerged by MIC determination. In all cases following to yeast addiction microbial loads appeared strongly reduced.
In conclusion, results obtained in this study confirmed the relative lack of antimicrobial activity of lysozyme on *Lactobacillus* strains already recorded during preceding surveys (Delfini *et al*., 2004). Nevertheless, in the chosen challenging experimental conditions, lysozyme activity proved to be a potential suitable tool to control lactic bacteria populations and to manage the start of the malolactic fermentation only if combined with a highly fermentative yeast, as already pointed out by several authors (Ribéreau-Gayon *et al*., 1998). Since lysozyme is a protein that partially remains in the white treated wine, further investigations are needed to highlight possible secondary effects and understand how to properly use it in this Sicilian wine production.

Keywords: lysozyme, white wine, *Lactobacillus*.

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Array-based transcriptional analysis of Clostridium sporogenes during its vegetative cycle, germination process and outgrowth

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Clostridium sporogenes, Gram positive bacterium usually involved in food spoilage and frequently isolated from late blowed cheese is genetically indistinguishable from proteolytic *Clostridium botulinum* and is the non-neurotoxinogenic counterpart often used as a model for the toxic subtypes. *Clostridium sporogenes* has been used as a model to study gene expression during the different steps of its life cycle.
The objectives of this work were (1) use an array-based transcriptional analysis to study gene expression in the different steps of Clostridium sporogenes life cycle: dormant spore phase, germination and outgrowth, (2) determination of the mRNA transcripts abundance and their role in dormant spores, (3) to obtain a first insight in the regulation of physiological, morphological and transcriptional stages of *Clostridium sporogenes*/*botulinum* life cycle.
Clostridium sporogenes UC9000, isolated from milk in our laboratory, was used for all the experiments. Cells and spores at different stages have been selected using OD measurements and scanning electron microscopy. Combimatrix microarray 12K was applied to all samples and conditions analysed.
The trascriptomic analysis we made, validated the ipothesis that also in *Clostridium sporogenes* dormant spores mRNAs are abundant and strikingly different from those present in growing cells. The transcripts we found in silent spores included numerous genes responsible of different functions. Only a few genes were overexpressed in germinating spores in comparison to dormant ones.
Spores were already equipped with a large amount of transcripts useful to front the next steps of outgrowth when environment conditions could be favourable. Meanwhile, a basic activation of polyfunctional genes happened in germinant spores. The different RNA fingerprint obtained between cells and spores make easier to distinguish the two cellular stages.

Keywords: *Clostridium sporogenes*, microarray, germination, spores.

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Use of Biolog System to evaluate the antimicrobial activity of volatile constituents of essential oils for contamination control in ready-to-eat salads

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The Biolog System (Biolog Inc., CA, USA) is a methodology for microbial metabolic characterization, where the microbial activity on specific carbon sources is spectrophotometrically measured by the absorbance variation at 590 nm of a Redox indicator. The carbon sources and the Redox indicator are contained in the microwells of Elisa type plates (Preston Mafham *et al*, 2002). In this work, the ability of this system to detect the antimicrobial activity of volatile constituents of essential oils against the natural microflora of ready-to-eat salads was evaluated in comparison with the results obtained by plate count method (Freitas dos Santos *et al*, 2002) .
Six compounds with possible antimicrobial properties were tested (Skocibusic *et al*, 2006; Lanciotti *et al*, 2004): eugenol, limonene, cinnamaldheyde, thymol, menthol and γ-terpinene. They were added in 2 concentrations 1) in the microwells of MT Biolog plates (where only the Redox indicator is present, without any additional carbon source) and 2) in bacteriology glass tubes, in both cases with a lettuce-juice nutrient broth inoculated with the microflora extracted from a ready-to-eat lettuce salad. Chloramphenicol, pimaricin, sterile broth and inoculated broth with no compounds were used as controls.
The plates were incubated at 25 °C in the dark and microbial metabolic activity was periodically measured by recording the absorbance variations in the wells at 590 nm during 168 h of incubation. Such variations were expressed as AWCD (Average Well Colour Development). The tubes were similarly incubated and sampled after 24 and 72 h for plate counting with 3M PetrifilmTM "Aerobic Count Plate" and "Yeast and Moulds Count Plate". Results were expressed as CFU/ml.
The AWCD values measured at 24 h and 72 h were used to classify the compounds and their concentrations according to their antimicrobial effects on metabolism of the inoculated microflora. Moreover, by fitting to proper equation models the curves of AWCD temporal evolution during 168 h of incubation, a finer evaluation of the inhibiting behaviour of the substances tested was allowed.
Growth and metabolic data resulted highly congruent: both CFU/ml and AWCD values treated by hierarchical cluster analysis provided the same groupings. In particular, a relevant inhibition was observed in eugenol (0.5 and 3 µl/ml), cinnamaldheyde (0.2 µl/ml) and thymol (0.5 mg/ml). Thus, Biolog metabolic profiling seems suitable for preliminary screening antimicrobial activity of natural substances.

Keywords: Biolog, antimicrobial activity, VOCs, lettuce microflora.

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Relationship between Biolog response and microbial contamination in minimally processed vegetables

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Quick, simple and inexpensive methodologies to determine the microbial load in food during industrial processes are fundamental tools for an accurate shelf-life estimation in fresh or minimally processed vegetables.
In this work, the potentialities of Biolog System as an indirect method of microbial quantification have been explored, with the use of ECO type microplates (ECOmp). In the Biolog methodology, the microbial attack of 96 different carbon sources contained in the microwells of ELISA type plates, induces the reduction of a redox indicator present in the wells that is measured as increase of optical density (OD) at 590 nm and used as a signal of microbial metabolic activity (Garland *et al.*, 2001; Preston Mafham *et al.*, 2002). Such metabolic signal was here exploited for the indirect quantification of microbial loads in a minimally processed salad.
The suitability of ECOmp to allow growth and activity of microbial groups characteristic of ready-to-eat salads was verified by trials on pure strains previously isolated from such products (*Pseudomonas fluorescens, Enterobacter* sp*., Lactococcus* sp.). To investigate the relationship (by linear regression) between microbial counts in suitable medium, OD values and the kinetic parameters of the OD-curve vs time obtained by multiple absorbance readings during 96h of incubation, ECOmp were inoculated with a dilutions-set of the pure strains and the total microflora extracted from the food under study. OD-curves vs time were fitted to mathematical models: Lindstrom's $y=k/[1+exp(-r*(t-s))]$ and Gompertz's modified $y=A*exp[-exp(\mu*e*1/A*(\lambda-t)+1)]$ functions.
Once evaluated the existence of good proportionality between cell inoculum density and instrumental response, a first validation of these results was made by the development of calibration curves for a specific minimally processed salad. These equations were applied for the microbial load evaluation in the same product, by comparing the CFU/g values calculated by plate-counting and those predicted by the calibration curves (Gardini *et al.*, 1997).
No statistically significant differences were observed between the microbial counts (CFU/g) obtained by traditional plate-counting and those predicted by the calibration curves constructed on the data of optical density measured between 24 and 48 hours of microplates incubation.
Investigations directed to decrease the time of incubation for the information achievement are in progress, along with trials to make such technique selective by modifing the composition of the detection media, by means of Biolog MT2 microplates, where only the redox indicator is present, without any additional carbon source, except those choosen by the operator.

Keywords: Biolog; minimally processed vegetables; microbial detection.

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Use of Lactobacillus plantarum strains with probiotic abilities as starters for Bella di Cerignola table olives

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In a preliminary phase 19 strains of lactic acid bacteria (LAB), identified as *Lactobacillus plantarum* through Rep-PCR and DNA-sequencing, were isolated from natural fermentations of Bella di Cerignola table olives. After a probiotic (adhesion to IPEC-J2 cells, survival under gastro-intestinal simulated conditions, antibiotic-resistance and antagonism against some food-borne pathogens) and a technological characterization (effect of salt, pH, temperature and phenolic compounds and biogenic amine production), 3 strains (labelled as c10, c18 and c19) were chosen and used throughout this study for their probiotic and technological performances.
These microorganisms were used as starters for Bella di Cerignola olives, produced through the Spanish style, focusing both on the effect of salt concentration (8 and 10%) and the addition of glucose (0.5%). The starters were inoculated in the brines at ca. 5-6 log CFU/ml and the fermentations were carried out in batches containing ca. 10 kg of olives and 10 l of brines; the amount of the starters was 30 ml. The evaluation of LAB and natural occurring microflora of olives and brines (Enterobacteriaceae, *Pseudomomas* spp., staphylococci, spore former and mesophilic bacteria and yeasts) were performed throughout 49 days, as well as the evaluation of the pH of the medium and the concentration of the lactic acid produced.
As expected, the starters prevailed on the natural occurring microflora of brines just after 7-14 days of fermentation; moreover, they showed the ability to adhere to the surface of olives up to a value of 8 log CFU/g. Regarding the interaction of the starters with the occurring microflora of olives, a promising result was the inhibition of yeast microflora throughout the 1st phase (after 7-14 days) and the reduction of their cell number in the latter step (after 42-49 days).
The combination LAB inoculum/addition of glucose decreased the pH of the brines to 4.3-4.5; otherwise, in the control batch (olives neither inoculated with the starters nor added with glucose) the final pH was 5.1. Finally, in the sample inoculated with the starters or added with the glucose the pH decreased up to the threshold value of 4.7-4.8.
In conclusion, the results of this research show a strong implication for olive production in Apulian region: the selected strains, in fact, could be used successfully as starters for Bella di Cerignola olives, as they decreased the pH of the brines and inhibited the spoiling microflora. Moreover, the use of the proposed strains shows at least two topics of great interest: the possibility of adding to the brines a low amount of inoculum (ca. 0.3%) and the use autochthonous strains acting at the same time as starters and probiotics.

Keywords: table olives, *Lactobacillus plantarum*, probiotic strains, starters.

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Alicyclobacillus acidoterrestris inhibition through cinnamaldehyde, eugenol and a thermal treatment: use of the desirability approach for the optimization of the technological process

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Alicyclobacillus acidoterrestris is an emerging spore-former microorganism, able to grow at low pHs (2.5-3.0) and high temperatures (55-60 °C). Originally isolated from fruit juices, nowadays several strains have been isolated from soil, tea and other acidic drinks; in the last years the spoilage incidents due to alicyclobacilli increased and different types of foods, like shelf-stable ice-tea, were contaminated. At present alicyclobacilli are more widespread than originally observed and they are becoming an industry-wide problem.
The thermal approach has been considered for a long time as the only way to inactivate this spoiling microorganism; however, several alternative approaches have been proposed and successfully used both in lab media and in foods (high hydrostatic and homogenization pressure, essential oils, lysozyme, nisin and other bacteriocins). This research proposes the use of cinnamaldehyde and eugenol, as effective hurdles to inhibit spore germination, through two steps: the use of cinnamaldehyde, combined with a mild thermal treatment, and the combination cinnamaldehyde/eugenol; the methodology of the central composite design was used, along with the desirability approach. The experiments were performed on spores, inoculated firstly in a lab medium (Malt Extract Broth, acidified to pH 4.5) and then in tomato and apple juices.
In the 1st step, cinnamaldehyde (0-160 ppm) was combined with the pH of the medium (3.5-5.5) and a thermal processing (4-12 min at 90 °C). A multiple regression procedure through the saddlepoint approach highlighted that the maximum inactivation of *A. acidoterrestris* spores occurred at pH 4.3, adding 97 ppm of cinnamaldehyde and processing the samples at 90 °C for 9 min; these results were validated in tomato juices, assessing that two "convenient combinations" are the following: 40 ppm of cinnamaldehyde/12 min or 80 ppm of cinnamaldehyde/4 min.
In the 2nd phase, cinnamaldehyde (0-80 ppm) was combined with eugenol (0-160 ppm), to reduce the amount of the aldehyde due to its strong impact. Cinnamaldehyde appeared as the most strong antimicrobial compound; otherwise, eugenol acted as a strengthening element and allowed a reduction of cinnamaldehyde in the system. Then, the experiments were performed in a commercial apple juice, thus highlighting that spore germination could be inhibited through the use of 80 ppm of eugenol, 40 ppm of cinnamaldehyde or alternatively through the combination of 40 ppm of eugenol with 20 ppm of cinnamaldehyde.

Keywords: *Alicyclobacillus acidoterrestris*, spores, essential oils, thermal treatment.

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Monitoring of yeast microflora during Gragnano DOC (Campania Region, Italy) winemaking and selection of autochthonous Saccharomyces cerevisiae strains

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Known since antiquity, appreciated and cited by Plinio with different definitions, Gragnano wine reached such great notoriety that all red wines products in the Naples area were identified as "Gragnano". Gragnano DOC (D.M. 03/10/1994, G.U. n. 238 - 12/10/1994) is made with different kinds of red grapes: Sciascinoso and Aglianico (20%), Piedirosso (40%) and other grapes (40%).
The study and the conservation of the biodiversity of wine yeasts have recently become object of growing interest. The maintenance of such biological patrimony is in fact essential both to obtain starter strains that are able to fully develop the typical flavours and aromas of wines originating from different grapevine cultivars and to ensure the conservation of gene pools of primary importance for the preservation of productive activities based on yeast-mediated processes (Pretorius, 2000).
In this study we monitored the natural yeast microflora during fermentation of the Gragnano wine, evaluated the genetic and technological diversities of *Saccharomyces cerevisiae* strains and selected some autochthonous *S. cerevisiae* strains suitable to be used as starters.
Yeast microflora of 4 different wine fermentations were identified and monitored (must-wine up to 130-140 days of fermentation) by means of molecular techniques: 5.8 S rDNA RFLP (Esteve-Zarzoso *et al.* 1999), 5.8 S rDNA sequencing and interdelta analysis (Legras and Karst, 2003).
Yeast microflora of this environment was represented by *Metschnikowia fructicola*, *Issatchenkia terricola*, *Brettanomyces bruxellensis*, *Candida zemplinina*, *Zygoascus hellenicus*, *Hanseniaspora opuntiae* and *S. cerevisiae*.
Among 79 *S. cerevisiae* isolates a total of 31 biotypes were individuated by interdelta analysis. In two wine fermentations a low diversity was found (3-4 biotypes); while, in the remaining two wine fermentations were characterized by a higher diversity (12 biotypes each). Some oenological traits (Zambonelli, 1998; Ranieri and Pretorius, 2000) of these 31 biotypes were also evaluated. Thirty percent of biotypes were able to grow in presence of 16% (v/v) ethanol and 100 mg/L SO2, 25% showed a fermentation vigour >5 (g CO_2/100 ml of Gragnano must), 50% showed a fermentation power >10 (g. CO_2/100 ml of Gragnano must), 21% were able to grow at 10 °C and 25% at 37 °C, 18% were able to produce killer toxin, 59% were resistant to 6.25 mM of Cu^{2+}, 75% showed a low production of H_2S. All biotypes showed beta-gucosidase activity on MUG, 50% on esculine and only one on arbutin. Thirty percent of biotypes showed a glycerol production higher than 8 g/L and 25% a acetic acid production lower than 0.20 g/L. Finally, after 21 days of fermentation, four biotypes were able to absorb > of 50% (50-54) of OTA in simile must contaminated with 5 ppb of OTA and three were able to release > of 130 mg/L (130-170) of mannoproteins in simil must after the same period.
In conclusion during this study it was possible to select some autochthonous *S. cerevisiae* biotypes harbouring important oenological traits and therefore suitable to be used as starter cultures.

Keywords: monitoring, Gragnano DOC, yeast microflora, *Saccharomyces cerevisiae* biotyping, starters.

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A genetic marker for distinguishing Lactococcus garvieae strains from different food environments

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Lactococcus garvieae is considered an emerging pathogen of increasing clinical significance in the field of fish farming and of veterinary and human medicine (Eyngor *et al*., 2004). On the contrary , this species was shown to represent a relevant positive component of the microbial population of artisanal Italian cheeses (Fortina *et al*., 2003, 2007). For these reasons, the obtainment of a specific marker for distinguishing *L. garvieae* strains coming from different ecological niches is of practical significance. Our genetic studies, carried out constructing different vectorette libraries, provide evidence that in fish isolates no genes involved in lactose utilization were present. For dairy isolates, a single system for the catabolism of lactose was found. It consists of a lactose transport and hydrolysis depending on a phosphoenolpyruvate-dependent phosphotransferase system combined with a phospho-β-galactosidase. The genes involved were highly similar at the nucleotide sequence level to their counterparts in *Lactococcus lactis* species; however, while in many *L. lactis* strains these genes are plasmid encoded, in *L. garvieae* are chromosomally located. Thus, in the species *L. garvieae*, the phospho-β-galactosidase gene, detectable in all strains of dairy origin, but lacking in fish isolates, can be considered a reliable genetic marker for distinguishing biotypes from the two different ecological niches. Moreover, we obtained information regarding the complete nucleotide sequence of the gal operon in *L. garvieae*, consisting of a galactose permease and the Leloir pathway enzymes.This is one of the first reports concerning the determination of nucleotide sequence of genes, other than the 16S rDNA gene, in *L. garvieae* and should be considered a step in a continuous effort to explore the genome of this species with the aim to know the real relationship between the presence of *L. garvieae* in dairy products and food safety.

Keywords: *Lactococcus garvieae*, dairy cultures, lactose genes, genetic marker.

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Yeast viability as a parameter to maintain artisanal beer quality features

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Despite brewing parameters in an artisanal brewery are often bound to empirical factors such as fermenter availability and consumer demand, brewer should keep on chasing reproducibility and process standardization in order to guarantee a constant high quality of the final product. In order to increase process standardization and to maintain the quality of the final product, yeast viability has been monitored in different steps of an artisanal beer production and has been correlated with fermentation activities from the first pitching throughout subsequent cycles of fermentation. Yeast viability was evaluated during seven fermentation cycles of the artisanal pilsner beer VIÆMILIA, single or double batch brewed. Fermentation activities were also evaluated determining glucose and maltose consumption and ethanol production. We observed that, standardization of procedures and parameters from raw materials throughout all fermentation batches, plays a key role in producing a more uniform and with constant features product. Yeast viability was found to be variable according to the selected parameters being affected by yeast handling before re-pitching In both single and double batch brewed trials, yeast inoculated with higher viability performed better in fermentation giving a faster sugar consumption for single batch brewed trials, or a greater consumption of sugars before the top up and a faster reaching of ethanol production and yield stability for double batch brewed trials. Studying how yeast viability could affect fermentation performance, we provide evidence to suggest that yeast viability should be taken into account for the development of an efficient program of yeast handling in order to give the consumer the product with the taste he knows and likes.

Keywords: artisanal beer, yeast viability, fermentation dynamics.

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AFLP technique as molecular typing of Streptococcus thermophilus strains

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Streptococcus thermophilus is a lactic acid bacteria (LAB) widespread used in milk fermentation processes as a starter culture. The biodiversity of bacteria involved in cheese production is considered a fundamental factor for the maintenance of the typical features of traditional cheese products and actually different techniques are available to characterize microorganisms at the strain level. Genotyping is a molecular tool widely used to study microbial diversity because it is able to fingerprint specific DNA patterns that are characteristic for a single strain.
In the present study we reported the optimization of an AFLP (Amplified Fragment Lenght Polymorphism) protocol in terms of primer combinations and experimental conditions to differentiate S. thermophilus strains at intraspecific level. Moreover the fingerprinting resolutions of RAPD (Random Amplified Polymorphic DNA) and AFLP were evaluated and compared. The overall data suggest that genotypic characterization performed by AFLP provide a better view of microbial diversity of S. thermophilus indicating that RAPD is less discriminating than AFLP.

Keywords: *Streptococcus thermophilus*, AFLP, biodiversity.

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Intraspecific biodiversity of Lactobacillus sanfranciscensis from traditional sourdoughs

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Lactobacillus sanfranciscensis, one of the most frequently found lactic acid bacterium in artisan as well as industrial sourdough fermentations, is known to possess a significant intraspecific variability, both phenotypic and genotypic (Foschino *et al*., 2001). Since many traditional baked products in Italy originate from sourdough processes, the identification at strain level of *L. sanfranciscensis* isolates is a key step in the characterization of the sourdough microflora, on which both quality and typical sensorial properties of each final product are dependent. In this work, several lactobacilli from three sourdoughs used to produce as many typical baked products were identified and characterized at strain level by molecular methods. Identification of the isolates by Ribosomal DNA Restriction Analysis (ARDRA) demonstrated that 69 isolates belonged to *L. sanfranciscensis* species, but the use of the restriction enzyme HinfI originated two different restriction profiles: ribotype I, that included a reference strain (DSM 20663), and ribotype II, that included most assayed isolates and the type strain ATCC 27651^T. Since sequence analysis of the amplified 16S rDNA from some strains representative of the two ribogroups did not show mutations at any HinfI restriction's site, the two ribotypes might have been originated by mutations on only few rRNA-operon copies. It is underlined that only one sourdough was characterized by the presence of both *L. sanfranciscensis* ribotypes. The characterization at strain level of all the *L. sanfranciscensis* isolates was carried out by Random Amplification of Polymorphic DNA (RAPD-PCR) using ten different primers, in both single and two-primer reactions. Only four sets of reaction mixtures proved to be useful for an adequate isolate discrimination. Numerical elaboration of the combined RAPD-PCR profiles of the 69 isolates by UPGMA analysis resulted in a dendrogram that confirmed a high degree of genetic variability, 64 out of 69 isolates grouping into 12 clusters at a similarity of 75%. None of these clusters included the *L. sanfranciscensis* reference strains. Since, at the same similarity level, the isolates from each sourdough grouped into at least three well distinguishable clusters not including isolates from other sourdoughs, a high degree of intraspecific biodiversity characterized the *L. sanfranciscensis* population inhabitating each sourdough. This finding was strengthened by taking into account only the isolates from the sourdough harbouring both *L. sanfranciscensis* ribotypes. Indeed, the UPGMA dendrogram obtained from only the RAPD-PCR patterns of these isolates showed, at same level of similarity (75%), five groups, each contaning only isolates possessing either ribotype, I or II. In any case, a certain relationship between strains and their isolation source was ascertained.

Keywords: *Lactobacillus sanfranciscensis*, sourdough, ARDRA, RAPD-PCR.

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Combined antifungal activity of eugenol, ph and water activity against Fusarium oxysporum

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Fruits and vegetables are very susceptible to attack by pathogenic fungi; for example, some species of Fusarium spp. cause the potato tuber dry rot, which is an important disease of stored potatoes. The application of higher concentrations of chemicals is in attempt to overcome this problem; however this approach increases the risk of high level toxic residues in the products, which is particularly serious because fruits and vegetables are consumed in relatively short time after harvest. Natural plants extracts, called Essential oil (EOs), may provide an alternative to these preservatives.
In this study the antifungal activity of eugenol, the major phenolic component of clove oil, against *Fusarium oxysporum*, was investigated.
In a preliminary phase the effect of different concentrations (0-2000 ppm) of eugenol on *F. oxysporum* growth, in a model system, was studied.
Duplicate Petri dishes of potato dextrose agar (PDA) with antifungal compounds for each eugenol concentration were centrally inoculated by pouring 10 µl of the conidia suspension (103 CFU/ml) to give a circular inoculum. Growth control without antifungal compounds was prepared as above. All plates were incubated at 25 °C and periodically observed to measure colony diameter.
The experimental data were modelling according to Gompertz equation and the derived parameters, such as. The maximum colony diameter attained during the experimental time (35 days), the maximal radial growth rate, the lag time (i.e. the number of days before the beginning of radial fungal growth) and the minimum detection time (MDT) defined as the time to attain 1 cm of colony diameter, were evaluated.
Eugenol showed antifungal activity; F. oxysporum growth was affected also by the lowest concentrations (0-140 ppm) by increasing of MDT and lag time. A reduction in maximum colony diameter attained within the experimental time, was also observed. At the highest concentration (> 140 ppm) eugenol showed a fungicidal activity against F. oxysporum: for these eugenol concentrations, it was not possible to calculate Gompertz parameters, because *F. oxysporum* was completely inhibited within the running time.
In a second experimental phase, the influence of different factors (pH, water activity, different concentration of eugenol) on the *F. oxysporum* growth at two inoculum levels (103 CFU/ml – 106 CFU/ml) was studied and the responses were estimated by statistical Central Composite Design (CCD) method.
The obtained results showed a positive correlation between eugenol concentration and mould inhibition; besides, the acidification of PDA significantly affected the fungal growth: the higher inhibition was observed at the highest eugenol concentration (100 ppm) and at the lowest pH value (2.5). On the contrary, different water activity values did not affect *F. oxysporum* growth.
In vivo studies may be required to confirm the applicability of the results obtained

Keywords: *Fusarium oxysporum*, eugenol, antifungal activity.

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The hsp16 gene of the probiotic Lactobacillus acidophilus is differently regulated by single and combined stress as revealed by reverse transcription quantitative PCR (qRT-PCR)

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Lactobacillus acidophilus is used extensively for the production of dairy foods and is increasingly applied in the area of health improvement, as probiotics, in the form of yogurts and dietary supplements. It is also being considered as a potential vaccine-delivery vehicle to the gastrointestinal tract (Sanders and Klaenhammer, 2001). The probiotic effects of this organism include the treatment of various types of diarrhoea, alleviation of Crohn's disease, and balancing of intestinal microflora through the growth modulation of bacteria present in the gastrointestinal tract (Pfeiler *et al.*, 2007). Because of the importance of this organism as probiotics, studies into different mechanisms of stress response may be useful in order to select or to improve *L. acidophilus* strains able to growth under harsh stress condition.
In this study, a reverse transcription quantitative PCR (RT-qPCR) was developed and used in order to quantify the transcript level of a small heat shock gene (*shs*) in *L. acidophilus* ATCC 4356 under stress conditions such as heat (45 and 53 °C), bile (0,3% v/v), NaCl (1 M and 2.5 M), and low pH value (pH 4). The *shs* gene of *L. acidophilus* ATCC 4356 was induced either by salt or by high temperature stress and repressed by bile stress. Interestingly, the *shs* gene was repressed when combined salt and high temperature stresses were applied.
The effect of overproducing the small heat shock protein (sHsp) was investigated in *L. acidophilus* ATCC 4356. Overproduction of the *shs* gene yielded a protein of approximately 16 kDa, as estimated from Tricine sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE) in agreement with the predicted molecular weight of sHsp. Small Hsp overproduction alleviated the reduction in growth rate triggered by exposing exponentially growing cells to heat shock (45 °C) and salt stress (1 M and 2.5 M). Therefore, the observations reported in our study could be of general interest in developing *Lactobacillus* strains with an improved resistance to multiple stresses for applications where growth under harsh stress condition would be an attribute.

Keywords: RT-qPCR, *Lactobacillus acidophilus*, small heat shock gene, stress, *ldhD.*

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Safety for celiac patients of breads made of wheat flour hydrolyzed during food processing

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Celiac disease (CD) is an inheritable disorder of the small intestine that affects ca. 1% of the world population. Nowadays, the only effective treatment for CD is the life-long gluten-free diet. More recently, it was shown that selected sourdough lactobacilli (Di Cagno *et al*., 2004, De Angelis *et al*., 2007) in combination with fungal proteases, routinely used in bakery, decreased the residual concentration of gluten to below than 12 ppm during food processing (Rizzello *et al*., 2007). This study aims to evaluate the safety of daily administration (60 days) to CD patients of breads made of wheat flour hydrolyzed during food processing. Previously selected sourdough *Lactobacillus alimentarius* 15M, *L. brevis* 14G, *L. sanfranciscensis* 7A, LS3, LS10, LS19, LS23, LS38, LS47 and *L. hilgardii* 51B, and fungal proteases from *Aspergillus oryzae* and *A. niger* were used to ferment wheat flour. Fermentation and bread making were carried out using the biotechnology standardized previously (Rizzello *et al*., 2007). Three type of breads were manufactured: (i) BY fermented by baker's yeast alone; (ii) S1, sourdough bread fermented with sourdough strains *L. alimentarius* 15M, *L. brevis* 14G, *L. sanfranciscensis* 7A and *L. hilgardii* 51B; S2 fermented with all sourdough strains of lactobacilli together with fungal proteases. Sixteen CD patients were enrolled and separated in three groups: (i) six patients consumed BY; (ii) four patients consumed S1; and (iii) six patients consumed S2. Each patient daily consumed 200 g of wheat bread and was subjected to serological, morphometric and immunohistochemistry analyses before and at 30 and 60 days. As determined by R5-ELISA analysis, BY, S1 and S2 breads had the following concentration of gluten: 80,127 ± 123, 2,480 ± 86 and 8 ± 2 ppm. All patients that consumed, for 60 days, BY bread showed CD symptoms (malaise, abdominal plain, diarrhoea) with increased level of anti-EMA, anti-tTG antibodies, lymphocytes infiltration and mucosa atrophy. None of the four patients challenged with S1 bread showed any CD symptoms but three of them had alterations of the small intestinal mucosa, increased CD3 and two developed subtotal atrophy. Five patients, challenged with S2 bread, showed no clinical symptoms, neither an increase of the serum anti-tTG antibodies nor a modification of the architecture or the grade of inflammation of the intestinal mucosa. The sixth patient interrupted the trial for reasons unrelated with the challenge. The complete exclusion of dietary gluten is difficult (Gobbetti *et al*., 2008). Other therapeutic options are under investigation, including the oral supplementation with microbial oligopeptidases. Nevertheless, most of these enzymes showed limitations since they may be irreversibly inactivated in the stomach by pepsin and acidic pH, thus failing to degrade gluten before it reaches the small intestine (Pyle *et al*., 2005). This is the first time that a bread made of wheat flour hydrolyzed during processing is shown to be not toxic after administration to CD patients for 60 days.

Keywords: celiac disease, gluten, sourdough.

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Effect of autochthonous lactic acid bacteria starters on shelf-life, health-promoting and sensory properties of raw vegetables

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Vegetables are strongly recommended in the human diet since they are rich in antioxidants, vitamins, dietary fibres and minerals. The major part of the vegetables consumed in the human diet are fresh, minimally processed, pasteurized or cooked by boiling in water or microwaving. Minimally processed and, especially, fresh vegetables have a very short shelf-life since subjected to rapid microbial spoilage and the above cooking processes (Zhang and Hamauzu, 2004). Among the various technological options, lactic acid fermentation may be considered as a simple and valuable biotechnology for maintaining and/or improving the safety, nutritional, sensory and shelf-life properties of vegetables (Buckenhüskes, 1997). The Department of PPAM of the University of Bari has developed some studies on the fermentation of raw carrots, French beans, and marrows (Di Cagno *et al*., 2008a), tomatoes (Di Cagno *et al*., 2008b) and red and yellow peppers (Di Cagno *et al*., 2009) by autochthonous lactic acid bacteria starters. Autochthonous lactic acid bacteria were identified from raw vegetables by partial 16S rRNA gene sequence and subjected to typing by Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) analysis. Single and/or mixed autochthonous starters were selected based on the kinetics of growth and acidification. Specific protocols to ferment vegetables were set up. Unstarted or raw vegetables fermented with allochthonous strains were used as the controls. As shown by RAPD-PCR analysis, autochthonous strains persisted during processing and storage. When fermented with the autochthonous starters, raw vegetables were characterized by rapid decrease of pH, marked consumption of fermentable carbohydrates, and inhibition of Enterobacteriaceae and yeasts. Fermentation with the allochthonous starters did not acidify to the same extent and did not inhibit bacteria and yeasts. Overall, unstarted and raw vegetables fermented with allochtonous starters showed marked decreases of ascorbic acid, glutathione and total antioxidant activity during storage with respect to those fermented with the autochthonous starters. The same was found for the indexes of colour and firmness. Sensory analysis differentiated raw vegetables fermented with the autochthonous starters. In particular, tomato juice fermented with the two selected autochthonous strains were characterized based on volatile components through Purge and Trap or Solid Phase Microextraction Gas Chromatography-Mass Spectrometry analysis. As shown by Principal Component Analysis a large number of volatiles belonging to various chemical classes markedly differentiated tomato juices fermented with autochthonous strains with respect to unstarted tomato juice or fermented with allochthonous strains. Fermentation of raw vegetables seems to be a simple biotechnology option but indispensible to maintain elevated nutritional, rheology and sensory properties as well as to ensure the hygiene. Protocols of fermentation have to be differently set up depending on the intrinsic characteristics of the vegetables. Starter lactic acid bacteria has to be inherently adapted to the characteristics of the raw materials. Although belonging to the same species, the metabolism of autochthonous strains was always preferred with respect to allochthonous ones.

Keywords: autochthonous lactic acid bacteria, raw vegetables, shelf-life, nutritional properties.

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Pecorino d'Abruzzo Raw Ewes' milk cheese: seasonal factors of biochemical and microbiological aspects

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Quality of artisanal cheeses is closely associated with a given territory and its traditions. These products contribute to preserve food biodiversity. Such instances must cop the respect of quality and hygiene levels: the use of particular microbial starters is a key factor in this context. "Pecorino d'Abruzzo" cheese is a semi-hard cheese of Central Italy with raw ewe milk.
This paper deals with the microbial, compositional and biochemical profiles during cheese ripening. Microbial isolates suitable as starter and adjuncts were characterized; their behaviour was investigated in response to variations of raw milk properties.
Samples of traditional Pecorino to study microbial and biochemical characters were taken from three different batches worked out is usual in May (Spring) and November (Autumn).
As much in 202 bacterial strains were isolated on M17 and MRS agar and identified by the combined use of physiological and biochemical assays, species-specific PCR and restriction analysis of the amplified 16S rRNA gene.
The majority of the lactic acid bacteria isolates were *Lactobacillus paracasei*, *Lactobacillus plantarum* and *Lactococcus lactis* subsp. *lactis*. *Enterococcus faecium* and *Enterococcus faecalis* were also isolated, mostly from cheese samples (46% of the coccal isolates), together with a lower amount of *Streptococcus thermophilus* (3.4%). A substantial presence of enterococci already report by Di Cagno *et al*. (2003) in other Italian ewe cheeses was confirmed. Spring batches showed a more complex microbial population: *Lactobacillus brevis*, *Lactobacillus curvatus*, *Lactobacillus paraplantarum*, *Lactobacillus pentosus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus fermentum*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus*, *Pediococcus pentosaceus*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis cremoris*, *Leuconostoc mesenteroides* were found, while in autumn batches only *Lactobacillus pentosus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus zeae*, *Streptococcus thermophilus*, *Lactococcus lactis* subsp. *lactis* were isolated.
A decrease in moisture content, together with an increase in total proteins and fats was observed during ripening. In the first 20 days of ripening, pH values around 5.1 were measured followed by a slight increase up to 5.7 by the end of ripening.
Urea-PAGE pH 4.6-insoluble fraction and RP-HPLC peptide profiles of the 70% (v/v) ethanol-soluble and ethanol-insoluble fractions of the cheeses demonstrated some proteolysis (O'Mahony *et al*., 2003). Urea-PAGE results showed extensive proteolysis of alfaS1-casein (CN). In autumn production alfaS1-CN was completely degraded in 20 days; while in spring cheeses it remained at low level and completely disappeared after 60 days. A slight hydrolysis of beta-CN with low level of gamma-CN was found; beta-CN was not completely hydrolyzed at the end of ripening for all cheeses. Formation of gamma-CNs from beta-CN was more evident in spring cheeses. RP-HPLC profiles 70% (v/v) ethanol-soluble and insoluble fractions showed several peaks, indicative of heterogeneous mixtures of proteolysis products evolving through ripening. Principal Component Analysis (PCA) of peak height data on covariance matrix was performed. PCA explained no more than 26.2% of cheeses variability as an effect of seasonality.

Keywords: Pecorino cheese, raw milk, ripening, microflora, biodiversity.

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Synthesis of γ-aminobutiric acid (GABA) by lactic acid bacteria: from functional foods to cosmetics

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GABA (γ-aminobutiric acid) is a non-protein aminoacid involved in nervous system functionality, and having ipotensive, diuretic and diabetes-preventive properties (Rizzello *et al*., 2008).
Twenty-two Italian cheese varieties, differing for type of milk, technological process and ripening, were analyzed for GABA concentration. More than 200 lactic acid bacteria strains, isolated from GABA-containing cheeses (114-391 mg/kg) were genetically identified and characterized for the synthesis of GABA and resistance to gastro-intestinal conditions. GABA-producing strains belonging to the species *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus brevis*, *Lactobacillus casei*, *Lactobacillus bulgaricus*, *Lactobacillus rhamnosus*, *Streptococcus thermophilus*, *Lactococcus lactis* and *Leuconostoc mesenteroides*, were found. GAD enzyme was studied using Gadb gene partial sequencing (Siragusa *et al*., 2007).
GABA-producing strains were used for sourdough fermentation of wheat (white and wholemeal) and rye flours. The fermentation conditions were optimized to allow a contemporary production of GABA and anti-ACE peptides, both involved in modulation of human hypertension states (Rizzello *et al*., 2008). The highest GABA concentration was found in wholemeal wheat flour sourdough with a dough yield (DY) of 160 (258.71 mg/kg), whereas semi-liquid conditions (DY 330) were found to be appropriate for the release of antihypertensive peptides. Fourteen anti-ACE peptides were identified in wholemeal wheat flour sourdough (IC50 of the purified extracts: 0.19-0.54 mg/ml).
Following the same approach, flours of amaranth, quinoa, buckwheat and chickpea were fermented with GABA-producing strains. Concentrations ranging from 600 to 1100 mg/kg of GABA were found during single fermentation of the above flours (DY 160) for 24 h at 30 °C. A sourdough manufactured by mixing the four above flours was started with *L. plantarum* C48 (ca. Log 7 CFU/g) and used for bread making, according to a traditional two-steps protocol. Breads were characterized by GABA concentration, antioxidant activities and glycemic index.
The possibility to produce GABA using lactic acid bacteria was also evaluated using agriculture by-products or surplus (e.g., whey-milk or grape must) as substrates. Optimal conditions for synthesizing GABA in diluted grape must were the following: initial pH 6.0, initial cell density of ca. Log 7 CFU/ml and addition of 3.38 g/l L-glutamate. After fermentation (*L. plantarum* DSM19463 used as starter), the diluted grape must was freeze dried and contained 8.9 g/kg of GABA, high levels of niacin, free minerals, total polyphenols, and ca. Log 10 CFU/g of viable cells (Patent n. Reconstructed®RM2007A000398). The preparation was applied to the SkinEthic Human Epidermis to determine the effect on human beta-defensin-2 (HBD-2) transcriptional regulation by RT-PCR and immunohistochemical analyses. With the treatment corresponding to 267 mg/l of GABA, the up-regulation of HBD-2 gene was shown (Di Cagno *et al*., 2009) The stimulation by an exogenous chemical compound cover a great interest for industrial application in cosmetics.

Keywords: γ-aminobutiric acid, lactic acid bacteria, sourdough, human beta-defensin-2.

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Different molecular strategies for detection of bacteria in Italian goat cheese

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The typical sensorial qualities of traditional goat cheese depend on several factors, including traditional cheese-making practices, feeding of goats, and the dynamics of microbial communities. The qualitative and quantitative composition of the microbial flora, its evolution, and its activity during manufacturing and ripening time play an important role in the development of hygienic and sensorial qualities. In order to better understand the functions of the microbial community a description of the microbial ecosystem is required.
Several bacterial fingerprinting techniques are available and are used to make the analysis of the taxonomic diversity in complex ecosystem as food matrix easier.
In this study four different approaches were used to monitor microbial species during manufacturing process and ripening of goat cheese with the objective to value their efficacy at typing and tracking microbial dynamics.
First were tested two hyper variable regions of 16S rRNA gene (V2 and V3) by both LH-PCR analysis and SSCP analysis. Next was tested the efficacy at discriminating of internal transcribed spacer (ITS) by Automated Ribosomal Intergenic Spacers Analysis. For each of all this techniques were created relative databases of pure strains isolated from differential and selective media. The isolates were essential for the identification in the fingerprints obtained with the three different techniques and as reference to have the proof of their effective presence in the community. Then we investigated the first three hyper variable regions of 16S rRNA gene (V1-V3) by PCR-DGGE fingerprinting. The fingerprints obtained by amplification of the V2 and V3 regions in LH-PCR analysis and by amplification of the intergenic transcribed spacer (ITS) gave different results: the V2 and most of all the V3 profiles had showed a lot of comigrations probably due to the low length heterogeneity in these regions among the bacteria in the communities. This coelution make the peaks identification less reliable. Instead in ARISA common peaks for different species were very rare and never interested the major peaks so a greater number of peaks could be univocally identified. This fact confirms that ARISA highlights the taxonomic diversity, evident from the marked variability in ribosomal spacer length, in the prokaryote genomes (Fisher *et al*., 1999; Kent *et al*., 2000; Ranjard *et al*., 2001) because ARISA explores microbial diversity at the intraspecific level (Cherif *et al*., 2003; Daffonchio *et al*., 2003). SSCP analysis allowed us to detect a greater number of species with respect to those obtained in ARISA analysis. The problem of comigrations still subsisted but it was less accentuated. In DGGE-PCR we recovered and indentified some bacteria that never have been found between the isolates. SSCP and DGGE techniques have revealed useful tools to rapidly give photographs about the microbial dynamics during the manufacturing process in cheese matrix.

Keywords: goat cheese, bacterial communities, LH-PCR, ARISA, SSCP, PCR-DGGE.

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Mycotoxinogenic activity of moulds isolated in traditional Korean sweet baked foods on the market

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Different moulds were isolated from traditional Korean sweet bakery products, which were singularly packaged in PET, kept in paper box. Each box included 25 sweets, were, as suggested by the facility, stored at room temperature. The label indicated the follow ingredients: flour type 0, malt, rice and chestnut flour and salt. No MAP presence was indicated on the label. Considering that the sweets were stored at room temperature and sold by internet, the aim of the work was to identify the moulds present on the sweets and evaluate their potential mycotoxigenic activity in "vitro". The moulds were present on the surface, because of the presence of air in the packaging. The main strains were *Eurotium amstelodami*, *Eurotium herbariorum*, *Penicillium aurantiogriseum*, *Aspergillus flavus* and *Penicillium commune*. *Penicillium olsonii*, *Penicillium viridicatum*, *Penicillium cyclopium*, *Penicillium chrysogenum*, *Penicillium expansum*, *Eurotium chevalieri*, *Eurotium repens*, *Aspergillus niger*, *Aspergillus ochraceus*, *Cladosporium cladosporioides*, *Rhizopus nigricans* and *Mucor racemosus* were also isolated. The spoilage seemed to depend on the Aw (0.85 ± 0.5) values of the products. *Eurotium* spp. had *in vitro* the Aw limit of 0.80, in contrast the other strains had 0.85 or 0.90, depending on the growth temperature and the strains. The toxic potential of the isolated strains was also investigated: *Eurotium* spp. did not produce any mycotoxins, in contrast OTA (Ochratoxin A) was produced by *A. ochraceus* and *A. niger*, aflatoxins were produced by *A. flavus* and cyclopiazonic acid was produced by *P. commune* and patulin by *P. expansum*.
From our data it seems that the presence of air in each packaging, the Aw over 0.85 have permitted the moulds growth. Sweets without visible moulds contained a concentration of 180 CFU moulds/g, too. It is possible that the contamination could come from post-cooking, during packaging, considering that mould spores and mycelium are killed by cook temperature (260 °C). To prevent moulds growth it was suggested to add preservatives as ethanol and to package in modified atmosphere, without oxygen added.

Keywords: moulds, micotoxins, sweets.

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In vitro antimicrobial evaluation of carvacrol against some spoiling microorganisms

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Consumer demand of natural, fresh, chemical-additive free and safe food products is increasing at present in Europe. Therefore, there is interest in the development of novel combinations of natural antimicrobial systems with reduced levels of traditional physical and chemical food preservation processes to improve the quality and safety of agroindustrial products.
Among these new tools, the use of natural antimicrobial compounds such as carvacrol could be a promising strategy. Carvacrol is a common component of the oils from oregano, thyme, marjoram and summer savory, generally recognised as a safe food additive. It is frequently used in several products as a flavouring and/or as an antimicrobial agent, showing a broad-spectrum of activities against bacteria, yeasts and fungi.
In this study, a quantitative investigation on the inhibitory activity of carvacrol against some microorganisms (one strain of *Alicyclobacillus* spp., two stains of lactic acid bacteria, two strains of yeasts and two strains of *Bacillus* spp.), that could represent a potential spoilage risk in foods is presented.
In order to assess potential biostatic or biocidal activity of carvacrol, both the growth kinetics and dose-response profiles were obtained and analyzed. Inhibitory data were produced using a macrodilution methodology based on a turbidimetric technique. The growth of microbial suspensions in the presence of carvacrol was evaluated using a growth index (GI) and the microbial response was modelled and analyzed in terms of Gompertz's parameters. Moreover, for each tested microorganism, the noninhibitory concentration (NIC) and the Minimal Inhibitory Concentration (MIC) were quantified. NIC and MIC were determined to investigate both the microbial sensibility and resistance toward carvacrol, and Gompertz's parameters were evaluated to assess the microbial response at each phase of growth cycle.
The obtained results suggested that the tested microorganisms showed differences in the microbial sensitivity to the carvacrol. In particular, all the microorganisms showed NIC levels under 100 ppm, except two strains of lactic acid bacteria which were characterized by a major resistance against carvacrol, in fact they showed a NIC level around 200 ppm.
The possibility of completely inhibiting microbial growth was related to the use of carvacrol at the MIC level. As expected, lower MIC level did not necessarily correspond to lower NIC levels for the same microorganism. The microorganisms which showed the greatest value of the NIC, were characterized also for a major value of the MIC. For the two strains of lactic acid bacteria, in fact, MIC levels reached 380 and 850 ppm, whereas, the other microorganisms tested showed a value of the same parameter under 200 ppm.
The microbial susceptibility and resistance were found to be linear dose related except for one strains of lactic acid bacteria. Moreover all the phases of the growth cycle were affected by carvacrol.

Keywords: carvacrol, minimal inhibitory concentration, not inhibitory concentration, spoiling microflora.

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Comparison of molecular and metabolomic methods as characterization tools of Debaryomyces hansenii cheese isolates

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Debaryomyces hansenii is one of the yeast species most frequently isolated from cheese and salty foods, however little is known about the phenotypic and molecular variability of its strains. *Debaryomyces hansenii* is a one of the prevalent yeast species isolated from cheese ripening and in limiting the growth of deleterious spoiling bacteria. Its high resistance to sodium chloride makes *D. hansenii* ideally suited to many salty environments, among which cheese is the best studied. In order to explore the possibilities of a large study on its biodiversity, some *D. hansenii* strains were selectively isolated from Pecorino cheese sampled in ten different Italian regions. The hypothesis explored in this paper is that *D. hansenii* strains may be natural and ubiquitous markers of cheese origin. This possibility is based on the presence of this yeast in all cheeses studied and on the fact that a high number of cells survive after ripening. All isolates were identified as *D. hansenii* on the basis of conventional and molecular taxonomic analysis (Cardinali *et al*., 2001). The D1/D2 domain sequences of the 26S-rDNA did not show any variation, confirming the extreme homogeneity of this species. PCR-duplex-RAPD banding patterns analyzed with PCoA showed interesting clustering related to the geographic areas of isolation, although some overlapping between strains derived from different districts could be observed. A FTIR (Fourier Transform Infrared Spectroscopy) metabolomic fingerprint produced groupings weakly related to those observed with RAPD and less associated with the isolation locales. The discriminatory power of metabolomic fingerprint was able to discriminate strains otherwise considered identical. This preliminary study showed that, in spite of the homogeneity at the 26S rDNA level, the *D. hansenii* strains exhibit high molecular and metabolomic variability somehow linked to the places of isolation.
Further studies will be necessary to better investigate on the link between terroir and strain variability, as well as on the relation between genotypic and metabolomic fingerprints

Keywords: *Debaryomyces hansenii*, cheese, FTIR.

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Thymol effect on total proteome of Salmonella enterica serovar Thompson

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Thymol is a natural biocide and component of some essential oils from herbs. Its inhibitory effect on the growth of different microorganisms is well known, while less known is its mechanism of action towards microbial cells. Of course that depends on the target microorganism as well as environmental conditions. The response of bacteria grown in presence of thymol has not been investigated satisfactorily although its known that a modification of membrane fatty acids composition is caused. However, it remains to be assessed whether the modifications in membrane composition really lead to an increased resistance or are just part of a general adaptive response.
A strain of *Salmonella enterica* serovar Thompson (MCV1), isolated from poultry, has been grown in presence of a sublethal concentration (0.01%) of thymol. After growth the cells have been processed for total proteins extraction and the extracts from treated and untreated cells were subjected to two-dimensional polyacrylamide gel electrophoresis (2D-SDS PAGE), followed by in-gel digestion of spot and subsequent MALDI-TOF analysis.
The analysis of gels showed many proteins that were either up- or down-regulated by the presence of thymol, with significant changes in proteins belonging to different functional classes: glucose and fatty acid metabolism, molecular chaperones, protein and aminoacid biosynthesis, transcription regulation, chemotaxis and cell adhesion.
The global response regulator protein (arcA), essential for resistance of the cells to RNI and ROI, and the Outer membrane protein X (OmpX) have been found up-regulated in treated cells. The concurrent up-regulation of these two proteins is associated with an increase of the resistance at different stress conditions. The regulation of these proteins plays an important role in the acid adaptation and general stress tolerance.
It can be concluded that the regulation of the listed classes of proteins concur to the adaptive response activated by the cells to withstand the adverse environmental conditions beyond resulting in promotion of cross-resistance to other environmental stresses.

Keywords: natural antimicrobial compounds, thymol, salmonella enterica proteome, stress response, 2D sds page.

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Improvement of wine quality by using non-Saccharomyces yeasts in mixed culture with Saccharomyces cerevisiae

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Spontaneous grape must fermentations are consistently characterized by a sequential colonization of the substrate according to which apiculate yeasts (*Hanseniaspora/Kloeckera*), that predominate at the beginning of the process, are quickly replaced by *Saccharomyces* yeasts. In addition, other yeasts such as those belonging to the genera *Candida*, *Torulaspora*, *Kluyveromyces*, *Zygosaccharomyces* and *Metschnikowia*, may be present during must fermentation (Fleet, 2003). These "wild" yeasts have been considered for a long time responsible for wine defects. Thus, to inhibit their development and ensure the production of wines with repeatable characteristics, grape must is commonly treated with SO2 and inoculated with selected cultures of *Saccharomyces cerevisiae*. However, quantitative studies on the composition of the microflora of fermenting musts indicate that wild non-*Saccharomyces* yeasts may persist during inoculated fermentations (Mora *et al*., 1990) and produce high amounts of different metabolites and enzymes able to carry out the transformation of the aroma precursors present in grapes (Fernandez *et al*., 2000). Thus, non-*Saccharomyces* yeasts may influence the perceivable characteristics of the final product (Romano *et al*., 2003).
In this context, the possibility "to mimic" natural fermentations, maintaining the control of the fermentative process, represents an interesting approach to the improvement of the final quality of wine. In this study 105 isolates of non-*Saccharomyces* yeasts, belonging to different genera, naturally involved in spontaneous fermentations and coming from grape-musts of different origins, were identified and evaluated for their principal oenological characters. Fifteen strains showing interesting oenological characteristic are currently evaluated in fermentation processes carried out by mixed culture with *S. cerevisiae*.

Keywords: wine, *Saccharomyces*, non-*Saccharomyces*, mixed-culture.

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Autochthonous Saccharomyces cerevisiae strains from local grapevine varieties to improve wine quality

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The final wine characteristics depend strongly on the involved microorganisms, and this justifies the recent interest in the study and development of microbial strains, so-called "autochthonous", somehow linked to the region of origin, capable to contribute to the diversification of oenological products. In the same time conservation and protection of grape local varieties is a powerful tool to avoid the progressive decrease of the genetic diversity (Scalabrelli, 2005). The geographical distribution of *S. cerevisiae* strains within specific wine-producing regions has been defined (Caruso *et al*., 2002; Redzepović *et al*., 2002), and several studies have been devoted to the changes and fluctuations in the composition of the population of yeasts in vineyards. However, even if no correlation between strain biodiversity and geographical origin has been demonstrated, it seems of high practical interest to continue these studies, in order to use and make available yeast strains capable of improving the typical enological productions.
In the present work 54 yeast isolates belonging to *Saccharomyces* spp. have been obtained from lab-scale vinification of grapes of local grapevine varieties including 'Abrusco', 'Oliva', 'Canina' and 'Grand Noir' grown in an experimental vineyard, located at Crespina, near Pisa (Tuscany) gently hosted by Castellani Winery.
Isolation in pure culture of *Saccharomyces* spp. was achieved through the use of selective media and molecular identification by RFLPs of ITS regions and sequence analysis.
Strain characterization was obtained by microsatellite multiplex PCR (Vaudano *et al*., 2008) and RFLPs of mitochondrial DNA analysis (Fernandez-Espinar *et al*., 2000). Selected strains were used in enological characterization, including fermentation power, fermentation rate, killer activity, production of hydrogen sulphide, ß-glucosidase and proteolytic activity, and as inocula in vinification trials. Analytical parameters and sensory tests to check the aromatic characteristics of wines produced, confirmed the capacity to assist the vinification process without revealing difficulties and giving also interesting qualitative traits to the obtained wines.

Keywords: autochthonous yeasts, grapevine varieties, enological productions.

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Study on growth stimulator of bifidobacteria produced by potential probiotic dairy propionibacteria

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The human gastrointestinal tract constitutes a complex microbial ecosystem comprising hundred different species of bacteria. Because most of these bacteria are either beneficial or harmful to the human health, it is of great importance to promote beneficial bacteria such as bifidobacteria and lactobacilli and suppress potentially deleterious bacteria such as clostridia and bacteroidaceae among the intestinal microflora. There are two separated approaches to increasing the number of health-promoting bacteria in the gastrointestinal tract. The first is the oral administration of live, beneficial microorganisms, termed probiotics, and the second is to supply those already present in the intestine with a specific dietary supplements named prebiotics. Propionibacteria are prominent in the human large intestine and have been reported to have beneficial effects in the intestinal tract, in fact they are capable to released a growth stimulator for bifidobacteria (BGS, Bifodogenic Growth Stimulator) playing an important role in regulation of a bifidobacterial population in colonic microflora.
This experiment comes into a greater study on the probiotic characterization of dairy propionibacteria; in fact, in a preliminary phase, tests of adhesion to IPEC-J2 cells, survival under gastro-intestinal simulated conditions and antagonism against some food-borne pathogens were conducted on four dairy propionic acid bacteria (PAB) strains, purchased from public collection (DSMZ). In this study all this potential probiotic strains were studied for their ability to produce a bifidogenic growth stimulator, which stimulated the growth of two *Bifidobacterium* strains.
The BGS was present in the cell-free hydrosoluble fraction (CFE, cell-free extract) of *Propionibacterium* cultures; cell-free extract was added as ingredient (1% v/v) into the growth medium, both concentrated and diluted (1:10 and 1:100). The effect of cell-free extract on *Bifidobacterium* strains was evaluated through absorbance measurement at 580 nm, monitoring the response of stimulated microorganisms in growth phase. Two parallel experiments were conducted inoculating both bifidobacteria in initial concentration like 103 CFU/ml and 107 CFU/ml.
A stimulant effect was observed for the cell-free extracts released from *Propionibacterium jensenii* and *Propionibacterium acidipropionici* against *Bifidobacterium bifidum* (103 CFU/ml); in particular a decrease of lag phase and an increase of maximal growth rate were registered. The most significant effect was observed for the most diluted cell-free extract (1:100) released from *P. jensenii*; after 14 hours of incubation, absorbance values like 1.39, 1.79, 2.12 and 2.13 were registered for *B. bifidum*, *B. bifidum* added of CFE, *B. bifidum* added of CFE (1:10) and *B. bifidum* added of CFE (1:100), respectively.
An inhibitory effect, expressed as lag phase prolongation, was observed for the cell-free extracts released from *Propionibacterium thoenii* and *Propionibacterium freudenreichii* subsp. *shermanii* against *Bifidobacterium longum* (103 CFU/ml).
These results were confirmed by co-culture tests among propionibacteria and bifidobacteria.
In conclusion the results suggest that, in our experimental conditions, a BGS was found in the cell-free extract released from dairy propionibacteria, and the stimulant effect exerted on bifidobacteria growth were observed to be strain-dependent as regards the stimulant strains and species-dependent as regards the stimulated bacteria.

Keywords: probiotic strains, bifidogenic growth stimulator, propionibacteria, bifidobacteria.

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Surface bacterial communities of rind active cheeses

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The composition of the bacterial consortia of the surface ripening Italian cheeses and their role on quality and safety is still poorly understood.
The objective of this study was to identify and characterize the bacterial communities present on the surface of five traditional Italian cheeses, Casera Valtellina, Scimudin, Formaggio di Fossa, Gorgonzola and Taleggio.
Direct DGGE analysis was performed from total DNA obtained from cheese surface to identify the dominant bacterial populations. Bacteria were isolated from selective culture media, subsequently identified by the use of DGGE, 16S rDNA sequencing and randomly amplified polymorphic DNA (RAPD).
The DGGE profiles obtained from cheeses surface, showed a mixture of prominent bands plus others of lesser intensity. Bands identified as *Staphylococcus*, *Micrococcus*, *Psychrobacter* species were present in all type of cheese, showing different intensity. Although Formaggio di Fossa, Gorgonzola and Taleggio present different sensorial properties and manufacture technology, DGGE profiles showed similar rind ecology. Major differences in the DGGE profiles were observed in Scimudin and Casera. Bands originated from *Enterococcus* and *Brevibacterium* species were also detected. Microbiological analysis of the Gram positive isolates from cheeses surface confirmed the complexity in terms of species and strains of these bacterial communities. The most numerous bacterial group isolated was *Staphylococcus* sp., follows by *Micrococcus* sp., *Macrococcus* sp., *Enterococcus* sp., *Lactobacillus* sp., *Carnobacterium* sp., *Leuconostoc* sp., *Brevibacterium* sp., *Corynebacterium* sp., *Brochothrix* sp., *Bacillus* sp. When a RAPD approach was used for typing *Staphylococcus*, it was possible to observe that strains belonging to the different cheese surfaces clusters together suggesting the presence of cheese surface adapted biotypes.
The poliphasic approach, based on culture dependent and independent analysis, allowed us to obtain information on the composition of surface bacterial communities of five traditional Italian rind active cheeses, and the isolation of new strains from these dairy environments.

Keywords: cheese surface, DGGE, bacterial communities.

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Investigation of yeast community of "Grillo" grapes and musts from Marsala wine production area

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The oenological interest in the autochthonous yeast applications has increased since they represents an important supplement to wine quality (Martinez *et al*., 1989; Moreno *et al*., 1991). Yeast populations harboured onto the surface of berries and in musts of "Grillo" grape variety were isolated and analyzed. In order to obtain a first blastomycetic mapping of Marsala wine production area, eight vineyards were chosen on the basis of different climatic and agronomic parameters, including altitude, exposure,vineyard age, grape biotype, grape cultivation system, vegetative vigour, pruning, green pruning, yield per plant, phytosanity state, irrigation and closeness to wood areas. Analysis of blastomycetic populations was performed by cell counts on specific culture media for yeasts. Cell concentrations were evaluated on grapes and unfermented musts and during spontaneous must micro-fermentations at different times (after 3 and 13 days). Furthermore, during micro-fermentations non-*Saccharomyces* populations were distinguished from presumptive *Saccharomyces* based on the appearance of colonies after growth onto Wallerstein Laboratory (WL) nutrient agar (Pallman *et al*., 2001). Non-*Saccharomyces* reached about 106-107 CFU/ml both at the third and at the thirteenth day, while *Saccharomyces* exhibited variable cell concentrations, in particular, the majority of experiments showed level around 104 CFU/ml at the third day and almost all samples reached level of about 106 CFU/ml at the thirteen day. *Saccharomyces* cell concentrations positively correlated with weight loss registered during fermentations, since higher weight loss values were found in samples with their higher levels. After colony morphology inspection, 54 isolates were collected from grapes and unfermented musts and 60 during must micro-fermentations after 3 and 13 days, forming a total of 114 isolates. They were clustered into eight groups and into nine groups by optical microscopic observation of cell morphologies. Strain typing and differentiation was carried out by randomly amplified polymorphic DNA (RAPD-PCR) (Moschetti *et al*., 1998) and the band patterns were analyzed by means of the unweighted pair group method using arithmetic average clustering algorithm (UPGMA). The representative strains of each group are being genetically identified. So far, analysis of D1/D2 region of the 26S rRNA gene revealed the presence of *Candida zampliinina*, *Hanseniaspora uvarum*, *Issatchenkia terricola*, *Metschnikovia pulcherrima*, *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*. Moreover, the study was specifically oriented to the *Saccharomyces* spp. strains, which were isolated from musts fermented for 21 days using a "modified ethanol sulphite agar" (MESA), prepared from ESY medium. A total of 42 cultures were collected and 28 presumptive *Saccharomyces* yeasts, as selected by microscopic observation, were confirmed to be *Saccharomyces* spp. through amplification of ITS-5.8S rRNA region (Esteve-Zarzoso *et al*., 1999). All the isolates were typed as above reported. The representative strains of each group were characterized for technological traits with interest in wine production such as hydrogen sulphide production, ethanol tolerance and potassium metabisulphide resistance. Strains showing the best performance were used to carry out "Grillo" must micro-fermentations lasting 13 days to select yeast starter cultures. The work is still in progress.

Keywords: autochthonous yeast, Grillo grape, Marsala wine, *Saccharomyces.*

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Oenological properties of Saccharomyces cerevisiae and Zygosaccharomyces rouxii strains inoculated in high sugar grape must for Vin Santo production

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Vin Santo is a dessert wine traditionally obtained in Tuscany from white or red grape varieties that are, at first, naturally dried for some months to concentrate the sugar content to values of 30-40% (w/v) and then are allowed to ferment for 2-3 years under the action of indigenous yeasts at low temperatures (10-20 °C) in small wooden barrels, named "caratelli". Because of high sugar concentrations in musts and low environmental temperatures, different non-*Saccharomyces* yeast genera including *Zygosaccharomyces*, *Candida* and *Torulaspora* can occur throughout the alcoholic fermentation and ageing, although *Saccharomyces cerevisiae* or *Saccharomyces bayanus* usually dominate the fermentative process (Domizio *et al*., 2007). Nevertheless, analysis of yeast population dynamics during several Vin Santo vinifications, carried out in one winery in Tuscany, evidenced that in some barrels *Zygosaccharomyces rouxii*, that is usually considered a spoilage yeast species, dominated alcoholic fermentations without conferring negative organoleptic characteristics to the final product. Therefore, in this study, oenological properties of one *Z. rouxii* strain from the above mentioned Vin Santo vinifications and of one *S. cerevisiae* strain previously isolated from barrels of the same winery were evaluated by laboratory scale fermentations carried out at 18 °C using must obtained from dried grapes of Malvasia and Trebbiano varieties and containing 445 g/L of sugar. The growth behaviour of two yeast strains, that were inoculated in duplicate at 3 x 10e6 UFC/mL, was determined by pour plate counts during four months and, at the end of this incubation time, metabolic compounds of oenological interest were determined by chromatography analysis. The strains of the two yeast species, which are usually known to show differences in their tolerance to osmotic stress and ethanol, didn't exhibited significant differences in their growth behaviour, both reaching maximum cell densities of about 8 x 10e7 UFC/mL after 14 days of fermentation. Moreover, the inoculated *Z. rouxii* strain dominated indigenous *S. cerevisiae* populations, which didn't exceeded 5 x 10e6 UFC/mL and showed a lower persistence. The strains of both species behaved as glucosophilic yeasts and yielded ethanol at average concentrations of about 14% (v/v). As concerns secondary metabolites, no significant differences were observed between *Z. rouxii* and *S. cerevisiae* strains, with the exception of acetic acid and ethylacetate that were produced in lower amounts in the fermentations carried out by *Z. rouxii* strain. These findings highlight that strains of *Z. rouxii* species, which is traditionally considered as a spoilage yeast in high sugar environments, might possess suitable oenological properties, so that they could be used in inducing alcoholic fermentation of Vin Santo or other wines obtained from grapes at high sugar concentrations, either alone or in combination with other yeast species.

Keywords: Vin Santo dessert wine, *Zygosaccharomyces rouxii*, *Saccharomyces cerevisiae.*

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Transcriptomic and phenotypic analysis of the food isolated UC7032

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Staphylococcus epidermidis UC7032 was isolated from food; it is a vancomycin heteroresistant strain, up to 32 mg l^{-1}. This food isolate produced enterotoxin C and is classified in the agr group II. *Staphylococcus epidermidis* UC7032 harbors genes coding for protein with adhesive function, involved in the first phase of biofilm formation (atlE) and involved in catheter-associated infections (fbe). Scanning electron microscopy showed that cells of *S. epidermidis* UC 7032 form a biofilm-like structure when grows in the presence of the glycopeptide antibiotic and on food matrix, like cheese. In this report, whole-genome microarray experiments were performed to define and to identify genes differentially expressed during growth in BHI media in presence and in absence of vancomycin and during biofilm formation on food surface.
Compared to *S. epidermidis* UC7032 grown in BHI, 140 genes were upregulated and 75 downregulated in presence of 32 mg l-1 of vancomycin. The upregulated genes included genes encoding cell wall metabolism and secretion machinery, transcriptional and virulence regulators, transport and stress response proteins. The downregulated genes included genes encoding amino acid transport and metabolism, transcription, energy production and signal transduction mechanism.
Similar effect on transcription were observed when *S. epidermidis* UC7032 was grown in food, 310 genes were upregulated and 345 downregulated.
Interestingly also the expression of secgene, coding for the staphylococcal enterotoxin C, was affected by food environment.
This report represents a novel study of global transcriptional gene expression profiling of *S. epidermidis* grown as biofilm in food and in presence of vancomycin.

Keywords: *Staphylococcus epidermidis*, food, biofilm, microarray.

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"Wild" yeast flora detection during the pre-fermentative stages in winemaking

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Wine quality is influenced by several factors such as grapevine variety, winemaking conditions and yeast strain used during fermentation process. Therefore, the role of "wild" yeasts on fermentation and sensorial properties of wine is acknowledged and several authors confirmed the involvement of these microorganisms in the final wine quality (Lambrechts and Pretorius, 2000; Fleet, 2003). However, uncontrolled "wild" yeast presence at the pre-fermentative stages could generate undesirable compounds. For this reason, it is very important for cellar operators to be able to promptly evaluate the level of "wild" yeast contamination of grapes and musts at the pre-fermentation stages. Nowadays, the tendency in winemaking is to limit the sulphur dioxide addition because of its toxicity. To reduce this antiseptic compound that inhibits the "wild" yeasts before inoculation of selected *Saccharomyces cerevisiae* strain, the detection of yeast contamination level could be significant.
The aim of the present research is the quantitative evaluation of viable microorganisms that colonise grape berries and musts, measuring the oxygen consumption in the samples at the pre-fermentation stages.
In this study we determine the viable wild yeast population before fermentation, using a mathematical relationship between viable cell number and oxygen consumption rate. This parameter was measured using a probe with a pO2 electrode chamber that is directly collected in the liquid must samples.
Results of the evaluation of natural grape must samples indicated that the oxygen consumption rate was related to the "wild" yeast flora. Using this methodology the extensive microflora contamination was easily detected. Since using this technique to estimate the "wild" yeast at the pre-fermentative stages the results were available in a short time, we concluded that this fast method could be used to determine the contamination level and reduce the risks of an uncontrolled fermentation.

Keywords: wild yeasts detection, oxygen consumption, winemaking.

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Experimental evidence of a general pattern for biogenic amine accumulation during wine-making

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Biogenic amines (BAs) are nitrogenous low molecular weight organic bases that can be found in several foods, especially in fermented foods, mostly as a consequence of the decarboxylation of their respective free precursor amino acids, through the action of substrate-specific microbial decarboxylases. BAs can cause different toxicological effects to humans, depending on the specific BA, its concentration and individual sensitivity. As other fermented foods, wine is a good matrix for BA formation because of the possible presence of both free amino acids and microorganisms with amino acid decarboxylating activity (Lonvaud-Funel, 2001). Several studies indicated malolactic fermentation (MLF) as the winemaking phase responsible of most BA production and accumulation in wines, at least for what concerns the most frequently found amines (histamine, tyramine and putrescine) (Vincenzini *et al*., 2009). In spite of this, very few studies were carried out taking into consideration both BAs and malolactic bacterial population during winemaking on an industrial scale. Therefore, this work was aimed to investigate on the time courses of BA accumulation and bacterial counts during and after 15 commercial spontaneous MLF, until viable cells of lactic acid bacteria were still detectable in the investigated wines. A noticeable BA accumulation occurred in 11 out of 15 investigated processes, total BA content being highly variable among the BA positive wines, with values ranging from 13 to 38 mg/L, but with histamine, tyramine and putrescine always as the most abundant amines. In order to obtain a possible generalization of the BA time course during and after MLF, all data concerning the amounts of each main amine produced during and after MLF were normalized to their respective maximum value, reached, in each industrial process, when viable bacterial cells were no more detectable. The normalized data demonstrated that tyramine production occurred both during and after MLF, without any significant difference between the two winemaking phases, whereas histamine and putrescine were mainly released after MLF, with the LAB population in the decline phase. Finally, since the wines showed different physico-chemical characteristics, a possible correlation between BA formation and some intrinsic parameters (free alfa-amino nitrogen content, pH, sulphur dioxide and ethanol) was also taken into consideration by calculating Pearson and Spearman rank ($p < 0.05$) correlation coefficients. According to these calculations, the increase in total BAs should be not significantly correlated to pH and sulphur dioxide concentration, negatively correlated to ethanol content and positively correlated to alfa-amino nitrogen content. As concerns the increase of individual amines, both statistical coefficients agree in indicating a significant positive correlation between alfa-amino nitrogen and histamine or tyramine. In conclusion, the results demonstrate that BA accumulation, when it occurs, follows a general pattern, the main BAs being mostly produced in the winemaking stage following MLF and depending on the presence of viable, even if declining, malolactic bacterial cells. Hence, to prevent BA accumulation to high concentrations, the results strongly advise to eliminate bacterial populations in wines suddenly after MLF completion, verifying the effectiveness of the method utilized to eliminate the bacterial cells.

Keywords: biogenic amines, lactic acid bacteria, malolactic fermentation.

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Lactic acid bacteria and yeasts involved in four different sourdoughs fermentation

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Four types of sourdoughs (L, C, B, Q) from artisanal bakeries in Northern Italy were studied using culture-dependent and culture-independent methods. In all samples, the yeast numbers ranged from 160 to 107 CFU/g, and the numbers of lactic acid bacteria (LAB) ranged from 103 to 109 CFU/g. The isolated LAB were sequenced, and a similarity was noted between two samples (C, Q), both in terms of the species that were present and in terms of the percentage of isolates. In these two samples, *Lactobacillus plantarum* accounted for 73% and 89% of the bacteria, and *Lactobacillus brevis* represented 27% and 11%. In the third sample (B), however, the dominant LAB isolate was *Lactobacillus brevis* (73%), while *Lactobacillus plantarum* accounted for only 27%. The fourth sourdough (L) was completely different from the others. In this sample, the most prominent isolate was *Weissella cibaria* (56%), followed by *Lactobacillus plantarum* (36%) and *Pediococcus pentosaceus* (8%). In three out of four samples (L, C and Q), all of the yeasts isolated were identified as *Saccharomyces cerevisiae*, yet only *Candida humilis* (90%) and *Candida milleri* (10%) were isolated in the fourth sample (B). The microbial ecology of the sourdoughs was also examined with direct methods. The results obtained by culture-independent methods and DGGE analysis underline a partial correspondence between the DNA and RNA analysis. These results demonstrate the importance of using a combined analytical approach to explore the microbial communities of sourdoughs.

Keywords: sourdough, yeasts, lactic acid bacteria, DGGE, culture-independent methods.

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Characterization of carvacrol treated bacterial cells by atomic force microscopy

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In the last years many authors reported the antimicrobial effect of essential oils from herbs and of some their main components (Ultee *et al*., 2000; Burt, 2004). However, only few researches have been conducted to point out the possible mechanism of action of these substances on microbial cells (Di Pasqua *et al*., 2007). The aim of our investigation was to evaluate the effect of carvacrol on food spoilage bacteria by investigating morphology and roughness of the cell surfaces by AFM (Atomic Force Microscopy) technique. The following strains were used: *Listeria innocua* 1770, *Pseudomonas fragi* 25P, *Pseudomonas fragi* 6P2, *Brochothrix thermosphacta* 1R2, *Escherichia coli* 32, *Afnia alvei* 53M, *Serratia proteamaculans* 42M, *Lactobacillus plantarum* 48M. The susceptibility of strains towards different concentration of carvacrol was determined in liquid medium. Furthermore, the cell integrity was determined using LIVE/DEAD® BacLight™ Bacterial Viability Kit and epifluorescence microscopy counting. All bacterial strains showed a growth inhibition in presence of 0.05% carvacrol. Furthermore, membrane damage was verified by viable staining. After 1 h contact with the antimicrobial agent, cell suspensions were placed on poly-L-lysine covered glass slides and analyzed by AFM (EasyScan 2, Nanosurf AG, Switzerland) in contact mode after drying. The image output results were submitted to visual analysis and the effects of carvacrol on cell surfaces were compared and discussed. The cells before the treatment evidenced a smooth and homogeneous surface topography in all the directions and no obvious damage was observed. By contrast, the image of bacteria treated with carvacrol exhibited a profound modification, indicating a change in the cell surface organization. The surfaces of carvacrol treated cells appeared wrinkled, jagged, with numerous granules and holes. The effect of carvacrol on Gram-positive bacteria was found to be quite different from Gram-negative ones. In particular, the cells of Gram-negative strains showed a bumpy surface and showed to be completely warped with respect to normal cells. Some strains showed leakage of a substantial amount of fluid, probably following the drying process, as indicated by the ring around the cells. A similar phenomenon was previously observed in the case of peptide-damaged cells (Meincken *et al*., 2005). AFM images were further examined by SPIPTM (Scanning Probe Image Processor, Image Metrology A/S, Lyngby, Denmark) software in order to get an objective roughness evaluation (mean roughness and surface area ratio) as well as an estimation of some biometric parameters. All the strains showed an increase of roughness parameters values after carvacrol treatment. However, the cell surface of *Afnia alvei* 53M, *Serratia proteamaculans* 42M and *Escherichia coli* 32 treated with carvacrol showed the maximum roughness values. In addition, all the treated cells showed a reduction in length, generally about 300 nm with respect to the control sample. In some cases (e.g. *Listeria innocua* 1770 and *Pseudomonas fragi* 25P), also the thickness of the cells appeared reduced after the contact with carvacrol.

Keywords: carvacrol, atomic force microscopy, roughness analysis.

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Inactivation of spoilage yeasts Dekkera/Brettanomyces using Low Electric Current Treatment (LECT)

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Among the many food preparation processes and/or conservation techniques electric treatments in recent years are used. The main objective of such treatments is to reduce or eliminate part of the often undesirable microflora. Microbial spoilage is a serious problem in the beverage industry, especially for wine production. *Brettanomyces bruxellensis* spoilage is one of the most for the wine industry. These yeasts are notorious for their ability to produce acetic acid, C8 and C14 fatty acids, volatile ethyl phenol, responsible of unpleasant odours and tastes that affects wine aroma, amines biogene active biologically. The organoleptic defects resulting from by *Brettanomyces*, as indicated generically "character Brett" have been found in wines produced and stored in the most different situations, from all over the world. Preliminary experiments were carried out with low-intensity electric current (LEC) with success in winemaking (Ranalli *et al*., 2002, 2006; Lustrato *et al*., 2003, 2006, 2009). In this research we focused on the effects of LEC on the cell viability and metabolic activity of *Dekkera*/*Brettanomyces* yeasts. Different LEC treatments (100-200 mA) were applied at lab scale on *Dekkera bruxellensis* 4481 strain in synthetic medium. Conventional cultural methods and bio-indicators were used as monitoring the effects of LEC. Parameters such as polarity, treatment duration (0-7 day) were varied one at a time to highlight their cause-effect relationships. Results demonstrated that LECT using MMO electrodes has a greater effect on the viable D. bruxellensis yeasts 4481 strain. LECT decreased the survival time and increased the death rate of *Brettanomyces* yeasts. The present study suggests the importance of applying an appropriate low electric current as a tool to limit yeasts spoilage caused by *Brettanomyces* yeasts.

Keywords: control yeasts spoilage, low electric current, *Dekkera bruxellensis.*

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Safety aspects in staphylococci isolated from food, workers and surfaces

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Staphylococci occur widely in food environments, both of animal and vegetal origin, fermented and non fermented (Rosec *et al*., 1997). Up to now, many food microbiologists have focused on the incidence and the characterization of *Staphylococcus aureus*, a well known foodborne pathogen that produces one or more heat-stable enterotoxins that have been implicated in food-poisoning outbreaks. Another issue concerns the technological function of some staphylococcal species, e.g. *Staphylococcus xylosus* and *Staphylococcus carnosus*, in fermented sausages, where they play a significant role in proteolysis, lipolysis and flavour formation. Until now very little is known about the incidence of coagulase negative staphylococci in foods and food environments, as well as on the incidence of safety-related properties within the staphylococcal species. Since the frequent occurrence of staphylococci in foods from catering, the aim of this investigation was to identify at species level staphylococci isolated from meals served cold and hot, catering surfaces and food workers at canteens, as well as to characterize the isolates for some safety-related aspects, namely disinfectants resistance, and adhesion- and biofilm-forming ability.
Among isolated strains the most predominant species was *S. aureus*, accounting for 26.8% of isolated strains, followed by *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. Other isolates belonged to the species *Staphylococcus pasteuri*, *Staphylococcus sciuri*, *Staphylococcus warneri* and *S. xylosus*, whose presence has been recently evidenced in fermented foods and ready-to-eat foods in restaurants (Simeoni *et al*., 2008). Some of these species have been isolated both from food and on workers' hands and work surfaces, suggesting a certain potential of cross-contamination by them. The staphylococci strains were tested for their resistance against eight substances/formulated products used as disinfecting agents in the food industry. As expected, results were quite variable among strains; however, some strains showed an high resistance towards QAC-based and oxidizing agents. Incidence of high adhesion ability of strains was frequent towards Telflon surfaces, while it decreased against stainless steel surfaces, probably caused by electrostatic forces involved in adhesion mechanisms. A moderate and strong ability to form biofilm on microtiter culture plates was showed by 14,2% and 6,5% of strains, respectively. In the latter group were included strains belonging to species other than *S. aureus*, in particular *S. epidermidis* and *S. pasteuri*. About this microbial species frequently present in RTE foods, this is the first report that evidences the adhesion and biofilm forming ability on surfaces widely used in the food industry. This capacity, along with the resistance to oxidizing agents, has to be regarded as a potential risk given that oxidizing agents are frequently used to eliminate microbial biofilms.

Keywords: Biofilm formation, adhesion, *Staphylococcus* spp., disinfectants resistance.

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Influence of autochthonous lactic acid bacteria on cheese microflora and free amino acids of sweet Pecorino Sardo PDO

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Pecorino Sardo Protected Denomination of Origin (PDO) is a half-cooked paste cheese produced only with full cream ewes' milk that can be thermised if necessary. Pecorino Sardo PDO is a cylindrical-shaped cheese with flat faces and a straight or slightly convex rim. Two typologies of Pecorino Sardo PDO are currently produced: ''sweet'' when the ripening time is short (20-60 days), and ''ripened'' when the ripening is longer than 2 months.
In this study we evaluated the influence of autochthonous lactic acid bacteria (LAB) on the microbiological and nutritional characteristics of the "sweet" type of Pecorino Sardo PDO. For this aim two cheese-making trials (experimental and control) were carried out on an industrial scale. Experimental Pecorino Sardo cheese was manufactured with the addition of an autochthonous starter culture prepared with *Streptococcus thermophilus* + *Lactobacillus plantarum* + *Lactococcus lactis* subsp. *lactis* + *Lactobacillus casei* subsp. *casei* while control cheese was manufactured with the addition of a commercial starter including *Streptococcus thermophilus* + *Lactococcus lactis* subsp. *lactis* + *Lactococcus lactis* subsp. *cremoris*.
The evolution of free amino acids (FAAs) and cheese microflora were then monitored in both cheeses at different ripening times. The addition of the selected autochthonous starter resulted in a substantial increase of the mesophilic lactic microflora, lactobacilli in particular, in the experimental curd and cheeses. The presence of spoilage microorganisms in the experimental cheeses, such as total and fecal coliforms, was significantly reduced compared to the control indicating a suitable activity of the starter during the fermentation phase. Moreover, the use of the selected autochthonous LAB allowed the production of experimental cheeses with a significantly higher amount of FAAs compared to Pecorino Sardo control cheeses. It is concluded that the selected starter used in the experimental trials is suitable for the production on a industrial scale of the "sweet" type of Pecorino Sardo PDO.

Keywords: Pecorino Sardo DOP sweet type, autochthonous starter culture, free amino acids.

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Microbiological characterization of Fruhe cheese

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"Fruhe" is the name of a fresh cheese traditionally manufactured in Sardinia (Italy) from sheep or goat milk. When the cheese is stored for long time in brine it is called "Merca". The Fruhe is a fermented curd characterized by a compact coagulum, a white shining paste and a soft texture. The taste is slightly sour and aromatic.
The Fruhe is manufactured from whole milk, raw or heat-treated, deriving from Sardinian farms. It is produced using the following general manufacturing process:
- the milk, brought at 36-38 °C, is inoculated with autochthonous lactic acid bacterial (LAB) cultures and coagulated with calf or lamb liquid rennet;
- this milk is now distributed in plastic containers that can have a variable volume;
- the cheese ripening takes place in 12-24 hours;
- the coagulum within the container can be cut in cubes of approximately 4 cm and the whey is usually partially discarded;
- the cheese is stored at 4 °C.
In the present study we investigated the microbial populations associated with this traditional product. For this aim twenty samples of Fruhe, manufactured from sheep and goat milk by using different cheese-making technologies, have been investigated. Microbiological analyses showed that total mesophilic bacteria counts of Fruhe samples were between 1.00E+06 and 2.70E+09 CFU/g. Lactobacilli and lactic streptococci were constantly present in a 1:1 ratio and in a few samples they reached significant numbers (around 1.00E+09 CFU/g) while in general the size of these populations was more limited (around 1.00E+06 CFU/g). This was attributed to an excessive acidification and post-acidification of the cheese. It is important to mention that the presence of a suitable number of LAB (at least 1.00E+07 CFU/g) is crucial for the nutritional quality of fresh dairy products. The presence of total coliforms and coagulase-negative staphylococci was limited and related to some extent to the reduced number of LAB in most of the samples. Heterofermenting LAB, faecal coliforms and coagulase-positive staphylococci were always absent while yeasts were isolated only from three Fruhe samples.
The study showed that lactic microflora associated to Fruhe cheese was substantially homogeneous in all the samples analyzed. However, the number of LAB was globally low in the majority of samples and quite different from other fresh cheeses.

Keywords: Fruhe, traditional fresh cheese, lactic acid bacteria (LAB), autochthonous microflora.

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Antibiotic resistance genes delivered by lactic acid bacteria isolated from fresh cut vegetables

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Fresh-cut produce may consist of peeled, sliced, shredded, trimmed and/or washed fruits or vegetables. These products are usually sealed within semi-permeable packages and stored at refrigeration temperatures. Fresh-cut products are subject to a minimal form of processing, which lacks any effective microbial stabilization, resulting in a food product which would naturally carry microorganisms, some of which may be potentially hazardous to human health. In recent years, studies on the selection and dissemination of antibiotic resistances have focused mainly on clinically relevant bacterial species. More recently, it was speculated that food bacteria may act as reservoirs of antibiotic resistance genes. Therefore, minimaly processed foods may be an important vehicles of enormous amounts of living bacteria that may delivered into the human body. These may carry transferable antibiotic resistances (AR), which might be transferred to commensal or pathogenic bacteria (Phillips *et al*., 2004). In this paper we report the dissemination of antibiotic resistance genes in lactic acid bacteria (LAB) isolated from fresh cut vegetables collected from local markets. Antibiotic susceptibility tests for the determination of either ampicillin, penicillin G, erythromycin, chloramphenicol, gentamicin, streptomycin, tetracycline, or ciprofloxacin resistance were performed. Moreover, genes associated with resistance to chloramphenicol, lactam antibiotics, macrolides and tetracycline were identified using a PCR and a Reverse Transcription (RT) PCR approaches. Our results suggest that antibiotic resistance is common in LAB isolated from minimaly processed fresh cut vegetables. In addition, multiple resistance to antibiotics such as chloramphenicol and tetracycline was observed.

Keywords: fresh cut vegetables, lactic acid bacteria, antibiotic.

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High pressure homogenization treatment of ready-to-eat food: review results within the HIGHQ-rte European project

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Novel non thermal processes such as pulsed electric field (PEF), high hydrostatic pressure combined with CO_2 (HHPCO) and high pressure homogenization (HPH) represent an alternative to thermal treatments to improve quality, safety and functionality of milk, eggs, milk-based and vegetable products (Diels *et al*., 2006). Among the alternatives proposed, the high pressure homogenization is one of the most promising and its potential has been especially applied to the dairy field both for the milk decontamination and product diversification (Lanciotti *et al*., 2006). Differently, there are very few experimental evidences for the decontamination of spoilage and pathogenic microorganisms in vegetable juices and soups.
In this work, performed within the European project "Innovative non thermal processing technologies to improve the quality and safety of ready-to-eat meals (HighQ RTE)", the potential of HPH to inactivate pathogenic species such as , in raw milk, eggs and milk based emulsion was evaluated both at lab scale and at industrial level. Also the HPH inactivation of spoilage microorganisms such as , and inoculated in vegetable soup and fruit juices was assessed together with modifications of food viscosity in relation to the pressure treatment applied. Moreover, the effects of HPH on the expression of opuCA, clpP, gadA, rpoB, clpC and groEL genes in when exposed to sub-lethal HPH treatments were studied. The data obtained were compared with those collected using traditional heat treatments.
In general the results were very promising. In fact, the results regarding the milk decontamination showed that the inactivation curves of were linear up to the 4th cycle at 100 MPa after which their slopes diminished. Also resulted to be sensitive to HPH. A reduction of 5 Log CFU/ml was obtained with 8 pressure pushes at 100 MPa.
Concerning to yeasts, the inactivation was related to the tested species and food matrix. The most interesting results were obtained for apricot juices, finding a positive relation with the increase of pressure up to the 5th cycles at 100 MPa.
The data obtained confirmed the industrial importance of this technology both for the production of novel food and product decontamination.

Keywords: high pressure homogenization, microbial inactivation, food safety, shelf-life.

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Preliminary exploration on the presence of yeast on the apple surface

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The microbial ecology of fruit surface has been not thoroughly studied, although a deeper insight in this field could shed light on the scientific basis of non chemical post-harvest control, especially to control moulds proliferation. Several factors are likely to influence the qualitative and quantitative presence of yeast on the fruit surface, such as the position of the orchard, the fruit handling and processing, the harvesting, storing and shipping procedures. It is currently known that yeast predominate on the fruit when it is still in the orchard, then moulds can take over and finally bacteria could cause the final spoilage of the fruit (Doores, 1983).
The work presented in this poster aims to study the distribution of yeast on apples collected from a restricted productive area and to test different treatments to increase the yeast vs. moulds ratio. Three treatments were considered: a) no treatment, b) washing in water and c) washing with glucose solution.
The yeast isolates were identified by sequencing the D1/D2 domain of the 26S ribosomal DNA, showing that at least seven species can be found and none appears to predominate. The Scanning Electron Microscopic pictures of the fruit surfaces showed a differential distribution of yeast on the different regions of the apple with some predominance in the peduncular region. The washing with glucose has shown to increase the frequency of yeast with an apparent decrease of moulds. An intriguing aspect of the microscopic investigation is the intimate connection between yeast and bacterial cells, the meaning of which deserves further studies.
This preliminary investigation is one of the few known attempts to characterize the yeast community with a combination of molecular, electron microscopic and microbiological means. Moreover, the possibility that mild treatments, compatible even with organic farming, can improve the yeast density and therefore the fruit shelf life, can provide new perspectives in non-chemical post-harvest control.

Keywords: fruit, fungi, apple and yeasts.

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Development of a specific Real-Time PCR assay for the detection of Brochothrix thermosphacta and evaluation of qPCR for its quantification in fresh and spoiled meat

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Brochothrix thermosphacta is a psychrotrophic bacterial species commonly involved in the spoilage of meat where it represents a significant component of the spoilage microflora (Russo *et al*., 2006). Numerous preservation methods have been applied, individually or in combination, to extend the shelf-life of meat during storage (packaging of meat under vacuum or modified atmosphere) and, since the spoilage never ceased to occur, the knowledge of the genera/species affecting meat spoilage is necessary to define a successful method for food preservation (Macian *et al*., 2004; Samelis *et al*., 2000).
The aim of this study was to define a PCR-based method for a rapid and species-specific detection of *B. thermosphacta* and to develop a Real-Time PCR method (qPCR) for its enumeration in fresh and spoiled meat samples, avoiding culturing steps. The specificity of the primers designed on the basis of the 16S rRNA gene sequences of *B. thermosphacta* was validated using the DNA extracted from strains belonging to different related species usually found in spoiled meat.
The qPCR assay was evaluated using *B. thermosphacta* genomic DNA and an artificially inoculated meat model to verify the influence of meat on the qPCR sensitivity. The standard curves obtained showed that there was an influence of the food matrix on the effectiveness of the DNA extraction and the model employed was a real food sample. Standard curve had a magnitude of linearity ranging from 2.2 x 102 to 2.3 x 107 CFU/ml, covering six orders of magnitude, while the reaction efficiency was equal to 1.0939. The qPCR assay was then applied to enumerate *B. thermosphacta* in 20 fresh and spoiled meat samples and the results were compared to those obtained by the count method on the selective medium specific for this species (STAA). With the exception of four counts, where the differences between the two methods were < 0.5 log, in sixteen cases the enumerations of *B. thermosphacta* by qPCR was lower than that obtained by plating. In particular, in 6 (30%) samples the difference was 0.5< log <1.5, in 7 (35%) samples it was 1.5< log <2 and in 3 (15%) samples it was > 2 log. The average difference between the viable counts and qPCR methods was 1.12 Log10 CFU/g and 1.51 Log10 CFU/g when applied to fresh or spoiled meat samples respectively; the Pearson correlation coefficient for the methods calculated on the total dataset was 0.89.
The quantification of *B. thermosphacta* by the Real-Time PCR method developed in this study is certainly simple and fast and could be useful for a reliable detection of the species in meat samples. However, considering the level of underestimation reached in most of the samples analyzed, the qPCR method cannot be recommended to accurately quantify *B. thermosphacta*, but only to approximately predict its level of contamination.

Keywords: *Brochothrix thermosphacta*, meat spoilage, plate counting, Real-Time PCR, quantitative analysis.

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"Challenge test" with Listeria innocua on a ready to eat turkey breast

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Reg. CE 2073/05 introduces tolerance limits for some pathogenic microorganisms such as . The attention is focused mainly on RTE (Ready To Eat) products that, since they are not processed before their consumption, require particular controls and major guarantees. "Challenge test" is an useful instrument to study spp. behaviour in food, because it allows to reproduce chemical, physical and microbiological processes that really occur in food products.
This work was made in collaboration with the "Istituto Zooprofilattico della Lombardia e dell'Emilia Romagna" and with the kindly support of a firm producing RTE foods. Our aim was to control the dynamics of growth of three different strains (as an alternative to the pathogenic species), voluntarily inoculated on some samples of turkey breast. In particular we wanted to verify the effectiveness of the post-packaging heat-treatment of the firm and to monitor the behaviour of during the whole shelf-life. The samples were stored at four different temperatures: 4, 10, 15 and 20 °C to understand the dynamics of the microorganism even under thermal abuse conditions.
The post-packaging heat treatment performed by the plant proved to be suitable, since a reduction ≥ 2 logarithmic cycles was always reached. During the storage at 4 or 10 °C the bacterial concentration increased of 4 log cycles in 18 or 7 days respectively. An exponential growth without lag phase was observed with an increase of 4 log cycles within 3 days at 15 °C or 2 days at 20 °C. RTE turkey breast showed to be an optimum medium for spp. growth, therefore it is recommended that the producer pay attention to the hygienic prevention, in order to minimize the initial contamination of the products' surface.

Keywords: challenge test, *Listeria innocua*, turkey breast.

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Survey of Shiga-like toxin producing Escherichia coli in foods of animal origin of the Piedmont region in Italy

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The purpose of this study was to investigate the presence of Shiga-like toxin *Escherichia coli* (STEC) in traditional food products of animal origin from the Piedmont region of Italy. A multi-phasic approach was used combining culture dependent and independent methods. Using an optimized quantitative PCR-based protocol, the genes *rpoB* (specific for the *E. coli* species), *stxI*, *stxII* and *eae* (virulence genes that determine STEC) were amplified from DNA extracted either directly from the food samples or after an overnight enrichment. This permitted the detection and quantification of total *E. coli* populations and of STEC sub-populations in the samples. In parallel, counts were determined on Sorbitol MacConkey Agar (SMAC). Colonies on SMAC were randomly selected and were identified as STEC by conventional multiplex PCR analysis targeting the three virulence genes mentioned above. Isolates that were identified as STEC were characterized by ERIC-PCR. This methodological approach was applied to 50 cheese and 52 sausage samples. The application of the qPCR for the detection and quantification of the virulence genes resulted in 53 samples positive for one or more of the determinants with the *stxI* being detected at a higher frequency compared to the *stxII* and *eae* genes. Overall, sausages presented a higher frequency of contamination. One of the samples, a seasoned cheese, harbored STEC population(s) carrying all three virulence genes at the level of 103 CFU/g. With the culture-dependent approach, STEC strains were isolated from 43 of the samples. According to the results of the identification of the isolates, sausages were contaminated with STEC at a higher frequency with respect to the cheeses. A significant diversification observed between sausages and cheeses was the higher frequency of isolation of strains from cheeses carrying the *stxII* gene and a more uniform isolation of strains carrying one of the three virulence determinants from the sausages. The two approaches employed for the survey of STEC in traditional products highlighted the significant level of contamination, particularly in sausage samples. The results obtained were not always in agreement but gave complementing information regarding the ecology of this pathogen in foods of animal origin.

Keywords: qPCR, STEC, *Escherichia coli.*

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Chemical composition and organoleptic characteristics of Vinsanto wine obtained by using different Saccharomyces strains

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The production of Vinsanto still relies on the utilization of traditional techniques in many viticultural areas of central and northern Italy. According to these, the techniques consist of an initial phase of maturation and drying of grapes on special mats in traditional room (the "fruttaio") before pressing, and the resulting high sugar containing must undergoes fermentation and biological aging (for two years or more) in wooden barrels placed in traditional room (the "vinsantaia") under ambient temperature and humidity. Just before barrel filling, the must is traditionally enriched with a percentage of sediment (known as "mother") collected from the barrels from the end of the ripening of the previous Vinsanto wine production. This traditional process, carried out without any effective control of the process variables, can leads to the production of excellent wines but their characteristics may vary dramatically from year to year (Domizio *et al*., 2007). For this reason some wineries today prefer not to use the "mother" of Vinsanto, and inoculate musts with commercial starter yeasts able to ferment musts containing high sugar concentrations.
The present work reports on chemical composition and perceivable characteristics of Vinsanto wines obtained using four different strain of *Saccharomyces cerevisiae* tested with and without mother addition and under ambient conditions (vinsantaia). Trials without starter strain were used as a control. Under the same conditions, the different *S. cerevisiae* strains showed different fermentation behaviours and produced wines with different compositional and organoleptic characteristics. The fermentative behaviour of yeast strains and the compositional and organoleptic characteristics of the wines were strongly influenced by the addition of the "mother". In particular, in the inoculated fermentations a higher alcoholic degree in wines was reached, especially in those fermentations conducted with "mother" addition. The wines obtained with yeast inoculation and without "mother" addition generally showed a lower content of acetaldehyde and higher alcohols. The wines produced in the different trials showed different organoleptic characteristics especially in terms of sweetness and bitterness.

Keywords: Vinsanto-wine, wine-aroma, yeasts, *Saccharomyces.*

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Individuation of the nutritional conditions able to induce dimorphic transition in Pichia fermentans

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The utilization of yeasts as bio-control agents against fruit pathogens may represent an effective alternative to the use of synthetic chemical fungicides. Recently, Giobbe *et al*. (2007) reported the case of the dimorphic yeast *Pichia fermentans* that is able to control brown rot caused by *Monilia* spp. on apple fruits but causes a rapid fruit decay and shows a pathogenic behaviour when tested on peaches. Interestingly, in both cases *P. fermentans* forms biofilm on fruit surfaces, but this is made of yeast-like cells on apple and pseudo-hyphae on peach fruits. In this context, it seems crucial to understand whether the pathogenic behaviour of *P. fermentans* is a consequence of pseudohyphal growth and, therefore, if the ability to form pseudo-hyphae is one of the risk factors to be evaluated in view of the utilization of microbial antagonists. In the present work, with the aim of elucidating the mechanisms involved in *P. fermentans* dimorphic transition, we looked for the nutritional conditions able to induce and separate yeast-like and pseudo-hyphal growth. While on urea-containing medium *P. fermentans* produces a biofilm made of yeast-like cells, on methionin, as the sole nitrogen source, the biofim consists merely of pseudo-hyphae. The role of methionin on filamentation will be discussed.

Keywords: *Monilinia fructicola*, *Pichia fermentans*, biological control, dimorphic transition, biofilm.

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Whey starter for Parmigiano Reggiano: culture independent approach

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Natural whey starter is a complex microbial association of lactic acid bacteria utilized for the production of Parmigiano Reggiano PDO cheese. One of the traditional features of this production is given by the continuous reutilization of this culture obtained by incubation at gradually decreasing temperature of the non acidified whey collected the day before. A polyphasic approach, combining a traditional culture method and different culture-independent methods such as FISH and LH-PCR, was used to investigate the microbial composition in eighteen natural whey starters and eighteen non acidified wheys from which they derived. The samples were collected from nine different dairies located in the province of Parma, Modena and Reggio Emilia belonging to the area of production of Parmigiano Reggiano cheese.
Plate counts were performed in a whey-based medium and total bacterial counts were carried out by using a dye-binding assay (DAPI). Direct total microbial counts of natural whey starters were 1 log unit higher than those of the cultivable population, confirming the inability to cultivate more than a small proportion of the entire microbial community identifiable by direct count procedures.
LH-PCR results highlighted a high variability in the composition of the different whey starters. The majority of the LH-PCR profiles showed comparable percentages of *Lactobacillus helveticus* and *Lactobacillus delbrueckii* subsp. *lactis* as dominant species while only two samples were characterized by *L. helveticus* as dominant species. Furthermore, in a few samples the percentages of *L. helveticus* and *L. delbrueckii* subsp. *lactis* were comparable to those of other species represented by non attributed peaks and *Streptococcus thermophilus*. FISH results were in agreement with those by LH-PCR. However a higher number of samples showed the presence of *S. thermophilus*.
Non acidified wheys showed LH-PCR profiles similar to those obtained in the subsequent whey starters. Conversely, other profiles revealed different dominant species with respect to the starter. This variability could be related to different incubation conditions used by each dairy for the production of natural whey culture. Discordance between FISH and LH-PCR results was found in a few of non acidified whey samples; that could be do to the different molecular target (RNA and DNA, respectively). Cooking could affect integrity of cells making some of them undetectable by FISH.
These methods showed a different picture of the same community; thus combining these two techniques, a more accurate analysis of both the community structure of Parmigiano Reggiano natural whey starters and its evolution from non acidified wheys was obtained. New light has been shed on Parmigiano Reggiano natural whey starters microbial composition, highlighting how a culture-independent approach could be used and improved for studying this and other food ecosystems.

Keywords: Parmigiano Reggiano natural whey starter, LH-PCR, FISH.

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Lactobacillus plantarum acidic stress response in terms isovaleric acid pathway

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The presence of *Lactobacillus plantarum* is associated with desirable properties in many fermented foods as it favourably influences the flavour of the final products by its metabolism. In addition to acids, this specie produces volatile compounds during the fermentation of carbohydrates, conversion of fatty acids by β-oxidation and the breakdown of proteins by proteolysis. When *L. plantarum* is under optimal conditions the concentration of these metabolites is constant, while during sub-lethal stresses, as acidic stress (pH 3.6 obtained with the addition of lactic acid), (Pieterse *et al*., 2005) *L. plantarum* responds with an alteration of the metabolic profiles, characterised by overproduction of compounds such as isovaleric acid (IVA). IVA is described as one of the most present aromatic compounds in fermented foods. The aims of this study were to elucidate the mechanisms of IVA over production in exposed to acidic stress (pH 3.6), identify the IVA precursors, as well as the over or under expression of specific genes and, subsequently, the proteins potentially induced by 2-DE. The results obtained from the Gaschromatography-Mass spectrometry-Solid phase microextraction (GC-MS-SPME) of the cultural fluids suggested that at pH 3.6 there was always an over production of IVA. This over production was linked more to fructose than to maltose consumption probably for the electron acceptor capacity of the first carbohydrate that can compensate a possible disequilibrium into NAD+/NADH under acid stress. In fact, the synthesis of IVA is associated with the branched chain amino acids metabolism. These reactions are NAD+ dependent and produce NADH. The results obtained were supported by the proteomic study (changing in the expression of some heat shock proteins) and gene expression study by real time PCR. All the analyses developed, pointed out the important role of IVA formation in the cells internal pH maintenances due to the need to maintain the ratio NAD/NADH.
This hypothesis is supported by the finding that under acid stress the IVA overproduction is accompanied by the overproduction of metabolites having good flavour attributes whose biosynthesis is NAD dependent.

Keywords: *Lactobacillus plantarum*, acid stress, isovaleric acid, flavour.

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Effects of nutritional and environmental conditions on Salmonella spp. biofilm formation

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Microbial biofilms are recognized as matrix-enclosed bacterial populations adhered to a surface. If food processing surfaces are not properly cleaned, biofilms can develop with growth and survival advantages over planktonic cells, experiencing enhanced resistance to hostile environmental conditions. Attachment of bacteria with subsequent formation of biofilms on food industry surfaces have important economic consequences, since they can serve as a potential source of contamination for food products, which may lead to food spoilage or transmission of diseases.
Salmonella spp. are one of the most important foodborne pathogens. More than 95% of cases of infections caused by these bacteria are foodborne and these infections account for about 30% of deaths resulting from foodborne illnesses. Several studies have lead to the discovery that these bacteria are capable of adhering and forming biofilms on different surfaces: the attachment of bacterial cells is affected by several factors, including the medium in which they are grown, motility, growth phase of the cells, type and properties of the inert material, presence of organic material (''conditioning film''), temperature, pH, length of contact time, and so on.
A better understanding of *Salmonella* spp. biofilms may provide valuable pathways into the prevention of their formation. Therefore, this investigation focused on the use of the response surface methodology to study the effects of temperature (20-40 °C), pH (4.5-7.5) and medium composition (0.5-2.5 g/L of peptone) on biofilm formation by *Salmonella* spp. Stainless steel was the surface chosen. To allow biofilm formation, jars containing sterile medium and stainless steel chips were inoculated with Salmonella spp. ATCC 35664 (initial inoculum ca. 103 CFU/ml). Incubation temperature, pH and composition of the media varied according to the experimental design. Viable and cultivable biofilms cells were enumerated after 8, 24, 48, 72, 96 and 120 hours after inoculum.
Results highlighted that the target strain was able to adhere on stainless steel, under all the conditions tested. To assess potential differences, the aptitude to biofilm formation (ABF), defined as the time necessary to start adhesion on the surface, was calculated by using the Gompertz equation. This parameter was modeled through a stepwise regression procedure and experimental conditions resulting in the greater ABF were: growth in poor media (1.0-1.5 g/L of peptone), incubation temperature of about 30 °C, pH close to 6.0.

Keywords: biofilm, *Salmonella* spp., environmental conditions, culture conditions.

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Determination of condition assays to identify glycosidase activity in probiotic bacteria

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The main beneficial intestine bacteria are *Bidifobacterium* spp. and *Lactobacillus* spp. Dietary sources of plant origin are very rich in bioactive compounds such as polyphenols, which possess a demonstrated role in prevention of cardiovascular diseases, cancer, neurodegenerative diseases, diabetes and osteoporosis (Scalberts *et al*., 2005). Flavonoids are biologically active polyphenols present in plants mainly as beta-glycosides. The human intestinal enzymes cannot remove the sugar attached to flavonoids, avoiding its uptake by the gut. However, some groups of bacteria can hydrolyze these beta-glycoside flavonoids to the aglycone form, which is easily absorbed. Some *Bifidobacterium* species of human intestinal origin have been described as capable of hydrolyzing the beta-glucosidic bonds of flavonoids present in common bean, without fermenting the aglycone form (Marotti *et al*., 2007). The final objective of this work is to extend this study to *Lactobacillus* species which are probiotics with wide applications in the food industry. The first step of this research was to optimize the beta-glucosidase assay in *Bifidobacterium* strains grown in TPY and in MRS medium in order to define the experimental conditions for the beta-glucosidase assay in a medium suitable for lactic acid bacteria growth, i.e. MRS, as some reports describe that beta-glucosidase activity may vary according to the growth medium used (Choi *et al*., 1996). In order to optimize the beta-glucosidase assay, two *Bifidobacterium* species were choosen: *B. pseudocatenutalum* for its high beta-glucosidase activity and *B. longum* for not possessing any detectable glycosidase activity (Marotti *et al*., 2007). The assay consists in growing the bacteria with p-NPG (p-nitrophenyl-beta-D-glucopyranoside) for 14-24 h and then determining the amount of pNP (p-nitrophenol) at 405 nm. The results show that maximal growth of each bacterium decreases at higher concentration of p-NPG added. The beta-glycosidase activity in *B. pseudocatenulatum* is not dependant on the medium (TPY or MRS) or cell density; instead it depended on substrate concentration, suggesting an operon regulation where the expression is affected by the levels of substrate and product present in the medium. This kind of regulation also could explain the effect of p-NPG concentration on maximal growth. Finally, we also observed that pNP could be used as substrate by these bacteria. If both bacteria are growing in MRS or TPY with 1 mM pNP, after 17 h of incubation no traces of pNP are detected, although the levels of pNP are conserved after 17 h of incubation in MRS or TPY. So, we suggest that even though *B. pseudocatenulatum* and *B. longum* can use pNP, it is probable that *B. pseudocatenulatum* could have a degradation rate of pNP slower than beta-glycosidase activity on pNPG. Meanwhile it cannot be argued if *B. longum* glycosidase on pNPG and on pNP has the same rate of beta-degradation, or if it does not have any glycosidase activity. Therefore the assay optimized in this work can allow to identify bacterial strains possessing high beta-glycosidase activity without possessing fermenting activity on the aglycone form. The next step is to extend the study to *Lactobacillus* species.

Keywords: *Lactobacillus*, *Bifidobacterium*, flavonoids, beta-galactosidase assay.

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Microbiology of two traditional sourdough production processes in Italy: Lagaccio biscuit and Panettone cake

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A very large number of traditional sweet-leavened baked products originates from sourdough processes, especially in Italy (De Vuyst and Neysens, 2005). Sourdough is a mixture of flour and water fermented by a stable association of yeasts and lactic acid bacteria, the resulting baked product being characterized by prolonged shelf-life and typical sensory quality. Most studies dealing with sourdoughs have focused their attention on the microbial diversity which characterizes each sourdough or on the metabolic interactions among microbial species during sourdough fermentation (De Vuyst and Neysens, 2005; Gobbetti, 1998). However, the rheological as well as the sensorial properties of a sweet product obtained by sourdough technology depend on the metabolic behaviour of the microbial community during both the propagation stages (two or more refreshments) and the final leavening stage. Indeed, the behaviour of the microbial populations in this final phase of the process, where ingredients other than flour and water are usually added, has been poorly investigated. Hence, a study was undertaken to investigate on the behaviour of the microbial populations throughout two Italian traditional sourdough production processes leading to two distinct sweet-leavened bakery goods: Lagaccio (typical Genoese dry biscuit) and Panettone (soft stuffed North Italian typical cake). In both cases, as it resulted from a preliminary investigation, the mother sponge was characterized by the dominant presence of the same microbial populations: *Lactobacillus sanfranciscensis*, *Candida milleri* and *Saccharomyces cerevisiae*. Dough samplings and platings were carried out at the factories, located near Genoa (Lagaccio production) and near Turin (Panettone production). Identification of microbial isolates was attained by molecular methods: PCR-RFLP of ITS region for yeasts and PCR-ARDRA for lactic acid bacteria. Microbial metabolites in sourdough samples were determined by HPLC analysis.
The two production processes differentiated each other not only for the type and quantity of ingredients added in the final dough, but also for the production cycle, that included three refreshments plus one final dough in the case of Lagaccio production and two refreshments plus a two-step final dough in the case of Panettone production. In spite of these differences, the growth behaviour of the microbial populations during the propagation stages was quite similar, the ratios *L. sanfranciscensis* to *C. milleri* to *S. cerevisiae* changing from 18:2:1 and 35:2.5:1 (mother sponge of Lagaccio and Panettone, respectively) to 30:2:1 and 75:4:1 at the end of the refreshments. During the final leavening stage, the yeast populations grew more easily in Panettone dough rather than in Lagaccio dough, whereas the lactic populations showed the same behaviour. Indeed, after ingredient additions, *L. sanfranciscensis* entered a death phase that, in both cases, reduced the number of viable cells by a factor of about 90%. The chemical composition of the two sourdoughs at the end of the leavening stage was coherent with the observed growth behaviours of the different populations, which, in turn, were significantly affected by the type and quantity of ingredients added to fulfil the typical sensorial characteristics of the baked product.

Keywords: sourdough, Lagaccio, Panettone, yeast, lactic acid bacteria.

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Characterization of stx converting bacteriophages induced from Shiga-toxin-producing Escherichia coli strains isolated in dairy products

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The emergence of Shiga-like toxin producing *Escherichia coli* (STEC) as food borne pathogens has become a worldwide public health concern. These strains are responsible for several human gastrointestinal diseases with water or bloody diarrhoea and, in some cases, they may lead to severe infections such as hemorrhagic colitis (HC) or haemolytic uremic syndrome (HUS). The most well-known serotype is O157:H7; however, especially in continental Europe, the importance of non-O157 STEC is being increasingly recognized (Kaufmann *et al*., 2006). These downstream sequels of the infection are the result of the action of toxins which have been found to be carried on lambdoid-like bacteriophages that have the ability to horizontally transfer toxin genes between *E. coli* strains (Schmidt, 2001). STEC strains can produce one or both of two distinct form of Shiga-toxins: Stx1 and Stx2, and the last group presents numerous variants lead by different bacteriophages.
The aim of this study was to isolate and characterize *stx*-carrying bacteriophages from unpasteurized and raw milk dairy products, which consumption is often correlated to human infections. Three strains belonging to serotype O157:H7 and 2 strains that were not typeable, isolated from milk and dairy product of cow and goat origin, were examined for the presence of *stx*-carrying bacteriophages in their genomes. The lytic cycle of the bacteriophages was induced by norfloxacin, and *stx*-carrying phages were isolated after plaque blot and hybridization with a digoxigenin-labelled *stx* probe. Genetic and morphological characterization of induced stx phages was carried out; infectivity and ability to obtain lysogens on different host strains was also investigated.
Results indicated that only 4 out of 5 strains carried an inducible stx phage, while other kind of phages were not detected; all of them had the *stx*2 gene, as confirmed by a PCR assay with specific primers. The induced viral particles showed morphological diversity as concern capside and tail dimensions. This variability was also reflected in their restriction fragment length polymorphism variation and genome size. Despite of this variability, all phages infected the four host strains analyzed, with the exception of MC1061 strain that was infected by only two phages.
To our knowledge this is one of the first work concerning the characterization of *stx*-carrying phages induced from STEC strains isolated in dairy products. The level of diversity and the ability to infect diverse host strains founded in these phages could enhance and promote the rapid spread of shigatoxigenic potential throughout *E. coli* and related bacterial populations in food and environment to which humans may be exposed.

Keywords: Shiga toxin, *Escherichia coli*, *stx*-carrying-bacteriophages, phages, dairy products.

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Proteomic analysis of Saccharomyces cerevisiae wine strains with different susceptibility to stuck fermentations

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The importance of lipid nutrition and lipid metabolism during must fermentation has been recently recognized by some authors (Belviso *et al.*, 2004; Mannazzu *et al.*, 2008; Zara *et al.*, 2008 and references therein). In particular it has been shown that during the first hours of fermentation in the absence of exogenous lipids, the induction of key genes in lipid metabolism is strongly correlated with the fermentative performances of *Saccharomyces cerevisiae* wine strains.
In this work we have analyzed the proteome of two *Saccharomyces cerevisiae* wine strains, with different susceptibility to stuck fermentation, during growth in the presence and absence of lipid supplements (ergosterol and oleic acid). The aim of the work was to further characterize at the proteome level, the molecular changes that industrial strains of *Saccharomyces cerevisiae* experiment during fermentation of lipid depleted musts. Total proteins extracted from yeast cells, collected 60h after the inoculum, were subjected to 2D Electrophoresis and the gels obtained were analyzed by means of the PDQuest software (Biorad). Results obtained showed that 50 proteins are differentially expressed in the two strains and that 2, in particular, are highly expressed in the strain resistant to stuck fermentation. Comparison of the 2DE maps obtained with those of *Saccharomyces cerevisiae* available on line (http://www.ibgc.u-bordeaux2.fr/YPM/carte.htm) allowed us to identify the two proteins as two members of the HSP70 family, heat shock proteins that are involved in the general stress response of *Saccharomyces cerevisiae*. The attribution of the differentially expressed proteins to Gene Onthology categories will be carried out to better understand the molecular basis of yeast adaptation to the lack of lipid nutrients.

Keywords: lipid nutrition, stuck fermentations, proteomic analysis, 2-DE electrophoresis, *Saccharomyces cerevisiae* wine strains.

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Air-liquid biofilm formation is independent on RAS2 gene in Saccharomyces cerevisiae flor yeasts

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In *Saccharomyces cerevisiae RAS2* gene switches on cAMP-PKA and MAPK pathways (Sengupta *et al*., 2006) that, in turn, regulate *FLO11* gene. This gene is involved in many cell-cell adhesion phenotypes like pseudohyphae formation, adhesive growth and biofilm formation (Reynolds and Fink, 2001; Zara *et al*., 2005; Fidalgo *et al*., 2006). The aim of this work was to assess the involvement of *RAS2* gene in the air-liquid biofilm formation by *Saccharomyces cerevisiae* flor strains. To do that *RAS2* gene was deleted in the *Saccharomyces cerevisiae* flor strain 3238-32 and both the wild type and the *ras2D* mutant were tested for biofilm formation and *FLO11* transcription levels. *ras2D* maintained the ability to form biofilm, while significant differences were observed in *FLO11* transcriptional levels. Finally, the absence of glucose and the presence of nitrogen enhanced air-liquid biofilm formation both in the parental and *ras2D* flor strains. Thus, *RAS2* deletion does not affect air-liquid biofilm formation.

Keywords: flor, *Saccharomyces cerevisiae*, *RAS2*, biofilm.

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Lab-scale biopile assessment for the evaluation of diesel fuel soil remediation

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A soil of north-eastern Italy was interested by a strong diesel fuel spill. A wetting system was used to periodically release a microbial inoculant, containing a dried consortium of bacterial strains proven effective by previous microcosm evaluation. Investigations indicated that oil degrading bacterial strains were shown not to enhance significantly the remediation when the polluting agent was present in the soil for many years (Basaglia *et al*., 2003).
To make a distinction between the role of the remediation activity of oil degrading bacterial inoculants and that of the aeration and nutrients application, four lab-scale biopiles were set up and monitored. This assessment was also necessary to dispose of suitable controls and make the right comparison between inoculated and not inoculated soil (Aichberger *et al*., 2005).
Two different soil types were used in two different sets of experiments:
set 1 - two lab-scale biopiles loaded with an aged polluted soil coming from a 30 years contaminated site: this soil was supposed to contain a well established oil tolerant and oil degrading microflora.
set 2 - two lab-scale biopiles loaded with an unpolluted soil, artificially contaminated with diesel fuel: this soil was supposed not to contain a well established oil tolerant and oil degrading microflora.
In both the above experiments the control was represented by a non inoculated column.
Soil microbial community composition, as well as that of the commercial inoculant used, was mainly determined by denaturing gradient gel electrophoresis (DGGE) (Muyzer *et al*., 1998). A number of microorganisms capable of living in the presence of diesel as the only carbon source were also isolated and identified by 16s rDNA analysis. Among the bacterial species isolated and identified in the active biopiles *Sphingomonas* sp*.* was almost constantly detected.
DGGE analysis performed on set 1 showed that the profile related to the inoculant did not cluster with any of the profiles obtained from the two biopiles, at any time. Since gas-chromatographic analysis indicated that both the biopiles were fully active in the remediation process, the biodegradation occurred was to be ascribed to the autochthonous microflora that experienced a strong and continuous selective pressure during the long period (more than 30 years) of continuous pollution. After so long time the microbial community evolved in a hydrocarbon resistant and actively degrading population.
The results obtained from the experiment set 2, instead, indicated that the community profile of the inoculated biopile clustered with that obtained from the inoculant. This indicates that the inoculated bacteria did colonize the soil. While GC analysis demonstrated that they effectively metabolized the hydrocarbons, very reduced degradation activity was detected in the not inoculated control.
The above results clearly suggest that while bioventing technology always stimulates the natural *in situ* biodegradation, bioaugmentation could significantly contribute to the remediation process only if rapidly applied after the contamination occurs.

Keywords: biodegradation, bioaugmentation; DGGE; biopile; hydrocarbon.

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Two autotrophic processes drive plant establishment in a polar desert

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Several mechanisms occurring in terrestrial environments are still unresolved like those driving soil formation and primary colonization by plants (Hodkinson *et al*., 2002). Some key processes in land reclamation in hot and cold deserts are yet poorly understood despite a challenge for the future is to limit the problem of desertification, established or emerging in many regions of our planet (Bashan *et al*., 2002). Glacial moraines are unconsolidated rock debris accumulated by glacier movements. The ongoing global warming is causing the melting of glacier fronts and exposes the formerly ice-covered rock debris to the atmosphere, leading to the start-up of the slow process of soil and biocenosis formation (Hodkinson *et al*., 2003). Polar moraines above the 75°N are classified as cold deserts since for most of the year water is immobilized as ice and precipitations are very sparse. These are ideal sites for the study of primary colonisation of the rock substrate. In the moraines formation of soil and plant biocenosis on the substrate recently released from permanent ice cover is slow and hampered by the harsh environmental conditions (low water availability and low temperature) and by nutrient paucity that limits microbial primary production, the formation of organic matter and plant establishment (Yoshitake *et al*., 2007). In polar moraines photosynthesis by cyanobacteria and microbial heterotrophic assimilation of organic materials released by animals or transported by wind are commonly considered the processes initiating soil formation and mediating plant colonization (Hodkinson *et al*., 2003). In this work, a multidisciplinary approach exploited molecular ecology methodologies, bacterial cultivation and soil geochemistry, allowing to describe an alternative autotrophic mechanism initiating soil formation in the glacier foreland of Midtre Lovenbreen glacier (78°56'N, Ny Alesund, Svalbard). In this site, the chemolithoautotrophic iron-sulphur oxidation of pyrite triggers early soil formation and promotes primary colonization by plants. Rock pyrite weathering mediated by *Acidithiobacillus ferrooxidans* determines acidity and corresponding fertility gradients, where water retention, cation exchange capacity and nutrient availability are increased. This is a new, previously unrecognized soil genesis and crop formation model with potential, past and present, terrestrial and extraterrestrial analogues.

Keywords: soil primary colonisation, rock microbial weathering, *Acidithiobacillus ferrooxidans*, iron-sulphur chemolitoautotrophy.

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Dynamic of denitrification activity in the soil of a wooded riparian strip assessed for nitrogen removal.

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Human activities drastically altered the nitrogen cycle on a global scale, mainly causing a strong increase of combined nitrogen release to freshwater ecosystems. Located at the interface between terrestrial and aquatic environments, riparian buffer zones have been proven very effective in nitrogen removal from surface and subsurface water flows (Hunter and Faulker, 2001; Spruill, 2004; Carline and Walsh, 2007), essentially due to the metabolic activity of soil bacterial communities (particularly denitrification process) and to the uptake and assimilation by plants and microbes (Pinay *et al*., 1993; Haycock *et al*., 1997). However, there is no agreement (Haycock *et al*., 1997) on the relative significance of these two processes in different landscapes, though denitrification is considered to be the base process because it permanently removes nitrogen from the soil bringing it back in the atmosphere in a gaseous form.
This research is focused on the comprehension of the main mechanisms which regulate denitrification in the soil of a wooded riparian strip. In order to reach this objective a relationship between the denitrification rate measures (effects) and the composition, biomass and distribution of bacterial community (causes) in different soil layers, was undertaken. To this objective, an appropriate experimental site which allows the total control of the irrigation water volume flowing through a forested area was designed and assessed in 1999 along the riparian strip of the Zero river, within the Venice lagoon watershed, on an area previously exploited as an arable land. The buffer zone, organized with an irrigation ditch carrying water from the Zero river, produces a difference in elevation between the irrigation and the drainage ditches, resulting in a sub-surface water flow running through the entire buffer strips. New selected plants were placed in the ground and organized in four parallel rows for each plot. In this way two processes, vegetation/microbial uptake and denitrification, can work together to provide an effective buffer zone to reduce excess of nitrogen from the aquatic ecosystem. Microbial denitrification, continuously monitored in the different zones and depths of the pilot site during a period of time of three years, was directly measured in situ, while the denitrification potential was evaluated on specifically treated soil samples by lab-scale experiments.
The results obtained indicated that i) the denitrification process is very active already during the first year from the conversion of the arable crop to the forested area, but it reaches the higher rates after 3 year; ii) the upper soil samples show lower denitrification rates due to the aerated nature of the soil environment; iii) the nitrate rich water pumped from the Zero river flows through the medium layer (40-55 cm) where maximum values of denitrification were recorded; iv) higher denitrification rates occur in summer and autumn but the process is active during all the year; v) the denitrification potential study indicated that the most limiting factors could be the availability of organic carbon.

Keywords: denitrification, riparian buffer zones, nitrogen removal.

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Calcium carbonate precipitation in natural habitats: a possible microbial origin

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Calcium carbonate precipitation is commonly considered to be abiogenic, in spite of the fact that microbes are present in CaCO3 substrates (Castanier *et al*., 1999; Barton *et al*., 2007).
Laboratory experiments involving CaCO3 deposition by bacteria isolated from stalactites, moonmilk, travertines and calcareous soils, confirmed their role in mineral precipitation (Cacchio *et al*., 2003; Cacchio *et al*., 2004). Under laboratory conditions, we demonstrated that:

1) cultivable bacterial cells isolated from carbonate substrates are abundant, ranging from 1.0 x 103 – 1.6 x 105 CFU g^{-1} sample dw, suggesting that their presence is not occasional;
2) a large proportion of these bacterial strains (75-100%), were able to form $CaCO_3$ crystals *in vitro*, at different temperatures, confirming the data of Boquet *et al*. (1973).
3) the relative abundance (R.A.) of each calcifying strain with respect to the total cultivable microflora showed that:
   - in calcareous environments, calcifying isolates are dominant, suggesting that calcification is of evolutionary concern in these habitats;
   - the most frequent strains produced *in vitro* the largest amounts of crystals, confirming that the ability to precipitate carbonate may therefore be advantageous and subject to evolutionary selection in karst environments;
4) control experiments without bacteria or inoculated with autoclaved bacterial cells precipitate no carbonate minerals: metabolic activity is necessary for precipitation.
5) the type of CaCO3 bioliths obtained *in vitro* depends on both growth temperature and bacterial strains;
6) X-ray analyses indicated that calcite was always the predominant carbonate polymorph produced;
7) none or a small percentage of the calcifying bacteria derived from caves or calcareous soils dissolves detectable amounts of carbonate, but 50% of the calcifying bacteria associated with moonmilk solubilise $CaCO_3$, confirming the role of this process in the origin of this particular calcareous formation.
8) SEM observations of purified crystals obtained *in vitro* showed calcified bacterial cells, bacterial imprints and microbial biofilms.

The culture experiments reported here support that *Bacillus* spp., *Kocuria* spp., *Burkholderia* spp., *Staphylococcus* spp., *Acinetobacter* spp., *Arthrobacter* spp. play a major role in carbonate deposition in natural habitats.

Keywords: calcifying bacteria, calcium carbonate precipitation, calcareous natural habitats, speleothem, moonmilk.

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Effects of three active ingredients on soil bacterial communities

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The use of bioactive molecules for medical purposes is an important field of industrial activity and gives rise to release a large amount of chemicals in the environment. Industrial farm producing such molecules are suitable places to investigate the possible release of contaminants and their effects on biological communities. Soil microbes are directly affected when active molecules reach environment and thereafter they are very suitable as experimental tool. Side effects on humans and animals require specific toxicological studies, to which the knowledge deriving from soil microorganisms can give benefits (Boxall, 2004).
Our research has been focused on the assessment of the effects of glimepiride, ramipril and pentoxifillyne, three active ingredients belonging to different therapeutic classes, on the functional and genetic diversity of the bacterial community in soil.
Soil samples of different physical and chemical characteristics were collected from areas exposed to the risk of contamination by active ingredients inside a pharmaceutical farm estate (I) and from a control (E). Aliquots of each soil (I, E) were placed separately in plastic boxes and amended respectively with 0, 5, 50, 500 mg/Kg of each active ingredient. Changes in microbial diversity were monitored by culture-dependent methods: bacterial enumeration (CFU), community level physiological profiles (CLPPs) measured by Biolog Ecoplate™ and a culture-independent technique DGGE, over a period of 3 months.
The DGGE profiles of treated and untreated soils were different during the incubation period. In particular, ramipril treated soils showed a noticeable decrease in bands number respect to the control soils after 60 and 90 days.
At 90 days, CFU isolated from soils (E), treated with three active ingredients were significantly smaller ($P < 0.05$) than the ones from control; soils (I) treated with pentoxifillyne (50 and 500 mg/Kg) and ramipril (5 mg/Kg) on the contrary gave higher CFU figures.
Results of cluster analysis performed on Biolog data showed that functional abilities of the soil microbial communities were altered by application of the active ingredients. In particular, a) CLPPs of the glimepiride and ramipril treatments, especially 500 mg/Kg, differed from the control; b) CLPPs of pentoxifillyne treated soils taken from pharmaceutical farm (I) were sharply different from control than pentoxifillyne treated soil taken outside (E). Soil characters were influential on bacterial community response to active ingredients.
Differences were found on substrate utilization in the Biolog Ecoplate™ between treated and untreated soil, mainly as to the intensity of substrate assimilation.
In conclusion, this study shows that both genetic and metabolic potential of soil microbial communities are affected by application of these active ingredients.

Keywords: Glimepiride, Ramipril, Pentoxifillyne, soil, bacterial community.

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Effect of indigenous bacterial community on arsenic mobilization in a degraded soil under submerged conditions

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Arsenic (As) is relatively abundant in soils because of contamination from anthropogenic sources in addition to its natural occurrence in minerals. Its primary forms are arsenate As(V) and arsenite As(III). Bioavailability and mobility of As are governed by physico-chemical and biological factors. Microorganisms have a strong impact on mobility and transformation of As due to both metabolic and detoxification reactions (Stolz *et al*., 2006). The objective of this research was to examine the effect of the bacterial activity on As mobilization in an As-contaminated soil under submerged conditions. The soil used was a mixture (approximately 1:1 w/w) of pyrite cinders from an industrial site and a mineral unpolluted loamy sandy soil. The soil mixture was physically and chemically characterized and As fractionation was provided by using a sequential extraction procedure (Wenzel *et al*., 2001). The total As was 450 mg/kg and the bioavailable As was low (1% of the total) because of the strong binding of As with solid phases, mainly amourphous and crystalline iron (Fe) oxyhydroxides. Fe content was high (600 g/kg) and it was present mainly as Fe-oxides. Microcosm experiments were carried out in static conditions in 100-ml tubes containing soil and water (1:1 w/v) with (0.2% w/w) or without glucose. Abiotic controls were also prepared. Time courses of pH, redox potential, bioavailable As and Fe, and the number of heterotrophic bacteria were determined at successive incubation times. Denaturing gradient gel electrophoresis (DGGE) analysis of 16S rRNA genes of the bacterial community of soil was also performed to evaluate the dynamics of the microorganisms related to the addition of a C source. In glucose-amended microcosms the increased microbial activity caused a reduction of pH and redox potential. Consequently, the content of bioavailable As and Fe increased (from 0.1 mg/kg to 1.2 mg/kg and from 1.2 mg/kg to 30.0 mg/kg, respectively). DGGE analysis highlighted changes in the DNA patterns of microcosms amended with glucose but not in those without glucose. In particular, bands belonging to *Flavobacterium defluvii*, *Flavobacterium johnsoniae*, *Flavobacterium flevense*, uncultured *Flavobacterium* sp. and to *Paenibacillus lactis* intensified; on the contrary an intense band of the soil profile, related to *Beggiatoa alba*, disappeared. This data indicated that the structure of the microbial community was modified by the occurrence of physico-chemical changes, among which the As mobilization. As-resistant strains were isolated in the course of incubation and characterized. The isolates showed high resistance to As(III) and they were mainly clustered into two classes of resistance: the first one resisted to 10 mM and the second to 5 mM of As(III). For some strains the ability to reduce As(V) or to oxidize As(III) was demonstrated. All the results evidence that the microbial activity, enhanced by the presence of glucose, play a role in the mobilization of As and Fe in submerged soils.

Keywords: pyrite cinders, arsenic-resistant bacteria, DGGE, redox potential, bio-leaching.

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Metabolomic characterization of the growth curve in the yeast Saccharomyces cerevisiae

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Metabolomics describes the physiological status of the cell, giving a global view of the cellular components. The possibility to work on intact cells, the relatively inexpensive and straightforward procedure are major advantages to employ this technique to depict complex phenomena such as the cellular growth. The alterations in the growth curve represent an useful tool to detect stressing environmental conditions.
Scientific works attempting to describe the growth curve by means of metabolomic tools are relatively few and focused on bacteria (Ede *et al*., 2004; Zeroual *et al*., 1994), nothing, to our knowledge, is currently available on yeast.
Aim of this work was to study the mode of variation of metabolites during microbial growth, by means of the metabolomic tool FTIR (Fourier Transform Infrared Spectroscopy), which produces as an output a spectrum ranging from 4000 to 600 cm^{-1}, containing information on the major classes of chemical compounds. The use of whole yeast cells, ensured that the spectra reflected all of the components of the cells, including the cell walls, cell membranes, internal structures, and the cytoplasm. The yeast employed in the study was *Saccharomyces cerevisiae* DBVPG 4297.
Cells were grown in four different media: YEPD (2% glucose), YEPD (20% glucose), YNB (2% glucose) and switch from YNB media at 2% glucose to YNB media 3% glycerol. In all conditions the spectra collected in different times showed significant changes throughout the growth curve. In order to provide a synthetic view of the variations, distance indexes were calculated with a series of algorithms written in the R environment (ww.cran.org) for this purpose. The major variations occurred, among others, in the carbohydrate spectral region, where two subregions reacted in opposite ways to the growth progress.
The perspectives and the possible applications of these findings will be presented and discussed.

Keywords: *Saccharomyces cerevisiae*, FTIR, metabolomics, stress response.

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Yeasts isolated from olive mill wastewaters: technological characterization and potential use for removal pollution

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The foodstuff processing industry based on olive oil extraction is an economically important activity for many regions of the Mediterranean Sea area. It is estimated that the annual world production of olive oil is about 2.5 106 tons with Spain, Italy, and Greece. The process results in large quantities of waste, about 3.0 107 m3. This waste has a high polluting power and a high antibacterial activity exerted, among others, by various phenolic compounds. Several expensive physico-chemical processes, including simple evaporation, reverse osmosis and ultra-filtration have been proposed to reduce the polluting effects of olive mill wastewater (OMW). However these systems do not produce acceptable results. Among the methods for the purification, biological systems show some advantages, including the inexpensiveness, that make them particularly suitable. In this work four different samples of OMW were analysed. The OMW samples were derived from four different olive processing industries. For each sample the growth of autochthonous yeasts was evaluated. In spite the high concentrations of phenolic compounds (from about 3 g/l to 19 g/l), a significant cell load increase was observed. In particular, the most cell growth and significant abatement in polyphenol concentration were observed for samples derived from cultivar "Peranzana". To study the yeasts diversity and the interaction occurred with certain phenolic acids, 104 yeast strains were isolated and characterized. The predominant yeast species was *Pichia holstii*. In order to evaluate the capability to metabolize polyphenols, the isolates were tested in agarized distilled water and YNB. Among the 104 strains, 30 were able to metabolize caffeic acid, 28 vanillic acid and 26 p-cumaric acid. The same yeast strains, when tested in agarized yeast nitrogen base, showed a lower ability to metabolize polyphenols. Pectolytic, xylanolytic, lipolytic, catalase activities, potassium nitrate, ethylamine chlorhydrate and reducing sugar metabolism were also evaluated. A statistical multivariate approach showed that yeast population of OMW is quite homogeneous. The tests carried out demonstrated that the isolates were able to metabolize polyphenols, pectins and polysaccharides suggesting a potential use of these microorganisms in olive mill wastewater to remove quickly and economically polyphenols and pollution.

Keywords: yeast, polyphenol, olive mill wastewater (OMW).

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Beneficial effects of Azospirillum carbon-limited populations on white poplar stressed micro-cutting

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The effects of the inoculation of forest tree with facultative endophytic plant growth promoting rhizobacteria have received increasing attention in recent years in order to improve carbon sequestration through biomass production on marginal soils. A recent work has also pointed out many putative mechanisms, including carbon source utilization that help plant endophytes to thrive within a plant environment and potentially affect development of their plant host (Taghavi *et al.*, 2009). *Azospirillum* is ubiquitous in most plant species and its plant interaction may result in endophytic and rhizospheric associative relationships depending on the type of soil environment and soil nutrient status. When in the endophytic state, members of the *Azospirillum* genus might directly contribute to plant nutrition by excretion of different substances such as amino acids, phytohormones and enzymes involved in plant growth regulator metabolism. Positive effects of tree inoculation with this bacterium have been reported and typical examples of *Azospirillum* interaction with trees include the enhanced micropropagation response on *Prunus* spp. (Russo *et al.*, 2008) and the improved establishment of oak seedlings under nursery conditions (Zaady and Perevolotsky, 1995).
The objective of this study was to examine the influence of inoculation with *A. brasilense* strain Cd on *Populus alba* micro-cuttings exposed, during the acclimation period, to a limitation of photosynthetates. In this context we also analysed the poplar micro-cutting promoting activity of *Azospirillum brasilense* subpopulations adapted to different stress factors and degrees of carbon limitation. Cellular subpopulations of *Azospirillum brasilense* strain Cd, selected in chemostat cultures under fructose limitation were evaluated for the ability to endure antibiotics and hydrogen peroxide, for the capability to release auxin-like compounds and for the potential to produce phytostimulatory effects on poplar stressed micro-cuttings.
When grown in presence of hydrogen peroxide and various antibiotics the slow-growing cells exhibited considerably greater sensitivity to these stress-agents than the control populations. A lower level of IAA excretion was also observed in all batch cultures supplemented with tryptophan and inoculated with slow-growing C-limited cells. On the contrary only the slow-growing cells were effective in development of stressed micro-cuttings even if the formation of adventitious roots were not detectable. The results might suggest some important technological implication for inoculant formulation and the use of *Azospirillum* as plant growth-promoting bacteria in white poplar trees.

Keywords: bacterial slow growth, PGPR, cuttings.

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Potential arsenate reduction of bacteria isolated from an iron-arsenic co-precipitation product

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Due to its frequent occurrence in the environment and to its high toxicity, arsenic (As) is the first among the toxic elements listed by the Agency for Toxic Substances and Diseases Registry (ATSDR). Arsenic contamination is a dramatic problem in many Countries around the world, especially in Bangladesh.
The most common oxidation states of arsenic in environment are the pentavalent As(V) and the trivalent As(III), more mobile and more toxic. As(V) adsorbs to the surface of minerals such as ferrihydrite and alumina or co-precipitates with iron oxides. In As-rich waters, such as in Bangladesh groundwaters, high concentrations of dissolved metals can be found, and the formation of co-precipitation products is documented. The microbial communities that can develop in such As-rich substrates are directly or indirectly involved in regulating As mobility. Bacteria can enhance the production of co-precipitation products mediating As or iron oxidation. The presence of As (or iron) reducing microrganisms, on the contrary, could promote an enhancement of the As mobility.
The aim of this study was to evaluate the potential of As (V) reduction to As(III) of bacterial isolates from a iron-arsenic co-precipitation product sampled from a tank for groundwater storage in Satkhira District, south-west Bangladesh. We evaluate the ability of such isolates to produce As(III) in laboratory experiment with different amounts of As(V) and nutrient inputs. We tested both aerobic and sub-oxic incubation conditions.
No bacteria were isolated that could utilize As(V) as electron acceptor, under the respective growth conditions tested, but 26 morphologically distinct arsenic-resistant heterotrophic bacteria were isolated that could grow in the presence of high concentrations of As(V) and that could release As(III) via detoxification pathway.
Analysis of the 16S rRNA gene sequence of these bacteria revealed that the isolate that reduced As(V) to As(III) more efficiently respect with to others in each experimental condition tested belong to genus *Acinetobacter*.

Keywords: arsenic, co-precipitation products, arsenate reduction, *Acinetobacter.*

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Monitoring of bacterial community during a bioremediation process of diesel-contaminated soil.

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Petroleum products are today the main resource of energy in the modern industrial world, but due of its production, transport, use and disposal, release of hydrocarbons in the environment is increasing.
Between petroleum contaminants, diesel is a frequently reported soil pollutant leaking from storage tanks and pipelines or released in the soil during accidental spills.
Bioremediation is considered the better approach for restoring contaminated soils and groundwater (Cookson, 1995; Eweis *et al.*, 1998). Indeed, the microbial ability to degrade these aromatic compounds is the success of this advance. However, it is known that several factors reduces the biodegradation rates in soil remediation processes. Low solubility and adsorption on soil organic matrix are two major features that limit hydrocarbons availability in the soil water phase where microorganisms are active.
The aim of this study is to investigate the evolution of bacterial community during the removal of hydrocarbons from soil using laboratory scale bioreactors and evaluating the influence of compost, surfactants (ß-Cyclodextrin) and bacteria consortium (previously selected from diesel-contaminated soil) addition. The evolution of bacterial community during hydrocarbon removal was evaluated using both classical culturable techniques and a molecular culture-independent method (DGGE analysis).
Results of culturable bacteria population indicated that the compost addition positively influenced presence and development of total heterotrophic bacteria. DGGE analysis showed that biodiversity improved with the increase of hydrocarbon removal. In addition, the restoration of biodiversity due to xenobiotic removal was positively influenced by adding bacteria consortium.

Keywords: bioremediation, diesel-contamined soil, bacterial community, DGGE.

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Wild legume root nodules as a potential reservoir for human pathogenic bacteria

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A previous finding by our group (Benhizia *et al*., 2004) shows that root nodules from wild legumes, besides their natural rhizobium symbionts, can host and multiply bacteria belonging to species pathogenic to humans. These include *Enterobacter cloacae, Enterobacter kobei, Escherichia vulneris, Leclercia adecarboxylata, Pantoea agglomerans*. As these taxa were repeatedly found in nodules from three plant species, differing by habitat ecophysiology, and harvested in independent natural sites which are spaced apart up to 150 Km from each other, we believe that the phenomenon can be a general feature and have potentially significant impacts for the epidemiology of bacteria of clinical interest. In the sole Italian territory nearly four hundred species of wild leguminous plants are known, whose microbiological interactions are largely unknown. These plants can nevertheless develop abundant root nodules, which are optimal sites for bacterial multiplication. Wild legume distribution can span over a series of habitats, ranging from urban-synanthropic, to agricultural, and to the majority of natural habitats. In light of the above findings, yielding five Enterobacterial taxa of potential danger to humans from the analysis of only three species of wild plants, one could envisage the biomass of wild legumes as possible strategic niche for the survival and active multiplication of clinical pathogens in hosts alternative to mammals.

Keywords: legume, human pathogenic bacteria.

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Development of in vitro screening of EPS-producing LAB for cereal based products

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The bacteria are capable of producing exopolysaccharides (EPSs), a long chain branches made of repeated units of sugars or their derivatives excreted outside the cell wall. The EPSs improve the rheology and texture of many fermented foods like yogurt and baked goods and seem have a positive effect on health. Today, consumer demand is increasingly directed towards products with low levels of additives and use of EPSs could be a viable alternative. The selection of EPS-producing strains is a fundamental step to allow their more scope in cereal-based food processes.
Qualitative test on solid media: several experiments were carried out to decide the optimal medium composition to promote the EPS production using 12 *Leuconostoc*, 8 *Lactobacillus* and one *Streptococcus* strains isolated from sourdough for sweet dough. LAB strains were inoculated on the following different media: modified Agar Chalmers (Pepe *et al.,* 2001); modified Agar Chalmers with sucrose (5% w/v), with and without $CaCO_3$. Since $CaCO_3$ resulted in inhibition of EPS production, Agar Chalmers without $CaCO_3$ was further tested with the addition (5%) of one of the following sugars: maltose, glucose, galactose, fructose, lactose. Subsequently, the EPS-producer strains were also tested on Agar Chalmers containing a mixture of sucrose (1.6 % w/v), fructose (1.6% w/v) and maltose (1.6% w/v). EPS production was detected by the presence of slime colonies and ropy behaviour of the positive strain (Vescovo *et al*., 1989).
Quantitative analysis: the best EPS-producing LAB strains were assayed in the quantitative analysis following an ad hoc method: 1 ml of the overnight culture was surface inoculated on the modified Chalmers agar with sucrose, fructose and maltose. The yeast extract influence was also evaluated during this experiment. After 48 h of incubation, the EPS produced were directly dissolved with 20 ml of distilled water and collected in sterile falcons. The cells were removed by centrifugation at 6500 rpm for 10 min at room temperature. EPS were precipitated by adding volumes of chilled 100% ethanol and after 1 h, the pellet was collected, freeze-dried and weighed using analytical balance. The EPS production was expressed in milligrams of EPS ml^{-1}. The most strains used in this study produced slimy colonies (EPS) only when propagated in modified Chalmers Agar with sucrose (5% w/v) as the only carbon source, for its capability to promote the EPS biosynthesis, and without $CaCO_3$ that, at the concentration used in this study (2%), inhibited EPS production. We tested also modified agar Chalmers with three different sugars with the aim to the selection of EPS-producers in a medium with a similar sugars composition of dough for sweet baked products. EPS quantification is often tested using broth complex media medium. The most common procedures provide TCA precipitation and protein removal by centrifugation, followed by ethanol or/and acetone precipitation and several steps of purification (Cerning *et al*., 1994). Therefore, the method applied in this study revealed simpler. We used a solid medium in order to avoid the interference of some proteins and/or other high-molecular mass molecules. This method of screening will be applied to putative EPS-producing LAB strains isolated from cereal based products.

Keywords: exopolysaccharides (EPS), LAB, screening.

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Preliminary exploration on the perspectives of soil metabolomics – a case study on desertification in Sardinia

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Metabolomics is one of the most promising high-through output techniques that allows a global view of the metabolites included in the whole cell, in crude extracts or even in a mix of microbial communities. One of these cases is represented by soil, in which the complex of metabolites is the result of a complex joint contribution by several different microbial species. One of the most performing metabolomic technique is the Fourier Transform InfraRed spectroscopy (FTIR) which is able to give a rapid and reproducible picture of the metabolome, with the additional possibility to individuate signals, which can be used as metabolomic markers, by means of mathematical signal deconvolution.
The case study presented was focused on the Piscinas area in south-eastern Sardinia. The presence of dunes, large areas with self-vegetation and others subject to agricultural activities, allowed to individuate different types of soil: desert, semi-desert, interface desert-vegetation, Mediterranean bush and agricultural. Samples were subject to FTIR analysis with a preparation technique set up during this study. After the mathematical deconvolution of the signal, FTIR spectra were subject to statistical analysis in order to individuate metabolomic markers able to indicate the incipient desertification of areas at the risk of losing fertility. Metabolomic markers are specific region of the FTIR spectrum whose signal is related or associated with the state of health of the soils under study.
The results of this study are expected to improve the knowledge on this topic and to provide an easy and rapid tool to individuate dry districts on which a timely action is required. Other expected outcomes regard the use of this approach in the environmental management, in the sustainable management of the territory and in the ecological risk assessment (Shepherd *et al.*, 2002).

Keywords: risk assessment.

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Analysis of microbial diversity in the soil of a wooded riparian strip

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This research is part of a project aimed at verifying the potential of a specifically assessed wooded riparian zone in removing excess of combined nitrogen from the Zero river flow for the reduction of nutrient input into Venice Lagoon. The experimental site was built on an area previously used for arable crops. 300 seedlings of the following plant species were then planted: *Quercus robur*, *Alnus glutinosa*, *Acer campestre*, *Salix alba*, *Coryllus avellana*, *Salix triandra*, *Prunus padus*, *Crataegus monogyna, Fraxinus ornus*, *Frangula alnus*. The buffer zone was organized with an irrigation ditch carrying water from the Zero river and drainage ditches, resulting in a sub-surface water flow running through the entire buffer strips. Soils were sampled in March, April, June and October 2008 from three different depths through the soil profile.
The analysis of the bacterial communities was performed by combined approaches involving cultivation on complex solid media to determine colturable bacterial cells, the use of di-chromatic fluorescent stain and CTC^+ assay to determine the viable and the metabolically active fractions of the community. Amplified Ribosomal DNA Restriction Analysis (ARDRA) and computer assisted electrophoretic ARDRA fingerprinting were performed to analyze microbial diversity through (a) the comparison between the microbial communities in the wooded riparian strip (internal) to that of soil external to the experimental site, (b) the fluctuation of the microbial communities with time by collecting samples at each season, (c) the characterization of bacterial populations colonizing the soil at different depths.
The number of total living cells did not follow significant and important seasonal fluctuations, but epifluorescence counts showed greater variations when surface soils was compared to medium and deep samples. The fraction of culturable bacteria did not show any significant variation between internal and external sampling; the main difference observed was the number of metabolically working cells, higher for the internal soil. These results indicate that the plants play an important role in supporting microbial growth in soil by overcoming the main limiting factor, almost always represented by the availability of organic carbon.
To characterize microbial diversity of the culturable fraction, several hundred colonies were then isolated from the wooded riparian strip and from the external site, 16S rDNA was amplified and *Hin*fI- and *Hpa*II-restricted. A variety of different DNA fragment patterns was revealed. ARDRA analysis from the wooded riparian strip discerned high numbers of OTUs (Operational Taxonomic Units) in all the seasons, both from the internal and external soils. From the 500 colonies analysed 271 OTUs were obtained and 55 of those were found to be always present in all the samples.
These preliminary results indicate that the bacterial community structure in the wooded riparian strip seems to be significantly affected by the presence of the plants, associated to a high combined nitrogen input coming from the river Zero. The community was affected not numerically, but in terms of microbial diversity found to be directly related to different soil layers and seasons.
The above observations could contribute to understand the bacterial population dynamic of a agricultural soil when transformed in a wooded strip and to provide key indications for the management of a phytoremediation site.

Keywords: bacterial diversity, soil, phytoremediation, riparian strip.

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Internal variability of the D1/D2 domain of rDNA in Saccharomyces cerevisiae

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The D1/D2 domain of the rDNA 26S locus is widely used in the molecular microbial taxonomy for the species identification as a very fast and reliable monophasic method (1-5).
That region is employed even in microbial phylogenesis to reconstruct the evolutionary traces followed by the different microbial species. Is not still clear how can the over 100 repeats of this region modify at the same time in the same position, moreover it is still scarcely understood how can the variations of this marker be synchronized with speciation.
In the species *Saccharomyces cerevisiae* there are about 120 copies of the ribosomal DNA operon (Chromosome XII). In case different nucleotides are present in the same position throughout the repeats, the peak more than one nucleotide is expected to appear in that particular place of the electropherogram. As a matter of fact, several such situations were individuated among the electropherograms collected for the routine identification procedure. These cases are characterized by a major peak and one or more secondary peaks, of less intensity, all situated in the same place.
Aim of this study was to clone individual D1/D2 domain repeats in order to compare the variability present within this locus in one strain of *Saccharomyces cerevisiae*.
The results obtained showed that indeed variations exist in places were indeed more than one peak could be detected. The extent of this variability and the implications in phylogenetics and taxonomy.

Keywords: *Saccharomyces cerevisiae*, HGT, rDNA, D1/D2.

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Analysis of rhizobacteria involved in arsenic cycle associated with a spontaneous grass plant growing on an arsenic polluted soil

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Arsenic is widely distributed in soils, released from both natural and anthropogenic sources. Most arsenic in surface soil and water exists both in the reduced (arsenite) and in the oxidized (arsenate) form respectively. The biogeochemical cycle, the mobility and the distribution of arsenic strongly depend on microbial transformations. Microorganisms have developed different resistance mechanisms to cope with the presence of arsenic compounds. The bacterial resistance consists of either detoxification reactions or redox reactions that conserve the energy for cell growth (Silver and Phung, 2005). Whereas microbial communities in arsenic-polluted soils have been studied, little is known about the composition of rhizospheric bacteria associate with plants growing in such soils. The primary goal of this study was to investigate which microorganisms inhabit the rhizosphere of a spontaneous grass plant (thistle) [*Cirsium arvense* (L.)], growing on a soil with a long history of arsenic pollution and to analyse the diversity of As-resistant bacteria potentially involved in aerobic arsenic transformation, with regard of their plant growth promoting traits (PGP).
FISH analysis of root-soil rhizobacterial community of *Cirsium* evidenced a similar general picture at the level of main phylogenetic microbial groups in the three root soil fractions (bulk, rhizosphere and rhizoplane). Particularly, α-, β-, and γ-Proteobacteria were the dominant groups whereas Cytophagaceae, Actinomycetales and spore-forming bacteria were 1-2 order of magnitude lower. A substantial proportion of the culturable bacterial resisted to arsenate and arsenite, indicating for these bacteria an inherent way of dealing with the oxyanions. Sixty four aerobic arsenic-resistant bacteria were isolated and identified. Almost all isolates had putative arsenic resistance genes: 68% of the arsenic-resistant isolates contained one gene related to ArsB/ACR3p(2) and 39% contained an ArsC related gene. A large portion of strains were possessed of PGP traits such as siderophores, IAA, and ACC deaminase production. While arsenite was oxidized by numerous isolates, arsenate was reduced by few strains.
The results provide new insights into bacterial diversity of the thistle root-soil system and into the exploitation of isolates and genetic information on arsenic resistance. Microorganisms that are both arsenic-resistant and efficient in producing plant growth-promoting compounds may be used for processes of re-vegetation and phyto-remediation of polluted soils.

Keywords: arsenic-resistant bacteria, ars genes, plant growth promoting trait, arsenite oxidizer.

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Metabolomic fingerprinting of Saccharomyces cerevisiae cells subject to different stressing conditions

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The concept that infrared technology can provide fingerprints of microorganisms was proposed in the early nineties and was later extended to strain identification at the species level and to typing, showing that there is a good relationship between this method and well established molecular procedures (Rellini *et al.*, 2009; Cardinali *et al.*, 2008; Kummerle *et al.*, 1998; Helm *et al.*, 1991; Naumann *et al.*, 1991).
In general, these studies could demonstrate that the spectrum includes the information to define the metabolic state of the cells, bringing the Fourier Transform InfraRed Spectroscopy (hereinafter referred to as FTIR) technique in the group of the metabolomic procedures.
FTIR spectroscopy was used to analyze the metabolomic alterations caused to yeast cells by four chemical compounds: ethanol, sodium hypochlorite, sodium chloride and sulphur dioxide each tested at four different concentrations. The complex of four stressing agents at different concentrations, inducing cell mortalities ranging from 1 to 100%, has given the opportunity to prove that FTIR can individuate the presence of a stress before the cells start dying.
A series of "Stress Indexes" were calculated with an expressly designed "R" script, to estimate the level of stress induced by the chemical agents at different concentrations. These estimations allowed the direct comparison of the stress induced by the four agents at different concentrations. The response spectra, calculated as difference between the spectrum of the cells under stress and that of the cells maintained in water, showed different shapes in the diverse experimental conditions, suggesting a specificity of the response and the possibility to classify it. The contribution of five different spectral regions (fatty acids, amides, mixed zone, carbohydrates and typing region) could be calculated separately, gaining additional information on the stressing effects. These preliminary findings suggest that FTIR technology and a series of simple algorithms can be employed to study response of cells to various stressing situations, not limited to chemical agents. The ease and rapidity of the FTIR analysis suggest that this approach could be used in pure research and in several application fields such as the early screenings of new drugs and in ecotoxicological or food toxicity bioassays.

Keywords: *Saccharomyces cerevisiae*, FTIR, metabolomics, stress response.

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Imbalance of the faecal microbiota and volatile organic compounds between treated and untreated celiac children

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Celiac disease (CD) is an inflammatory disorder of the small intestine that affects genetically predisposed individuals when they ingest gluten from any *Triticum* species and similar proteins of barley and rye, and their crossbred varieties. CD is associated with maldigestion and malabsorption of nutrients, vitamins and minerals (Niewinski, 2008). The inflammatory milieu caused by gluten antigens could lead to imbalances of the gastro-intestinal (GI) microbiota of CD patients. GI microbiota plays a key role in health and disease (Neish, 2009). Health effects are direct, providing a stable microbiota resistant to incoming potential pathogenic microbes, and/or indirect, through cross-talk with the gut-associated lymphoid tissue. GI microbiota metabolizes nutrients, and activates both innate and adaptive immunity. The role of bacteria during development and CD treatment should be elucidated (Sanz *et al.,* 2007). Recently, the Department of Plant Protection and Applied Microbiology of the University of Bari hypothesized that lactic acid bacteria including probiotics (e.g., *Lactobacillus* and *Bifidobacterium*) may hydrolyze peptides involved in the CD also under GI conditions (for review see Gobbetti *et al.,* 2007). The present study aimed at investigating the faecal microbiota of CD children before (untreated CD; U-CD) and after gluten-free diet (treated CD; T-CD), and healthy children (HC). HC mainly belonging to the same family unit of T-CD were enrolled to avoid interference due to genetic factors and dietary components. PCR-DGGE analysis by universal primer V3 showed unique profiles for each faecal sample. PCR-DGGE analysis by group- or genus-specific 16S rRNA gene primers showed that *Lactobacillus* sp. varied between groups. T-CD and HC or T-CD and U-CD had the highest levels of similarity. On the other side, U-CD and HC had the lowest level of similarity. In comparison to HC, the ratio between cultivable lactic acid bacteria-*Bifidobacterium* and *Bacteroides-Enterobacteria* was lower in T-CD and, especially, U-CD. The percentage of strains identified as lactobacilli differed: HC (ca. 38%) > T-CD (ca. 17%) > U-CD (ca. 10%). *Lactobacillus brevis, Lactobacillus rossiae* and *Lactobacillus pentosus* were identified only in faecal samples of T-CD and HC. *Lactobacillus fermentum, Lactobacillus delbrueckii* subsp. *bulgaricus and Lactobacillus gasseri* were only identified in several faecal samples of HC. In comparison to HC, the composition of *Bifidobacterium* species of T-CD and, especially, U-CD varied. Forty-seven volatile organic compounds (VOCs) belonging to different chemical classes were identified using gas-chromatography mass spectrometry/solid-phase microextraction (GC-MS/SPME) analysis. Median concentrations markedly varied between HC and U-CD. In comparison to U-CD, the concentration of short chain fatty acids (SCFAs) was higher in faecal samples of T-CD and HC. Overall, r2 values for VOCs data of brothers and sisters were equal or lower than those found for unrelated HC and T-CD children.
This study shows the effect of CD pathology on the faecal microbiota of children. The gluten-free diet restores in part the balance within the faecal microbiota of CD patients. *Lactobacillus* and *Bifidobacterium* strains isolated from HC could be of interest as probiotic treatment to restore the balance of the GI microbiota in T-CD and, especially U-CD.

Keywords: celiac disease, faecal microbiota, lactic acid bacteria, bifidobacteria.

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Chemical and microbiological analysis in potable water: preliminary results

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Water is essential to sustain life, and a satisfactory supply must be available to all. Improving access to safe drinking-water can result in tangible benefit to health.
The health concerns associated with chemical constituents of drinking-water differ from those associated with microbial contamination and arise primarily from the ability of chemical constituents to cause adverse health effects after prolonged periods of exposure. The use of lead pipes can result in elevated lead levels in drinking-water if it is associated with acid water. Changes in the normal appearance, odour or taste of a drinking-water supply may signal changes in the quality of the raw water source or deficiencies in the treatment process and should be investigated.
In general terms, the greatest microbial risks are associated with ingestion of water that is contaminated with faecally derived pathogens. In addition to faecally borne pathogens, other microbial hazards may be present in drinking-water. *Legionella* spp. are ubiquitous in the environment and can proliferate more commonly in hot and warm distribution systems.
The purpose of this work was to evaluate the quality of the drinking water taken from several apartments around the city district of Bologna (Italy).
Water samples were processed for chemical and microbiological assessment based on the Italian Law (Legislative Decree 31/2001). Samples are subjected to chemical analysis like pH, conductivity, heavy metals (Pb, Cu, Fe), odour, taste and turbidity.
Water samples were also analysed using standard microbiological methods for drinking water including total viable counts at 22 and 37 °C, total coliform count, detection of *Salmonella* spp., *Legionella* spp. and *Escherichia coli*.
Data processing of chemical and microbiological results was developed to obtain histograms; these represent the frequencies, the number of times the event occurred in samples set. Among the chemical parameters, smell and taste were divided into10 classes and there are five for the odour and 4 for the taste; in all the samples analysed the class 0 (without odour and taste) is the most represented.
The pH is ranging between 7.0 and 8.1, while the conductance is in a range between 306 e 1024 microS/cm. The heavy metals, Pb, Cu and Fe are all below the legal limits with the exception of five samples where Pb exceeds the 10 microg/L. Total viable counts at 22 and 37 °C as expected, are related to each other and the first is in a range between 0 and 1300 CFU/mL and the second one between 0 and 1275 CFU/mL. Some samples were positives for the presence of *Legionella* spp. and total coliforms.

Keywords: drinking water, chemical and microbiological analysis, *Salmonella*, *Legionella.*

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Analysis, determination and cultivation of endophytic fungi associated with the orchid Spiranthes spiralis

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Mycorrhizas can sustain plants in most habitats through mutualistic interactions with fungi. The presence of these in internalized location can be due to true symbiotic relationships or to different levels of endophytism. In the case of orchids the fungal assistance is crucial throughout the seedling stage and only established plants begin to return organic carbon to the symbiont. Fungi from orchids are often difficult to isolate and their cultivation depends on seasonal and physiological conditions. The submediterranean species *Spiranthes spiralis* (L.) Chevall. is the latest-blooming native orchid in western Europe. Its rosettes appear in summer to die off in the following spring, while stalks flower in September (Willems and Dorland, 2000). The putative mycorrhizal fungi were isolated in culture from the surface-sterilized *Spiranthes spiralis* roots and their taxonomical identity was assessed by molecular techniques upon amplification of diagnostic ribosomal DNA regions. In parallel, DNA was isolated directly from the root tissues for comparison. Selective PCR amplification using ITS1-ITS4, ITS1F-ITS4 primers was carried out. Additionally, using fluorescence and confocal microscopy on acridine orange-stained freehand sections we observed a diffuse cortical colonization by intracellular hyphae. Their further ultrastructural details were resolved by electron microscopy.

Keywords: *Spiranthes spiralis*, orchid mycorrhiza, fungal endophytes.

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Effect of iron-porphyrin treatment on soil microbial communities

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Intensive agricultural management have led to an increasing transfer of carbon from soil organic matter (SOM) to atmospheric CO_2. It is noteworthy that the flux of CO_2 from the soil is ten times greater than fossil fuel emission from industrial and automotive activities.
Piccolo and co-workers (1999) suggested that hydrophobic humic components in soil exerted hydrophobic protection towards easily degradable compounds. They postulated that associations of apolar molecules deriving from plant degradation and microbial activity incorporate more polar molecules, thus preventing their otherwise rapid microbial degradation and enhancing their persistence in soil. Moreover, synthetic metal-porphyrins were shown to significantly decrease CO_2 emission from soil due to an in-situ catalysis of oxidative polymerization of soil OM (Piccolo *et al*., 2002).
The present work was conducted to investigate the effect of iron-porphyrin amendments on soil microbial communities in agricultural soils. Field experiments took place at three Italian locations strongly differing in pedological, chemical and climatic characteristics: Naples, Turin and Piacenza. Soils were treated with synthetic iron-porphyrins (POR) for three consecutive years. Soil and rhizosphere samples from plots under wheat and maize cropping were sampled at different time and the composition of microbial groups directly implicated in OM mineralization, such as actinobacteria, fungi and cellulosolytic bacteria, as well as microbial groups involved in key bio-geochemical processes (e.g. aerobic free-living N_2-fixing bacteria and ammonia-oxidizing bacteria) was estimated and the structure of cultivable populations was examined.
The iron-porphyrin treatment showed a different effect on microbial populations in bulk soil, wheat rhizosphere, and maize rhizosphere, but in all cases this was a long term effect. Indeed, during the first two years of treatment there was no significant difference between POR treatment and control without porphyrin (NO POR) in experimental fields.
The effect on bulk soil in the three different Italian locations was found to be the same. However, results were different from expected. In fact, POR treatment showed a significant increase in microbial groups directly implicated in OM mineralization if compared with NO POR control.
By contrast, the effect on rhizosphere communities was different. In maize rhizosphere (site location Piacenza) POR treatment showed a significant decrease in microbial populations involved in the turn-over of OM comparing with NO POR control. While, in wheat rhizosphere (site locations Naples and Turin) there was no significant difference between POR and NO POR treatments. Finally, POR treatments did not significantly influenced the aerobic free-living N_2-fixing and ammonia-oxidizing bacteria populations in all experimental fields. In conclusion, the influence of iron-porphyrin treatments on microbial communities depended on crop, but not on pedological, chemical, and climatic characteristics of experimental soils.

Keywords: microorganisms, soil carbon sequestration, iron-porphyrins.

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New environmental-friendly approaches against detrimental biofilms

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For decades, abatement of microbial growth has commonly been achieved by applying biocides to surfaces. In recent years impending environmental regulations both in European countries and elsewhere have severely restricted the use of biocides. The concern about the use of biocides is that eventually they are released into the environment, and because they are generally not specifically targeted against biodeteriorating microorganisms, they are potentially dangerous for human health and the environment. In addition, organic biocides might act as nutrients, and therefore they must be carefully used. Finally, killed cells might provide nutrients for subsequent colonization, and therefore leaving dead cells on the surface is not advisable.
Some environmentally-friendly alternatives have been recently proposed (Flemming *et al*., 1996). Natural non toxic compounds represent an attractive strategy. Such antifouling compounds have been isolated mainly from marine organisms which are not colonized by microorganisms. An interesting antifoulant agent is zosteric acid (p-sulfooxycinnamic acid), a natural extract from the eelgrass *Zostera marina* that prevents biofouling by some organisms, such as algae, barnacles, and tubeworms, at nontoxic concentrations (Barrios *et al*., 2005).
Capsaicin (8-methyl-N-vanillyl-6-nonenamide), a pungent compound of hot peppers, the fruit of the Capsicum plant, is considered by many researchers to be a compound able to prevent the growth of various bacteria, including *Pseudomonas* and *Bacillus* spp. (Molina-Torres *et al*., 1999; Dorantes *et al*., 2000).
The main goal of our research has been the development of this innovative, green and cost-effective technology for the prevention of detrimental biofilms. The results obtained by us so far with zosteric acid and the synthetic analogue of capsaicin indicate that indeed this technology may be a powerful tool (Cappitelli *et al*., 2006).

Keywords: antifouling, detrimental biofilm, zosteric acid, capsaicin.

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