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SHORT COMMUNICATION

Genomic analysis of type strain *Paenibacillus aceti* L14^T, a highly efficient producer of pyrazines

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Abstract *Paenibacillus aceti* L14^T (CGMCC 1.15420 = JCM 31170) is a novel species isolated from the solid-state acetic acid fermentation culture of traditional Chinese vinegar. The strain is able to biosynthesize the pyrazines, including 2,3-diisobutylpyrazine, 2-isobutyl-3-methylpyrazine and 1-(5-isobutyl-2-pyrazinyl)-1-propanone. Genome sequencing of L14 was performed to gain insights into the genetic elements involved in the biosynthesis of pyrazines. The genome of L14 contains 5,611,962 bp with a GC content of 47.92 mol%, 5147 protein coding genes, 92 tRNAs, 20 rRNAs and four sRNAs. The strain L14 also contains complete biosynthetic pathways of valine, leucine and isoleucine, and contains genes for encoding threonine dehydratase and ketol-acid reductoisomerase. This genome sequence provides a basis for elucidating the possible mechanism for the biosynthesis of pyrazines.

Keywords *Paenibacillus aceti* · Genome · Pyrazine · Leucine · Threonine dehydratase

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Findings

Pyrazines occur ubiquitously in nature and can be biosynthesized by several bacteria. Pyrazines have paramount biological activities and are important flavour additives in the food industry. However, knowledge about the biosynthesis of pyrazines is very limited. Nowadays, two different pathways for the biosynthesis of alkyl-substituted pyrazines have been proposed (Beck et al. 2003): (i) the pyrazine ring structure is formed by the condensation of the amidated amino acid with an α,β-dicarbonyl compound and, followed by methylation reaction, leads to alkyl- and methoxy-substituted pyrazines (Murray and Whitfield 1975); (ii) pyrazine biosynthesis is the condensation of two amino acids to a cyclic dipeptide, and then converted into the pyrazine molecule (Cheng et al. 1991).

The genus *Paenibacillus* was firstly proposed as a new group of bacilli by Ash et al. (1993) based on the analysis of 16S rRNA gene sequences. Members of this genus are generally Gram-positive, rod-shaped, endospore-forming, aerobic or facultatively anaerobic bacteria. Nowadays, numerous members of the genus *Paenibacillus* have been isolated from various environments with very diverse biochemical functions (Zhang et al. 2013; Xie et al. 2014).

In our previous study, we isolated a novel species of the genus *Paenibacillus* from the traditional solid-state acetic acid fermentation culture of Chinese cereal vinegar and designated as *Paenibacillus aceti* L14^T (Li et al. 2016a). To further characterise several features of strain L14, the strain *P. aceti* L14 was initially pre-inoculated with 5 mL tryptic soy broth (TSB; Difco) at 37 °C for 24 h to obtain seed culture. Afterwards, 1 mL of seed culture was inoculated in 100 mL tryptic soy broth (TSB; Difco) and the flasks were shaken at 37 °C and 200 rpm for 24 h. The control culture was designed as follows: 1 mL tryptic soy broth was inoculated in 100 mL tryptic soy

broth (TSB; Difco) and was also treated under the same conditions. The supernatant culture was obtained by centrifuging at 8000×g and 4 °C for 5 min. The aroma compound in the supernatant was detected by headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC-MS) (Li et al. 2016b). According to the results of HS-SPME-GC-MS, this strain is able to biosynthesize the pyrazines (1.68 g/L), including 2,3-diisobutylpyrazine, 2-isobutyl-3-methylpyrazine and 1-(5-isobutyl-2-pyrazinyl)-1-propanone (Fig. 1).

In order to gain insights into the genetic bases in the biosynthesis of pyrazines, the genome of *P. aceti* L14 was sequenced on an Illumina HiSeq 2500 sequencing platform. A 500-bp paired-end library and a 6-kbp mate-pair library were constructed using the TruSeq DNA PCR-Free Sample Preparation Kit (Illumina) and the Nextera Mate Pair Sample Preparation Kit (Illumina), respectively. This strategy produced 2053 Mb of paired-end data with about 339-fold coverage of the genome. After data processing and assembly by applying SOAPdenovo 2.04 (Luo et al. 2012), the *P. aceti* L14 chromosome consisted of 11 scaffolds comprising 64 scaffolded contigs. The genome has a size of 5,611,962 bp, featuring a GC content of 47.92% (Table 1). The coding sequences were predicted by GeneMarkS (Besemer et al. 2001) and annotated using public databases, including the non-redundant (NR), KEGG, COG, GO and Swiss-Prot databases. The tRNA, rRNA and sRNA genes were identified by tRNAscan-SE (Lowe and Eddy 1997), RNAmmer (Lagesen et al. 2007) and the Rfam database (Gardner et al. 2009). In total, 5147 protein coding genes, 92 tRNAs, 20 rRNAs and four sRNAs were determined (Table 1).

Table 1 Genome features of *Paenibacillus aceti* L14

Feature	Value
Genome size (bp)	5,611,962
GC content	47.92%
Gene total length (bp)	4,731,522
Number of scaffolds	11
Number of contigs	64
Largest scaffold (bp)	5,572,757
Largest contig (bp)	773,790
Protein-coding genes	5147
tRNA genes	92
rRNA genes	20
sRNA genes	4
Minisatellite DNA	100
Genes with predicted function	4789

Ammonium was found to be the precursor for the biosynthesis of alkylated pyrazine (Zhu and Xu 2010). We annotated 50 CDSs for peptidase and four CDSs for amino acid dehydrogenase, which can degrade proteins and peptides into amino acids and produce ammonium from amino acids, respectively. Valine, leucine and isoleucine were the precursors of the isobutyl- or isopropyl-substituted pyrazines (Beck et al.

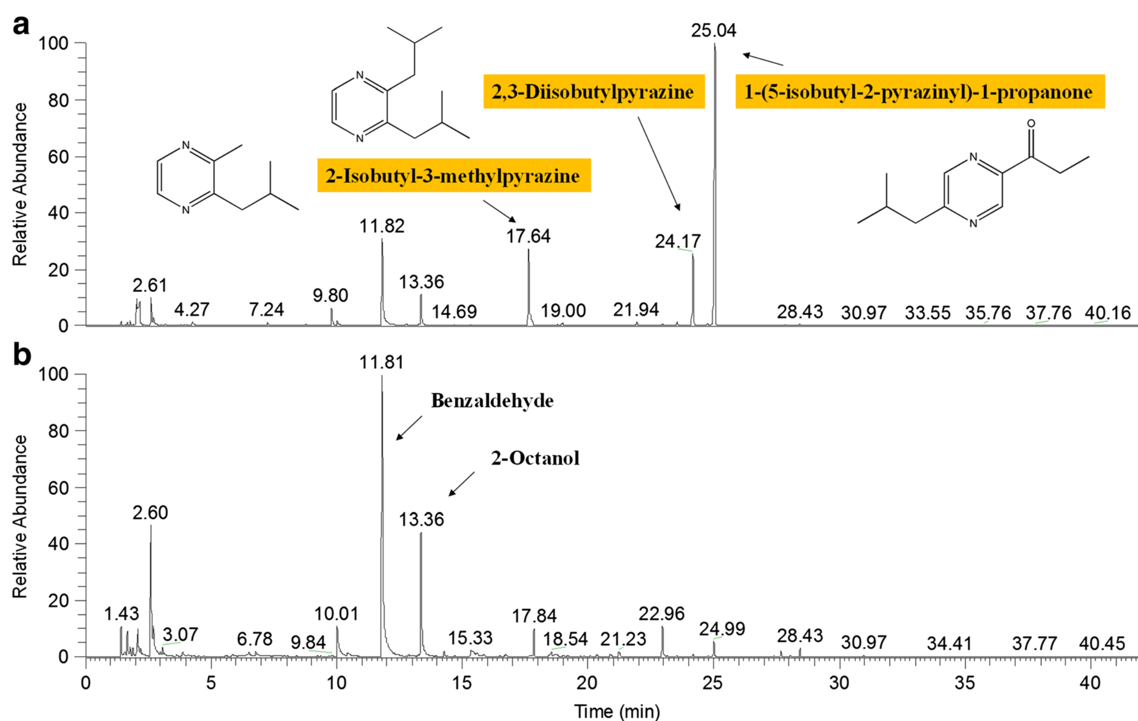

Fig. 1 Gas chromatography–mass spectrometry (GC-MS) chromatograms of fermentation culture (**a**) of *Paenibacillus aceti* L14 and control culture (**b**) for volatile compounds obtained by headspace solid-phase microextraction (HS-SPME). 2-Octanol was used as the internal standard

2003). Notably, the complete biosynthetic pathways were annotated for the production of valine, leucine and isoleucine in strain L14. The genes predicted for encoding threonine dehydratase and ketol-acid reductoisomerase, which are required for the formation of 2-oxobutanoate and (S)-2-acetolactate, have proven to be indispensable for the biosynthesis of pyrazines through the valine and leucine biosynthetic pathway in *Corynebacterium glutamicum* (Dickschat et al. 2010).

In conclusion, the genome data of *P. aceti* L14 is helpful for uncovering the possible mechanism for the biosynthesis of pyrazines, and will facilitate its potential applications as starter cultures in the food industry.

Strain and nucleotide sequence accession numbers

This whole genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number MDDO00000000. The BioProject designation for this project is PRJNA338379. The strain is available at the China General Microbiological Culture Collection Center and the Japan Collection of Microorganisms under the accession numbers CGMCC 1.15420 and JCM 31170, respectively.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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