Ann Microbiol (2013) 63:547–553
DOI 10.1007/s13213-012-0502-z

ORIGINAL ARTICLE

Comparative mycelial and spore yield by *Trichoderma viride* in batch and fed-batch cultures

Abiodun A. Onilude · Bukola C. Adebayo-Tayo · A. Olubusola Odeniyi · Damilola Banjo · Emmanuel Oluwaseun Garuba

Received: 22 December 2011 / Accepted: 20 June 2012 / Published online: 12 July 2012

Abstract The effects of cultural parameters such as carbon and nitrogen source and environmental factors including temperature and pH were investigated on spore and mycelial yield of *Trichoderma viride*, which has potential as a biocontrol agent against species of *Fusarium* in batch culture and fed-batch culture where there was limiting nutrient. The results obtained indicated that growth and sporulation of *T. viride* were greatly influenced by various carbon and nitrogen sources, and by environmental factors such as pH and temperature. Mannitol, wheat bran and rice bran as sole carbon sources appear to stimulate high mycelial growth and spore yield in fed-batch culture. Growth and sporulation were also favoured by $NaNO_3$, peptone and NH_4SO_4 as the nitrogen sources in fed-batch and batch cultures. Maximum growth and sporulation was between pH 4.5 and 6.0. Temperatures between 30 and 37 °C were good for mycelium growth of *T. viride* while temperatures between 30 to 45 °C were good for sporulation. The amount of spore and mycelium produced and the time required for attainment of maximum spore yield increased with increasing carbon and nitrogen source in batch culture. The final spore yield obtained in fed-batch culture was two times higher than the apparent spore-carrying capacity of batch culture. These results show that *T. viride* is capable of growing and sporulating with varied nutritional and environmental conditions, and, therefore, this strain of *T. viride* may be useful as a biocontrol agent under diverse physiological and environmental conditions.

A. A. Onilude · B. C. Adebayo-Tayo · A. O. Odeniyi · D. Banjo
Microbial Physiology and Biochemistry Laboratory, Department of Microbiology, University of Ibadan,
Ibadan, Nigeria

E. O. Garuba (✉)
Department of Biological Sciences, Bowen University,
Iwo, Nigeria
e-mail: oluwaseungaruba@live.com

Keywords *Trichoderma viride* · Spore yield · Biocontrol agent · Mycelial growth · Carbon · Nitrogen

Introduction

The use of biological control agents (BCAs) in which organisms play an important role is increasingly replacing chemical means of disease control (Whipps and Lumsden 2001). Of the various groups of organisms used, the fungal-based BCAs have gained wide acceptance next to bacteria (mainly *Bacillus thuringiensis*) primarily because of their broader spectrum in terms of disease control and high production yield (Coppings and Menn 2000). Of the various fungi used, *Trichoderm*a sp. have been the cynosure of many researchers who have been contributing to the pursuit of biological control through the use of fungi (Ahmed and Baker 1987; Benhamou and Chet 1993). This is because it can easily establish itself in different pathosystems, has moderate effects on soil balance and does not harm beneficial organisms that contribute to pathogen control. Furthermore, it has no known harmful effect on humans, wildlife and other beneficial organisms (Whipps and Lumsden 2001).

In the use of the various BCAs (*Trichoderma* sp. inclusive), spores are the most useful propagule (Churchill 1982). These must then be processed in large quantities quickly, inexpensively and efficiently, if BCAs are to be able to compete favourably with chemical control agents (Lisansky and Hall 1983). Fungal spores are normally mass-produced in large liquid culture fermentation (Churchill 1982), and information on the effects of manipulating liquid culture conditions to maximize the efficiency of spore production is of potential value. Reduction of the mycelium in the liquid culture would also be desirable, since it creates separation and disposal problems (Cascino et al. 1990).

There is, however, a paucity of information on the studies regarding culture conditions like fed-batch culture in a fermenter that allow finer control of substrate concentration, solids concentration, C:N ratio, the proportion of total biomass produced as spores (the relative spore yield) and single/multiple nutrient addition. Investigation of these parameters in relation to sporulation is thus necessary in order to maximize spore production by *Trichoderma* sp. for use as a BCA. This paper therefore reports on limiting conditions which affect spore formation in batch and fed-batch cultures and which may be adapted to large-scale fermentations.

Materials and methods

Microorganism

Trichoderma viride with antagonistic effect on species of *Fusarium*, especially *Fusarium solani* (unpublished data) used in this study was obtained from The Culture Collection Centre of The Department of Microbiology University of Ibadan, Nigeria.

Inoculum preparation

Spores used as inoculum were prepared according to the method of Nahar et al. (2008).

Batch culture fermentation

Batch fermentation was in 250-ml Erlenmeyer flasks containing 50 ml of the liquid medium described by Al-Taweil et al. (2009), which contains (g/l) ammonium chloride (2.0), sodium potassium tartrate (2.0), $MgSO_4 \cdot 7H_2O$ (4.0), K_2HPO_4 (14.0), $CaCl_2$ (0.2), KH_2PO_4 (4.0), yeast extract (4.5), trace element (2.0 ml), [$ZnSO_4.7H_2O$ (0.0014), $FeSO_4.7H_2O$ (0.005), $MnSO_4$ (0.0016), $CoCl_2$ (0.002)], glucose (7.5), $NaNO_3$ (6.0), and corn steep liquor (5.0). The liquid medium was adjusted to pH 5.5 using citrate buffer. The medium was inoculated with 1 ml suspension of the spores of *Trichoderma viride* and incubated at ambient temperature in static mode for 7 days.

Fed-batch experiments

Fed-batch experiments were done using the fermentation medium above and inoculated with 1 ml of the spore suspension and incubation carried out at ambient temperatures in the dark for 7 days. Then, 4 ml of the limiting nutrient, which was yeast extract (0.05 mg/ml), was added every 12 h. Each fed-batch culture was sampled periodically for spore counting by aseptically removing 4 ml of the culture with a sterile syringe every 12 h. At the final harvest, the spore concentrations of 50 ml subsamples were determined.

Determination of spore concentration

For the determination of the spore concentration, the content of the flasks was filtered through a sterilized double-layer muslin cloth to separate the harvestable spores from the mycelium. The stock suspension was kept in a Rotary Shaker Flask for 2 min, and 3 ml of the suspension was added into a cuvette. The equipment was calibrated with 3 ml of blank solution (liquid medium). The spore count was determined at a wavelength of 550 nm using a Perkin Elmer Lambda 25 UV Spectrophotometer.

Determination of mycelia weight

The modified method of Al-Taweil et al. (2009) was used to determine the fungal biomass with the mycelium being filtered through a pre-weighed muslin cloth. It was washed two times with distilled water. The washed mycelium was dried at 80 °C to constant mass, and dry weight was calculated by difference.

Effect of different carbon sources on fungal mycelium and spore yield

The carbon sources used in this study were glucose, mannitol, starch, wheat bran and rice bran. The fermentation medium was supplemented with each of the carbon sources at the rate of (g/l) 2.5, 5.0, 7.5, 10.0, and 15.0 in 250-ml Erlenmeyer flasks separately before autoclaving. The pH of the liquid medium in each flask was adjusted using citrate buffer to pH 5.5. Thereafter, sterilisation was carried out and the liquid medium was inoculated with 1 ml of spore suspension of *Trichoderma viride* in both batch and fed-batch cultures. The flasks were incubated at room temperature for 7 days after which the mycelium weight and spore concentration was determined.

Effect of different nitrogen sources on fungal mycelium and spore yield

The nitrogen sources investigated were $NaNO_3$, NH_4SO_4, peptone, soy meal preparation and casein. The nitrogen sources were supplemented at the rate of (g/l) 1.0, 3.0, 5.0, 7.0, and 9.0 in sets of 250-ml Erlenmeyer flasks containing 50 ml of liquid medium. The pH of the liquid medium was adjusted as described earlier. Thereafter, sterilisation was carried out and the liquid medium was inoculated with 1 ml of spore suspension of *Trichoderma viride* in both batch and fed-batch cultures. Flasks were incubated at room temperature for 7 days after which the spore concentration and mycelial weight were determined.

Effect of pH on fungal mycelium and spore yield

Different pH levels selected for the study were 3.0, 3.5, 4.0, 5.0, 5.5 and 6.0, and 50 ml of liquid medium were prepared in sets of 250-ml Erlenmeyer flasks. The pH of the medium was adjusted with citrate buffer in triplicate sets before autoclaving. After autoclaving, the cooled medium was inoculated and incubated as described earlier. Mycelial weight and spore concentration were determined as previously described.

Effect of temperature on the spore yield and mycelium growth

Fifty millilitres of the liquid medium adjusted to pH 5.5 using citrate buffer was dispensed into 250-ml Erlenmeyer flasks and sterilized. The flasks were then each inoculated with 1 ml of spore suspension of *Trichoderma viride*. Four different incubation temperatures, 25, 30, 37 and 45 °C, were used to cultivate the *Trichoderma viride* for spore production in both batch and fed-batch cultures for 7 days so as to study the effect of temperature on the spore yield and mycelium growth.

Results

The results of the effects of different carbon sources on spore yield and mycelia production in batch and fed-batch experiments are presented in Figs. 1 and 2. The effects of different carbon sources on spore formation in batch cultures revealed that mannitol at a concentration of 15.0 g/l supported the highest spore yield of 0.69±0.15 (± SEM) followed by starch at 7.5 g/l with 0.52±0.08 and glucose with 0.48±0.01 at 5.0 g/l. The lowest spore yield of 0.15±0.03 was recorded when 2.5 g/l starch concentration was used as the sole carbon source (Fig. 1a). Similarly, mannitol at 15.0 g/l gave the highest mycelial weight of 19.63±0.43 g/l closely followed by 10.0 g/l mannitol concentration (15.51 g/l mycelial weight) and starch (14.81±0.72 g/l mycelial weight) at 15.0 g/l concentration. The lowest mycelial weight of 6.78 g/l recorded in this study was from 2.5 g/l wheat bran concentration (Fig. 1b). Results of Fed-batch experiments are presented in Fig. 2a, b and revealed that wheat bran at 7.5 g/l supported the highest

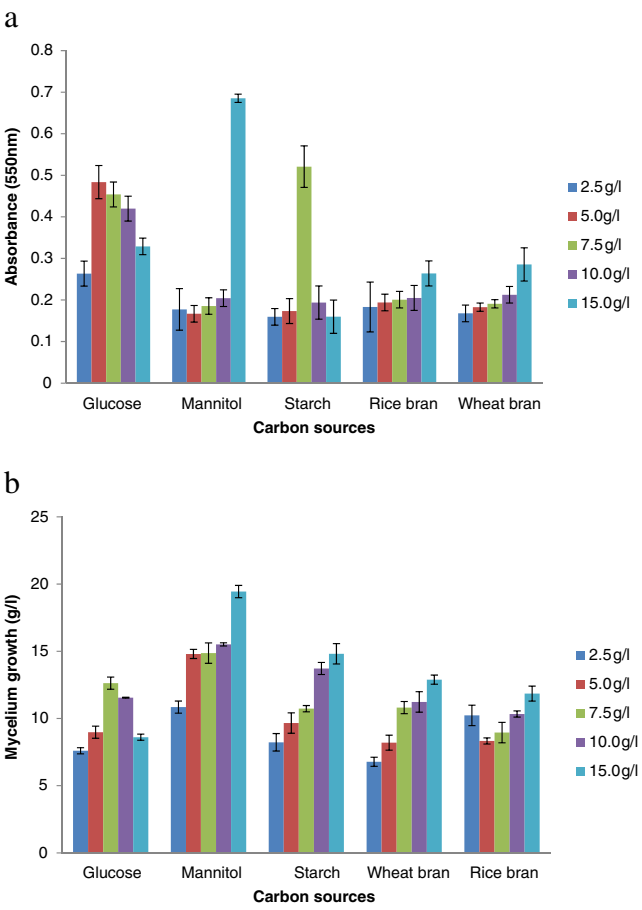

Fig. 1 Effect of carbon sources on spore yield (**a**) and mycelium growth (**b**) by *Trichoderma viride* after 7 days in batch culture. Data are given as means ± SEM, $n=3$

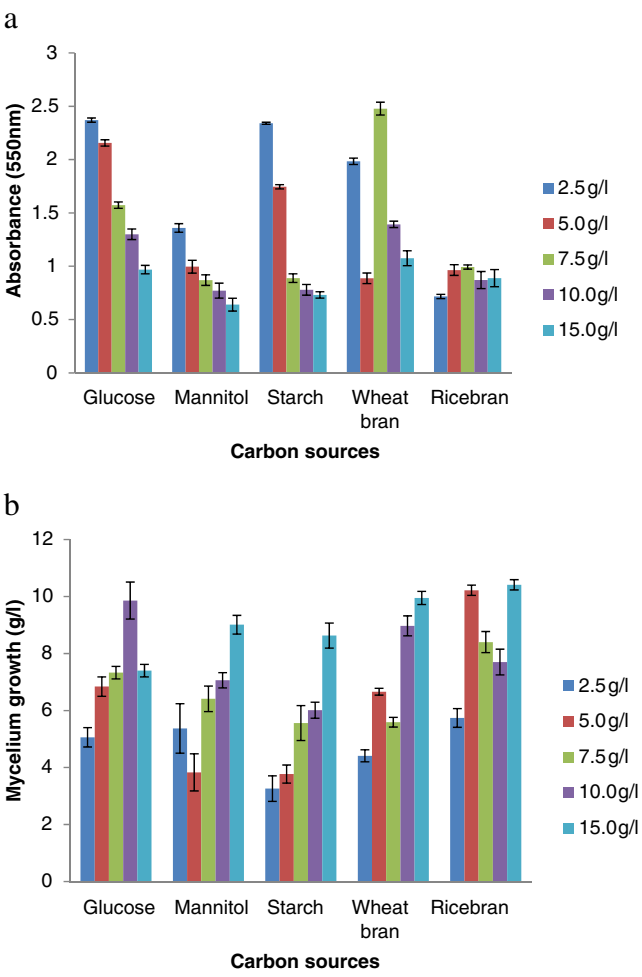

Fig. 2 Effect of carbon sources on spore yield (**a**) and mycelium growth (**b**) by *Trichoderma viride* after 7 days in fed-batch culture. Data are given as means ± SEM, $n=3$

spore yield of 2.48±0.01 closely followed by glucose (2.37±0.07 at 2.5 g/l). Glucose is followed by starch (2.15±0.12 at 5.0 g/l) while the least spore produced (0.64±0.04) was recorded in mannitol-containing medium at a concentration of 15.0 g/l (Fig. 2a). Rice bran at 15.0 g/l stimulated the highest mycelial weight of 10.41 g/l followed by rice again at a concentration of 5.0 g/l (10.22±0.13 g/l). Wheat bran extract at 15.0 g/l also gave a high mycelial weight of 9.95 g/l while a 2.5 g/l starch concentration stimulated the least amount (3.26 g/l) of mycelia in this study (Fig. 2b).

Effect of nitrogen sources

Results of the investigation of the effects of different nitrogen sources on spore yield and mycelia production by *T. viride* used in this study are presented in Fig. 3a, b for batch cultures and revealed that the medium containing $NaNO_3$ as the nitrogen source appears to stimulate the highest spore yield at all concentrations relative to all other nitrogen sources investigated. At 1 %, $NaNO_3$ gave a spore yield of 1.81±0.05 followed by 0.65±0.06 at 5 %, and 0.52±0.03 at 7 %. Following $NaNO_3$ is the soy meal preparation, which gave a spore yield of 0.48±0.07 at 5 % concentration and 0.44±0.02 at 7 %. The lowest sporeyield (0.14±0.04) was, however, recorded in a medium containing NH_4SO_4 (3 %) as the nitrogen source. The best mycelial growth (22.08±0.45 g/l) was produced in the medium that contained NH_4SO_4 as the nitrogen source, followed by peptone which gave a mycelial growth of 22.06±0.37 g/l in batch culture (Fig. 3b). Fed-batch experiments showed that *T. viride* gave the best spore yield (3.13±0.06) in the medium that contained $NaNO_3$ as the nitrogen source, followed by casein which gave a spore yield of 1.78±0.02. The best mycelial growth (12.89±0.47 g/l) was produced in the medium that contained peptone as the nitrogen source, followed by $NaNO_3$ which gave a mycelial

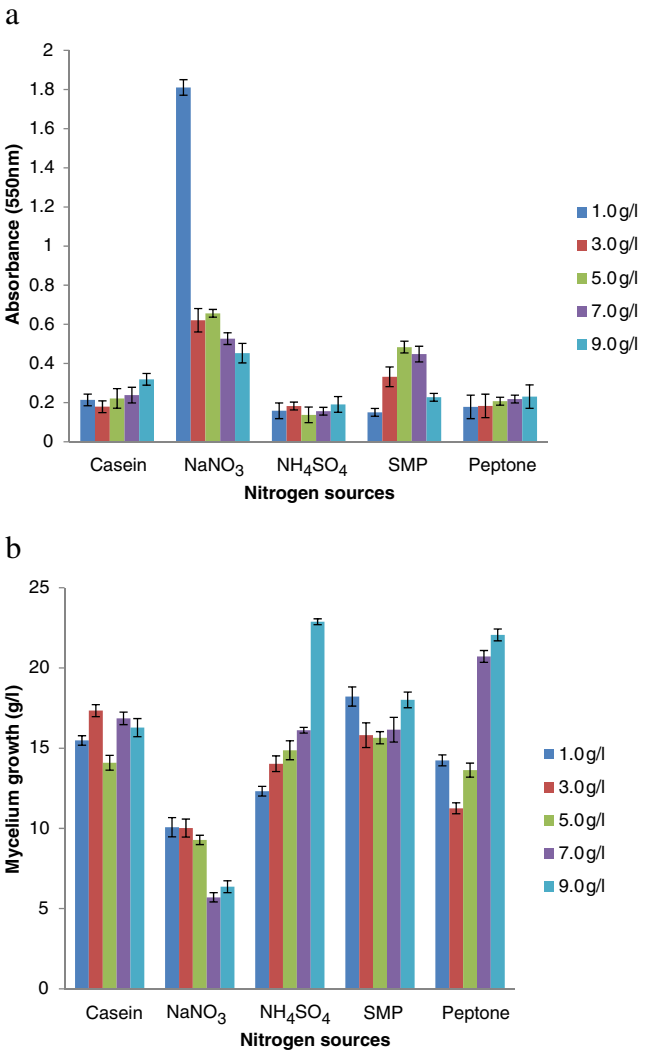

Fig. 3 Effect of different nitrogen sources on spore yield (**a**) and mycelium growth (**b**) by *Trichoderma viride* after 7 days in batch culture. Data are given as means ± SEM, $n=3$

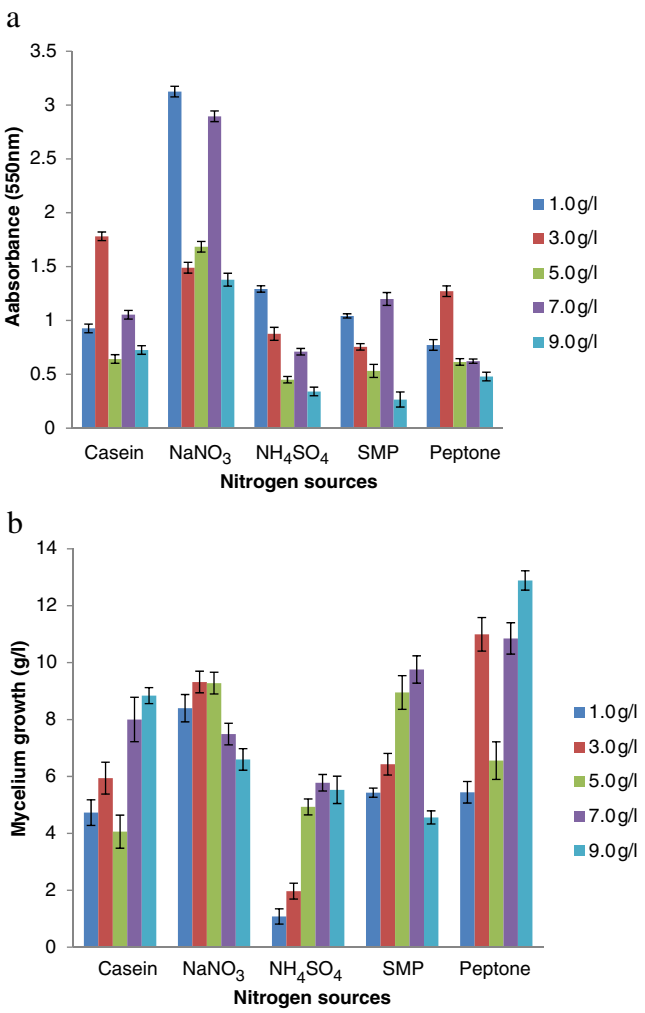

Fig. 4 Effect of different Nitrogen sources on spore yield (**a**) and mycelium growth (**b**) by *Trichoderma viride* after 7 days in fed-batch culture. Data are given as means ± SEM, $n=3$

growth of 9.32±0.29 g/l in fed-batch culture. Soy meal preparation gave the least spore yield of 1.04±0.03 and mycelial growth of 6.25±0.62 g/l (Fig. 4a and b).

Effect of medium pH on spore yield and mycelia formation

Five different initial pH levels, 3.5, 4.0, 5.0, 5.5, and 6.0, were established in the liquid medium and the fermentation was carried out for 7 days in both batch and fed-batch cultures. Results obtained are shown in Fig. 5a, b. Optimum pH recorded in this study for spore yield in batch culture by *T. viridae* was pH 4 (0.80±0.10). This was followed by pH 5 which gave a spore concentration of 0.58±0.07 while the least spore yield was recorded (0.19± 0.09) at pH 3 and 3. 5. The highest mycelial yield of 15.13± 0.02 g/l was obtained at pH 5.0 followed by a mycelial weight of 14.43±0.09 g/l at pH 4, while the lowest mycelial weight of 10.4±0.10 g/l was recorded at pH 6 (Fig. 5b). However, in fed-batch culture, pH 6 was the optimum pH for spore yield (1.62±0.06) followed by pH 4 (1.46±0.03), and the lowest spore yield (0.28±0.05) was recorded at pH 3.0 (Fig. 5a), while pH 4 was the optimum pH for mycelium growth (13.79±0.04 g/l) followed 11.85±0.04 g/l at pH 5. *T. viride* gave the lowest mycelial weight of 4.49±0.03 g/l at pH 6.0 (Fig. 5b).

Effect of incubation temperature on spore yield and mycelial growth

The results of the investigation into the effects of temperature on mycelial growth and soprulation by *T.viride* in both batch and fed-batch cultures are presented in Fig. 6a, b. The results showed that, in batch cultures, 25 °C was optimum for spore production with a spore yield of 0.54± 0.02. This is closely followed by 30 °C which gave a spore yield of 0.50±0.06, while the least spore yield (0.34 ±0.06) was recorded at 45 °C (Fig. 6a). The highest mycelial weight (12.61±0.08 g/l) was obtained at 30 °C followed by 11.96±0.08 g/l at 37 °C, and the least mycelial weight of 5.66±0.03 g/l was obtained at 45 °C (Fig. 6b). In fed-batch cultures, the optimum temperature for spore production was found to be 45 °C with a spore yield of 1.84±0.06 followed by 0.98±0.01 at 37 °C, while the least spore yield (0.56±0.05) was recorded at 25 °C (Fig. 6a). Mycelia growth in fed-batch cultures revealed that a temperature of 30 °C is optimum for mycelia production (13.27±0.01 g/l) followed by 37 °C (11.70± 0.06 g/l) while temperature of 45 °C stimulated the lowest mycelial growth of 8.51±0.2 g/l (Fig. 6b).

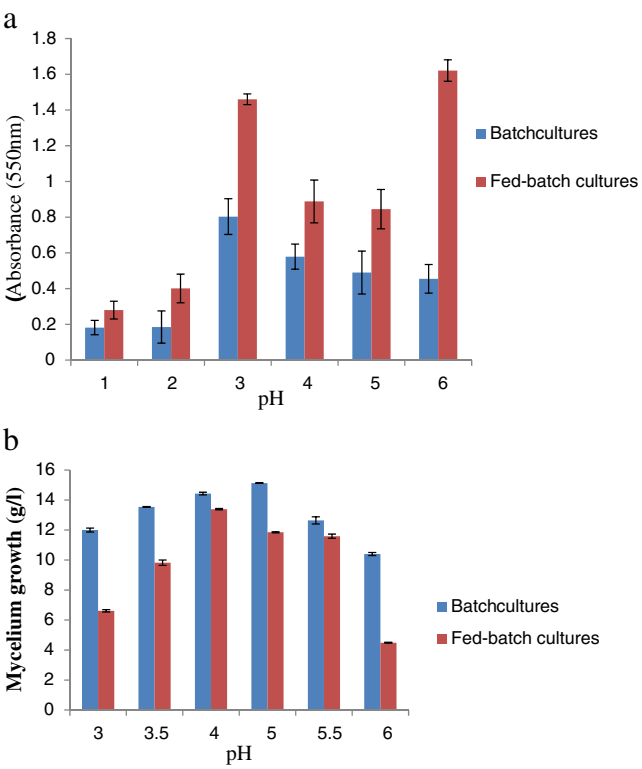

Fig. 5 Effect of initial pH on spore yield (**a**) and mycelial growth (**b**) by *Trichoderma viride* after 7 days in batch and fed-batch cultures. Data are given as means ± SEM

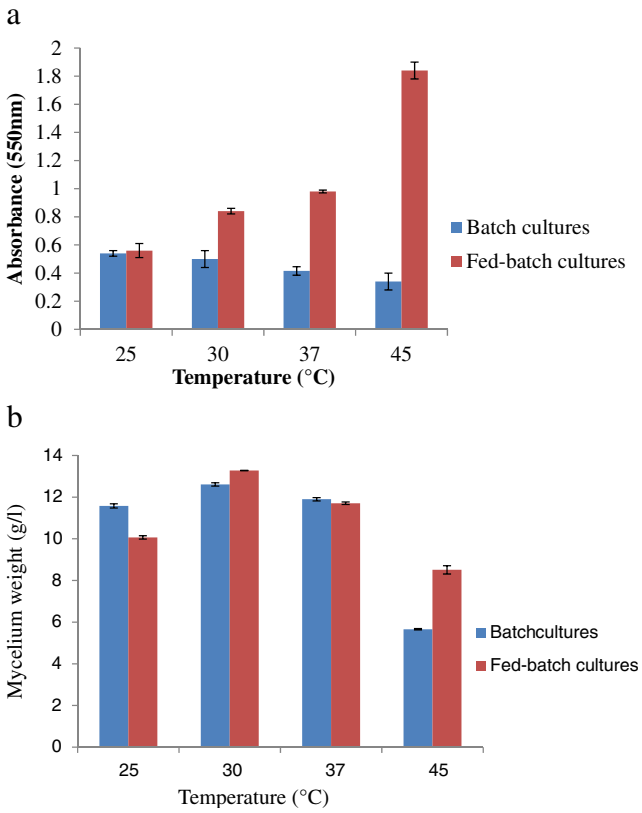

Fig. 6 Effect of incubation temperature on spore yield (**a**) and mycelial growth (**b**) by *Trichoderma viride* after 7 days in batch and fed-batch cultures. Data are given as means ± SEM

Discussion

The results of this study showed that this *Trichoderma viride* has the ability to use a variety of carbon and nitrogen sources for mycelial growth and spore production at different levels. This ability has also been reported by Papavizas (1995). This ability has been suggested as the main reason for the ubiquitous nature of *Trichoderma* sp. (Roussos et al. 1991). Mannitol stimulating the highest mycelial weight and spore yield by *T. viride* in this study has also been reported by Jayaswal et al. (2003) in batch culture. This could be as a result of it being easily transported across the cell membrane and oxidizing to generate energy (Schlegel 2002). Abundant mycelial growth and sporulation on medium containing wheat bran and rice bran in fed-batch cultures observed in this study are in agreement with the reports of Roussos et al. (1991), Ibrahim and Low (1993) and Sharma et al. (2002). Wheat bran and other agro-industrial residues contain an adequate amount of other nutrients like protein, fats, fibre, ash, Ca, Mg, P, K, etc. with various amino acids and porosity for oxygen supply which also help in growth and sporulation.

For optimum spore and mycelial production, $NaNO_3$ and NH_4SO_4 were found to be appropriate relative to other nitrogen sources investigated in batch cultures, while $NaNO_3$ and peptone were found appropriate for spore and mycelium, respectively, in fed-batch cultures. This could be as a result of the ease with which these compounds diffuse quickly into the cells (Nicholas 1965). Better growth of *T. viride* with ammonium sulphate and other forms of inorganic nitrogen could also be due to the fact that uptake of ammonium nitrogen reduces the pH of the surroundings, thereby creating a slightly acidic pH which is ideal for fungal growth (MacNish 1988). Ammonium sulphate and sodium nitrate supporting sporulation and mycelial growth may be important when considering the use of this *T. viride* as biocontrol agent in relation to agricultural practices, because of the use of ammonium and other inorganic fertilizers. The use of peptone which also supported high mycelial yield has been reported by Esan and Oancea (2010). This could be attributed to it being a complex mixture of peptides and amino acids containing some water-soluble vitamins (Cochrane 1958).

The results of the study carried out on the effect of initial pH on spore yield of *T. viride* in batch cultures showed that pH 4 was the optimum pH for spore yield while pH 5 gave the maximum mycelial growth, and pH 6 was optimum for spore yield and pH 4 was optimum for mycelial growth in fed-batch. Generally, *T. viride* has been reported to grow and sporulate well between pHs 4 and 6 (Aube and Gagnon 1969; Lewis and Papavivaz 1983; Bastos 2001; Steyaert et al. 2010). The pH of the growth medium has been identified as a factor which affects the permeability of the cell wall. Hence, maximum growth and sporulation at the optimum pH could be because the permeability of the cell wall reaches its optimum allowing the easy diffusion of nutrients needed for growth into the cell Grffin (1994)

Investigation of the effect of temperature showed that this *T. viride* has a broad range of temperature tolerance as regards growth and sporulation. A similar observation was recorded by Jayaswal et al. (2003). Maximum spore yield and mycelial growth at the optimum temperatures in both batch and fed-batch cultures could be because it also affects their metabolic activity especially the production of volatile antibiotics and enzymes (Tronsmo and Dennis 1978).

In this present study, the fed-batch cultures were initiated with a high spore density as a convenient experimental starting condition, and were established to meet the requirement for a continuous flux of substrates through a limiting substrate (yeast extract) pool, by adjusting the rate of substrate input to be lower than the approximately maximum (substrate-unlimited) rate of substrate demand by the fungal population. The yeast extract of the medium composition has been reported to be essential for microbial cultivation as it offers some additional growth factors like vitamins and amino acids, as well as some organic nitrogen compounds with high bioavailability (Esan and Oancea 2010). Mycelial growth and sporulation observed in both batch (when available nutrient was non-limiting) and fed-batch cultures (when available nutrient was limiting) in this study have also been reported by Morton (1961). According to him, the most general condition for induction of sporulation is the reduction or exhaustion of assimilable nitrogen while carbohydrate is still available. Andrew and Harris (1997), explained that the sporulation initiated response to nutrient limitation involves reorganization of the endogenous resource as well as the use of exogenous substrate.

Conclusively, this work demonstrated the possible production of spores of *T. viride* in fed-batch cultures using cheap and readily available raw materials. This is of great importance when considering the production of *T. viride* for use as a biocontrol agent. However, scale-up trials using small- and large-scale bioreactors under the conditions we have found optimal is recommended to determine the suitability of the organism (in terms of quantity) for industrial application.

References

Ahmed JS, Baker R (1987) Competitive saprophytic ability and cellulolytic activity of rhizophere-competent mutants of *Trichoderma harzianum*. Phytopathology 77:358–362

Al-Taweil HI, Osman MB, Aidil AH, Yussof WMW (2009) Optimizing *Trichoderma viride* Cultivation in Submerged State Fermentation. Am J Appl Sc 6(7):1277–1281

Andrew JH, Harris RF (1997) Dormancy, Germination, Growth, Sporulation, and Dispersal. In: Esser K, Lemke PA (eds) The Mycota IV. Springer, Berlin, pp 3–13

Aube C, Gagnon C (1969) Effect of carbon and Nitrogen nutrition on growth and sporulation of *Trichoderma viride pers Ex fries*. Can J Microbiol 15:703–706

Bastos CN (2001) Effect of temperature, pH and nutrition on growth and *sporulation of Trichoderma stromaticum* sp. nov; An antagonist of cocoa witches broom pathogen. Summa Phytopathology 27:73–76

Benhamou N, Chet I (1993) Hyphal interctions between *Trichoderma harzianum* and *Rhizoctonia solani*: ultrastructure band gold cytochemistry of themycoparasitic process. Phytopathology 83:1062–1107

Cascino JJ, Harris RF, Smith CS, Andrew JH (1990) Spore yield and Microcycle Conidiation of *Colletotrichum gloeosporioides* in Liquid Culture. Appl Environ Microbiol 56(8):2303–2310

Churchill BW (1982) Mass production of microorganisms for biological control. In: Charudattan R, Walker HL (eds) Biological control of weeds with plant pathogens. Wiley, New York, pp 139–156

Cochrane VW (1958) Physiology of fungi. Wiley, London

Coppings LG, Menn JJ (2000) Biopesticides: a review of their astion, applications and efficacy. Pest Manag Sci 56:651–676

Esan TE, Oancea F (2010) *Trichoderma viride* pers. – Experimental model for biological and biotechnological investigations of mycromyceta with importance in obtaining Plant protection bioproducts. J Plant Dev 17:49–62

Grffin DH (1994) Fungal Physiology, 2nd edn. Wiley Liss, New York

Ibrahim YB, Low W (1993) Potential of Mass Production and Field Efficacy of Isolates of the Entomopathoghenic Fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* on *Plutella xylostella*. J Invert Pathol 39:222–232

Jayaswal KR, Singhl R, Su-Lee Y (2003) Influence of Physiological and Environmental Factors on Growth and Sporulation of an Antagonistic Strain of *Trichoderma viride* RSR 7. Korean Soc Mycol 31(1):36–41

Lewis JA, Papavivaz GC (1983) Production of chlamydospores and conidia by *Trichoderma* sp. in liquid and solid growth media. Soil Biol Biochem 15:351–357

Lisansky SG, Hall RA (1983) Fungal control of insects. In: Smith JE, Berry DR, Kristiansen B (eds) The filamentous fungi, vol 4. Fungal technology. Edward Arnold, London, pp 327–345

MacNish GC (1988) Changes in take-all (*Gaeumannomyces graminis* var. *tritici*), rhizoctonia root rot (*Rhizoctonia solani*) and soil pH in continuous wheat with annual application of nitrogenous fertilizer in Western Australia. Aust J Exp Agr) 28:333–341

Morton, AG (1961) The induction of sporulation in mould fungi. Proc R Soc Lond B 153:548-569

Nahar S, Hossain F, Feroza B, Halim MA (2008) Production of glucoamylase by *Rhizopus* sp. in liquid culture. Pak J Bot 40 (4):1693–1698

Nicholas DJD (1965) Utilization of inorganic nitrogen compounds and amino acids by fungi. In: Ainsworth GC, Sussman AS (eds) The fungi, vol 2. Academic, New York, pp 349–376

Papavizas GC (1995) *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biological control. Annu Rev Phytopathol 23:23–54

Roussos S, Raimbault M, Viniegra-Gonzalez G, Saucedo-Castraneda G, Lonsane BK (1991) Scale-up of cellulases production by *Trichoderma harzianum* on amixture of sugar cane baggase and wheat bran in solid state fermentation system. Micol Neotrop Apl 4:83–98

Schlegel GH (2002) General Microbiology, 7th edn. Cambridge University Press, Cambridge, pp 246–249

Sharma S, Gupta RBL, Yadava CPS (2002) Selection of a suitable medium for mass multiplication of Entomofungal pathogens. Ind J Entomol 64(3):254–261

Steyaert JM, Weld RJ, Stewart A (2010) Ambient pH intrinsically influences *Trichoderma* conidiation and colony morphology. Fungal Biol 114:198–208

Tronsmo A, Dennis C (1978) Effect of temperature on antagonistic properties of *Trichoderma* species. Trans Br Mycol Soc 71:469–474

Whipps JM, Lumsden RD (2001) Commercial use of fungi as plant disease biological control agents: status and prospects. In: Butts T, Jackson C, Magan N (eds) Fungal biocontrol agents, problems and potential. CABI, Wallingford, pp 9–22