



ORIGINAL ARTICLE

Characteristics of *Planococcus antioxidans* sp. nov., an antioxidant-producing strain isolated from the desert soil in the Qinghai-Tibetan Plateau

Binglin Zhang^{1,2}  | Ruiqi Yang³  | Gaosen Zhang^{2,4} | Yang Liu^{2,4} | Dongming Zhang² | Wei Zhang^{2,4} | Tuo Chen¹ | Guangxiu Liu^{2,4}

¹State Key Laboratory of Cryospheric Sciences, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou, China

²Key Laboratory of Extreme Environmental Microbial Resources and Engineering, Lanzhou, China

³College of Geography and Environmental Engineering, Lanzhou City University, Lanzhou, China

⁴Key Laboratory of Desert and Desertification, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou, China

Correspondence

Tuo Chen, State Key Laboratory of Cryospheric Sciences, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou, China.
Email: chentuo@lzb.ac.cn

Guangxiu Liu, Key Laboratory of Extreme Environmental Microbial Resources and Engineering, Lanzhou, China.
Email: liugx@lzb.ac.cn

Funding information

Bureau of International Cooperation, Chinese Academy of Sciences, Grant/Award Number: 131B62KYSB20160014; National Natural Science Foundation of China, Grant/Award Number: 41801045 and 31570498; CAS "Light of West China" Program

Abstract

Strain Y74^T was an isolate from the sandy soil in the town of Huatugou, Qinghai-Tibet Plateau, China. An analysis of this strain's phenotypic, chemotaxonomic, and genomic characteristics established the relationship of the isolate with the genus *Planococcus*. Strain Y74^T was able to grow between 4 and 42°C (with an optimum temperature of 28°C) at pH values of 6–8.5 and in 0%–7% (w/v) NaCl. The dominant quinones were MK-8 and MK-7. The polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and an unknown phospholipid. The majority of the fatty acid content was anteiso-C_{15:0} (28.8%) followed by C_{16:1} ω7c alcohol (20.9%) and iso-C_{14:0} (13.4%). The 16S rRNA gene sequence similarity analysis demonstrated a stable branch formed by strain Y74^T and *Planococcus halotolerans* SCU63^T (99.66%). The digital DNA-DNA hybridization between these two strains was 57.2%. The G + C content in the DNA of Y74^T was 44.5 mol%. In addition, the morphological, physiological, and chemotaxonomic pattern clearly differentiated the isolates from their known relatives. In conclusion, the strain Y74^T (=JCM 32826^T = CICC24461^T) represents a novel member of the genus *Planococcus*, for which the name *Planococcus antioxidans* sp. nov. is proposed. Strain Y74^T was found to have potent antioxidant activity via its hydrogen peroxide tolerance and its 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity. The DPPH radical-scavenging activity was determined to be 40.2 ± 0.7%. The genomic analysis indicated that six peroxidases genes, one superoxide dismutase gene, and one dprA (DNA-protecting protein) are present in the genome of Y74^T.

KEYWORDS

antioxidant, *Planococcus antioxidans*, polyphasic taxonomy, Qinghai-Tibetan Plateau

Binglin Zhang and Ruiqi Yang contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 The Authors. *MicrobiologyOpen* published by John Wiley & Sons Ltd.

1 | INTRODUCTION

The accumulation of free radicals in living organisms can lead to many diseases, such as cancer and neurodegenerative diseases (Fischer & Maier, 2015; Lin & Beal, 2006). Thus, it may be possible to reduce and prevent these chronic diseases by decreasing the presence of free radicals and increasing the intake of antioxidants (Bonda et al., 2010; Fischer & Maier, 2015). Microorganisms are an abundant source of bioactive metabolites (Berdy, 2005; Velho-Pereira, Parvatkar, & Furtado, 2015). Therefore, in order to prevent the toxic effects of free radicals, potent natural antioxidants have been an important target for researchers. Recently, exploring new taxa for new antioxidants has been one of the effective strategies employed in this search.

The Qinghai-Tibet Plateau is the highest plateau in the world, where the average altitude is above 4,500 m (Zhang et al., 2019). Because of the stressful conditions, such as low air temperatures, high UV radiation, and low atmospheric oxygen content, the organisms have had to adapt to survive on this plateau (Zhang et al., 2018; Zhang, Tang, et al., 2016). This environment is a potential source of genetic diversity and is an ideal place to search for antioxidant-producing microbes (Zhang, Wu, et al., 2016).

The genus *Planococcus* was originally described by Migula (1895). There were 16 valid species in the genus *Planococcus* until recently: *P. citreus* (Migula, 1895), *P. kocurii* (Hao & Komagata, 1985), *P. antarcticus* (Reddy et al., 2002), *P. maritimus* (Yoon, Weiss, Kang, Oh, & Park, 2003), *P. maitriensis* (Alam, Singh, Dube, Reddy, & Shivaji, 2003), *P. rifietoensis* (Romano, Giordano, Lama, Nicolaus, & Gambacorta, 2003), *P. columbae* (Suresh, Mayilraj, Bhattacharya, & Chakrabarti, 2007), *P. donghaensis* (Choi et al., 2007), *P. salinarum* (Yoon, Kang, Lee, Oh, & Oh, 2010), *P. halocryophilus* (Mykytczuk, Wilhelm, & Whyte, 2012), *P. plakortidis* (Kaur et al., 2012), *P. soli* (Luo et al., 2014), *P. faecalis* (Kim, Kang, Yu, Kim, & Lee, 2015), *P. ruber* (Wang et al., 2017), *P. salinus* (Gan, Zhang, Tian, et al., 2018), and *P. halotolerans* (Gan, Zhang, Zhang, et al., 2018). Due to their phenotypic properties, menaquinone profiles, fatty acid composition and G + C content in the DNA, the species *Planococcus mcmeekinii* (Yoon et al., 2001), *Planococcus okeanoikoites* (Yoon et al., 2001), *Planococcus alkanoclasticum* (Dai, Wang, Wang, Liu, & Zhou, 2005), *Planococcus psychrophilum* (Dai et al., 2005), and *P. stackebrandtii* (Jung, Kang, Oh, Yoon, & Kim, 2009) were reclassified to genus *Planomicrobium*, and *Planococcus halophilus* was cataloged to the genus *Marinococcus* (Hao, Kocur, & Komagata, 1984; Novitsky & Kushner, 1976). The known features of the genus *Planococcus* are that the species are Gram-positive, aerobic, non-spore-forming and have cell shapes that include cocci, short rods, or rods (Gan, Zhang, Zhang, et al., 2018). The genera *Planococcus* and *Planomicrobium* are close phylogenetic neighbors. Dai et al. (2005) found that the specific difference in the 16S rRNA gene sequence between genus *Planococcus* and *Planomicrobium* at sites 183 and 190 (*E. coli* numbering) was that *Planococcus* contained the signature nucleotides T and A, whereas the *Planomicrobium* species contained C and G (Dai et al., 2005).

According to our research, a new *Planococcus* species strain, Y74^T, was isolated from the desert soil in the Qinghai-Tibetan Plateau, China. Strain Y74^T demonstrated a strong antioxidant activity, which has potential antioxidant applications.

2 | MATERIALS AND METHODS

2.1 | Bacteria isolation

The desert soil samples were obtained from the town of Huatugou, Qinghai province, China. Strains Y74^T was isolated with modified 216 L agar medium (per liter distilled water: 1.0 g sodium acetate, 10.0 g tryptone, 2.0 g yeast extract, 0.5 g sodium citrate, 0.2 g ammonium nitrate, 0.5 g nutrient broth medium, 20.0 g agar, pH 7.6) and incubated for 7 days at 20°C, after which it was preserved at -80°C in 20% (v/v) glycerol (Wang, Wang, & Shao, 2010).

2.2 | Genome sequencing and analysis

Genomic DNA was extracted with a bacterial genomic DNA extraction kit (Omega Bio-tek, Inc.), according to the manufacturer's instructions, and the sequence was determined by the Illumina HiSeq 2000. The reads from the sequencing were assembled de novo using the Velvet 1.2.10 program. The genomes of the type strains that were similar to Y74^T were retrieved from GenBank. The average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) were used to assess the degree of similarity of each pair. The ANI was calculated with the JSpeciesWS (Richter, Rossello-Mora, Glockner, & Peplies, 2016). The ANI could be divided into ANI_b and ANI_m, depending on the BLASTN (Basic Local Alignment Search Tool) algorithm or the MUMMER ultra-rapid aligning tool. The dDDH was computed by an online tool, GGDC 2.0: the results of this computation were obtained using the recommended formula 2 (Meier-Kolthoff, Auch, Klenk, & Goker, 2013). The genome of strain Y74^T was annotated using IMG Annotation Pipeline v.5.0.3 (Chen et al., 2019). The G + C content of the DNA of strain Y74^T was deduced from the genomic data. Y74^T horizontal gene transfer analysis by the method of Bertelli, Laird, and Williams (2017).

2.3 | Phylogenetic analysis

The closely related type strains of Y74^T were obtained by comparing their 16S rRNA gene sequences, retrieved from the genome in the EzTaxon-e database (Kim et al., 2012). Phylogenetic trees based on the 16S rRNA gene sequences were generated utilizing neighbor joining (Saitou & Nei, 1987), maximum parsimony (Tamura et al., 2011), and maximum likelihood (Felsenstein, 1981) algorithms in MEGA X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). The sequences were aligned with ClustalW (Larkin et al., 2007). The remaining parameters followed the model of Jukes and Cantor (Jukes & Cantor,

1969), and the bootstrap value was 1,000 re-samplings (Felsenstein, 1985). A phylogenetic tree based on 25 housekeeping genes nucleotide sequences was generated using neighbor joining algorithms. The parameters were the same as for the phylogenetic tree based on 16S rRNA gene sequences. The sequences of 25 housekeeping genes were obtained from genomes of 19 type strain of genus *Planococcus*, *Planomicrobium*, and 1 outgroup strain (*Lysinibacillus sphaericus* IAM 13420^T) after annotated using Rapid Annotations using Subsystems Technology (RAST) (Brettin et al., 2015). The sequences of 25 housekeeping genes were concatenated in the following order: CTP synthase, DNA primase, DNA-directed RNA polymerase beta subunit, LSU ribosomal protein L11p, LSU ribosomal protein L13p, LSU ribosomal protein L16p, LSU ribosomal protein L20p, LSU ribosomal protein L27p, LSU ribosomal protein L3p, LSU ribosomal protein L4p, LSU ribosomal protein L5p, LSU ribosomal protein L6p, phosphoglycerate kinase, ribosome recycling factor, SSU ribosomal protein S10p, SSU ribosomal protein S11p, SSU ribosomal protein S13p, SSU ribosomal protein S2p, SSU ribosomal protein S3p, SSU ribosomal protein S5p, SSU ribosomal protein S9p, tmRNA-binding protein *SmpB*, transcription termination protein *NusA*, translation elongation factor Ts, and translation initiation factor 3 (Gil, Silva, Pereto, & Moya, 2004).

2.4 | Morphological and physiological analysis

Cell size and morphology were determined by scanning electron microscopy (JSM-5600, JEOL) utilizing cells immobilized after gold sputtering for 60 s. For scanning electron microscopy, the strain Y74^T was fixed on 4% glutaraldehyde for 8 hr. Subsequently, the cells were dehydrated in an ethanol series (15%, 30%, 50%, 70%, 80%, 90%, and 100%) for 10 min each. Colony color was evaluated on LB (Lysogeny Broth) agar (Oxoid).

Gram staining was tested using the Solarbio Gram staining kit. Growth temperatures (4, 10, 15, 20, 25, 30, 35, 40, 42, and 45°C) and NaCl concentrations (0%–10%, w/v, intervals of 0.5%) were determined on 216L medium. The pH range for growth was examined with strains cultured at 28°C in 216L medium, where the buffering system (KH₂PO₄/HCl, KH₂PO₄/K₂HPO₄, and K₂HPO₄/NaOH) was injected to adjust the pH value from 5 to 10 at 0.5 pH unit intervals. Oxidase activity was detected with 1% (w/v) tetramethyl-p-phenylenediamine. Starch and gelatin hydrolysis, nitrate reduction, catalase activity, methyl red, and Voges–Proskauer tests were performed according to the description of Kurup and Schmitt (1973). A carbohydrate utilization test was performed as described previously (Zhang et al., 2018). Additional enzyme activities were detected by API ZYM systems.

2.5 | Chemotaxonomic analysis

For the chemotaxonomic analysis, cells were collected by centrifugation from strains cultured at 28°C in TSB medium (per liter

distilled water: 17.0 g tryptone, 3.0 g soy peptone, 2.5 g D-glucose, 5.0 g sodium chloride, 2.5 g monopotassium phosphate, pH 7.3) for 3 days and then washed twice with distilled water. The cell-wall peptidoglycan was analyzed by the method of Schleifer and Kandler (1972). The whole-cell sugars were analyzed by the methods of Lechevalier and Lechevalier (1970). The quinones and the polar lipids were analyzed by the method of Collins et al. and HPLC (Collins, Pirouz, Goodfellow, & Minnikin, 1977; Kroppenstedt, 1982) and by the method of Minnikin et al. (1984), respectively. The methylation, extraction, and analysis of the fatty acids were based on the methods of Sasser (1990) and identified in the TSBA 6.0 database of the Sherlock Microbial identification (MIDI) system (Kämpfer & Kroppenstedt, 1996).

2.6 | Antioxidant activity analysis

The effect of hydrogen peroxide on the growth of strain Y74^T was tested as follows: an inoculum of 100 μl of strain Y74^T within the exponential growth phase (OD₆₀₀ = 0.6) was mixed with 50 ml LB medium containing 0, 1, and 5 mM H₂O₂ and then incubated at 30°C for 48 hr. The cell concentration was monitored by spectrophotometer (absorbance at 600 nm). All experiments were performed in triplicate. The growth fitting curves of strain Y74^T were drawn with Origin 2018 (logistics nonlinear fitting).

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity was tested in steps. First, an inoculum of 1 ml of strain Y74^T within the exponential growth phase (OD₆₀₀ = 1.0) was centrifuged at 5,300 g for 10 min, after which the supernatant was discarded, and the precipitate was resuspended with 500 μl PBS. This process was repeated three times. The resuspended precipitate was then mixed with 500 μl 0.4 mmol/L DPPH•ethanol (the control group used an equal volume of distilled water), after which the mixture was allowed to react in at low-light area for 30 min at room temperature and subsequently centrifuged at 5,300 g for 10 min. The absorbance of the supernatant was measured with a spectrophotometer at 517 nm. The DPPH free radical-scavenging rate was calculated as follows: scavenging activity (%) = $[1 - (A_s - A_b)/A_c] \times 100\%$, where A_b is the absorbance of the blank group, A_c is the absorbance of the control group, and A_s is the absorbance of the sample set.

3 | RESULTS AND DISCUSSION

3.1 | Phylogenetic analysis

The entirety of the 16S rRNA gene sequences was extracted from the genome of strain Y74^T (1,512 bp, KU601236). The genome of strain Y74^T was deposited at DDBJ/EMBL/GenBank with the accession number RCWH000000000.

Compared with the EzTaxon database, type strain *Planococcus halotolerans* SCU63^T (99.66%), *Planomicrobium okeanoikoites* IFO 12536^T (98.43%), *Planomicrobium flavidum* ISL-41^T (98.30%),

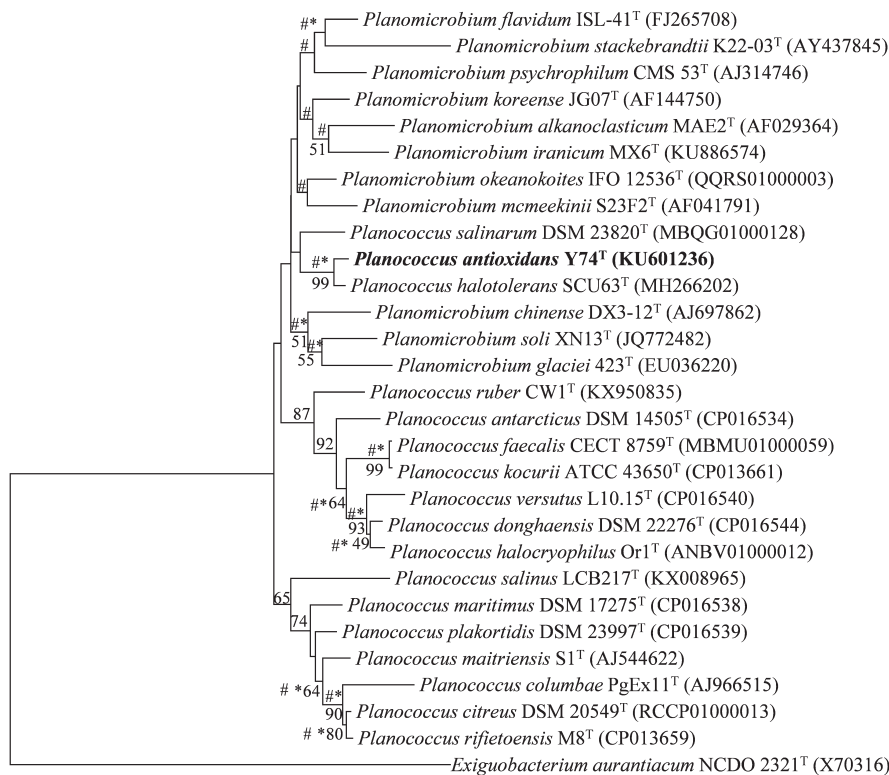


FIGURE 1 Neighbor joining phylogenetic tree, based on nearly complete 16S rRNA gene sequences, showing the relationships among strain Y74^T and their related species. Numbers at nodes are bootstrap values based on 1,000 re-samplings (only values above 50% are shown). Asterisks and hash marks indicate that the clades were also recovered in the maximum parsimony and maximum likelihood trees

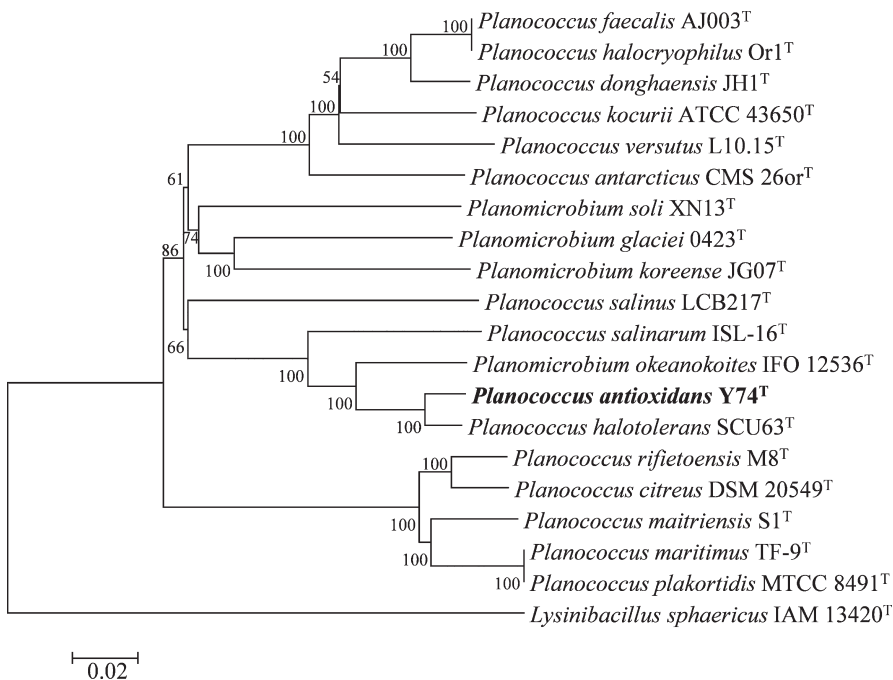


FIGURE 2 Neighbor joining phylogenetic tree based on 25 concatenated housekeeping genes of strain Y74^T and their similar related type strains. Numbers at nodes are bootstrap values based on 1,000 re-samplings (only values above 50% are shown)

Planococcus maitriensis S1^T (98.23%), and *Planomicrobium mcmee-kinii* S23F2^T (98.16%) were found to show high degrees of similarity with strain Y74^T (Kim et al., 2012). The three 16S rRNA gene phylogenetic trees indicated that Y74^T and *Planococcus halotolerans* SCU63^T formed a stable clade (Figure 1). However, many adjacent clades were not stable. Therefore, a more stable phylogenetic tree was constructed, which was based on 25 concatenated housekeeping genes of strain Y74^T and their related type

strains (Figure 2). According to this phylogenetic tree, strain Y74^T should be a member of the genus *Planococcus*. The exact position of *Planomicrobium okeanokoites* IFO12536^T could not exactly be resolved within the analysis. The dDDH between strain Y74^T and *Planococcus halotolerans* SCU63^T, *Planomicrobium okeanokoites* IFO 12536^T, or *Planococcus maitriensis* S1^T were 57.2%, 30.5%, and 19.1%, respectively. The ANIb values between strain Y74^T and *Planococcus halotolerans* SCU63^T, *Planomicrobium okeanokoites*

IFO 12536^T, or *Planococcus maitriensis* S1^T were 94.15%, 85.43%, and 72.19%, respectively, with ANIm values of 94.66%, 87.45%, and 83.50%, respectively (Table 1). These values were below the species demarcation threshold in prokaryotic species, the generally accepted species boundary for ANI and dDDH values were 95 ~ 96% and 70%, respectively (Chun et al., 2018; Kim, Oh, Park, & Chun, 2014; Meier-Kolthoff et al., 2013). For all of these reasons, *Planococcus antioxidans* sp. nov. Y74^T was designated as a novel species in the genus *Planococcus*.

3.2 | Morphological and physiological characteristics

Strain Y74^T was determined to be Gram-positive, whose cellular shape was cocci, short rods, or rods, and whose colony color was all white (Figure 3). The growth temperature range of Y74^T was 4–42°C (optimum temperature 30°C) with a pH range of 6–8.5 and a NaCl tolerance of up to 7% (w/v) (Table 2). Strain Y74^T had a wide range of growth temperatures and a high salt tolerance, which was similar to other closely related type strains of *Planococcus* or *Planomicrobium* (Gan, Zhang, Zhang, et al., 2018; Jung et al., 2009). Strain Y74^T could utilize D-fructose, D-galactose, D-glucose, D-lactose, or D-maltose as sole carbon sources, weakly utilize D-cellobiose, D-lactose, D-mannitol, D-mannose, D-melibiose, D-raffinose, L-rhamnose, D-sorbitol, D-trehalose, or myo-inositol, and not utilize starch or sucrose (Table 2). However, *Planococcus halotolerans* SCU63^T was able to utilize sucrose but could not utilize D-sorbitol or melibiose (Gan, Zhang, Zhang, et al., 2018).

TABLE 1 The genome comparisons of strain Y74^T and the related type species

Species	16S	dDDH	ANIb	ANIm
<i>Planococcus halotolerans</i>	99.66	57.2	94.15	94.66
<i>Planomicrobium okeanoikoites</i>	98.43	30.5	85.43	87.45
<i>Planococcus maitriensis</i>	98.23	19.1	72.19	83.50
<i>Planococcus citreus</i>	98.10	19.0	72.34	83.74
<i>Planococcus koreense</i>	98.10	19.6	73.37	83.96
<i>Planococcus salinarum</i>	98.03	24.9	80.89	84.83

There were some distinctions with other reference type strains. Strain Y74^T showed positivity for catalase and for gelatin hydrolysis and negativity for oxidase, nitrate reduction, methyl red, and the Voges-Proskauer tests (Table 2). The result of the API ZYM test showed that strain Y74^T was weakly positive for weak positive for α -glucosidase, cystine arylamidase, esterase lipase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, and valine arylamidase.

3.3 | Chemotaxonomic characteristics

The whole-cell hydrolysate of Y74^T contained ribose. The peptidoglycan type was L-Lys-D-Glu. The dominant quinone compounds of Y74^T were MK-8 (76%) and MK-7 (24%) (Table 2). Table 2 shows that the predominant isoprenoid quinone compound of the type strains of *Planococcus* and *Planomicrobium* were all MK-8 and MK-7. The polar lipids of Y74^T were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and an unknown phospholipid (Figure A1). The major fatty acids of Y74^T were anteiso-C_{15:0} (28.8%), C_{16:1} ω 7c alcohol (20.9%), and iso-C_{14:0} (13.4%) (Table 3). These results conformed to the characteristics of the *Planococcus* genus (Gan, Zhang, Tian, et al., 2018; Kim et al., 2015; Wang et al., 2017).

3.4 | Antioxidant characteristics

Strain Y74^T showed potent antioxidant activity. The strain grew in LB medium supplemented with 1 or 5 mM hydrogen peroxide. The growth curves of Y74^T in LB medium supplemented with 0, 1, and 5 mM hydrogen peroxide were similar. Strain Y74^T reached a stable growth phase after approximately 18 hr under three conditions. However, the cell concentrations were significantly different, with the highest cell concentration observed in the LB medium with 0 mM hydrogen peroxide and the lowest cell concentration seen in the LB medium with 5 mM hydrogen peroxide (Figure 4). The DPPH radical-scavenging activity was 40.2 \pm 0.7%. There were many studies on antioxidants of genus *Lactobacillus*, which could produce antioxidants and had extensive application (Das & Goyal, 2015; Le & Cao, 2010; Lee et al., 2010). The strain Y74^T had the significant resistant ability to hydrogen peroxide than many species of *Lactobacillus*,

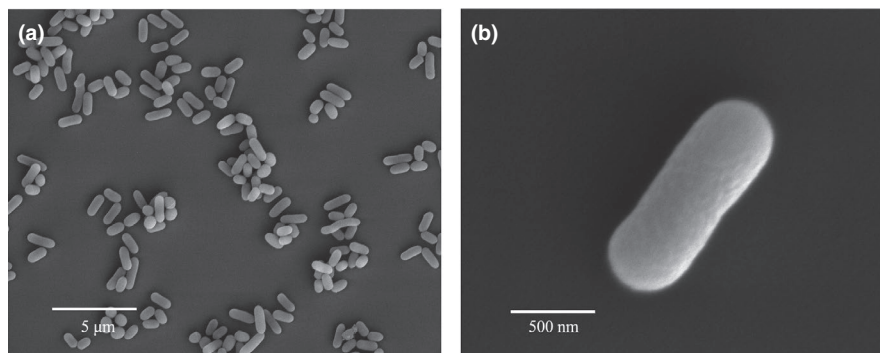


FIGURE 3 Scanning electron micrograph of strain Y74^T (a, b) cultivated on LB medium

TABLE 2 Comparison of the characteristics of strain Y74^T and the closely related type species

Characteristics	1	2	3	4	5	6	7	8
Cell shape	C, SR, R	C, SR	R	C, SR	C	C, SR	C, SR, R	C,SR,R
Cell length	0.8–3.4	0.4–1.4	1.0–20	2.7–3.3	1–2.0	0.8–1.0	0.5–2.8	0.8–5.0
Cell width	0.8–1.1	0.4–0.6	0.4–0.8	0.4–0.8	1–2.0	0.8–1.0	0.4–0.8	0.4–0.8
Gram stain	Positive	Positive	Positive to variable	Positive to variable	Positive	Positive	Positive to variable	Positive
Colony color	White	Moderate orange	Bright yellow-bright orange	Light yellow	Orange	Yellow orange	Yellow orange	Pale yellow
Spore formation	–	–	–	–	–	–	–	–
NaCl (%)(w/v)	0–7	15	0–7	0–14	0–12.5	0–10	0–7	0–13
pH	6–8.5	6.5–9.0	7.0 ^a	6.0–8.0 ^b	6.0–12	5.0–10	5.5–10	6–7.5 ^b
Temperature	4–42	0–40	20–37 ^c	4–37	0–30	10–45	4–38	4–38
Catalase	+	+	+	+	+	N	+	+
Oxidase	–	+	W	+	–	–	–	+
Nitrate reduction	–	–	–	–	+	+	–	–
Gelatin hydrolysis	W	–	+	+	+	+	+	–
Utilization as carbon sources								
D-cellobiose	W	+	–	–	–	–	+	–
D-fructose	+	+	+	+	+	N	–	+
D-galactose	+	+	–	–	–	N	–	–
D-glucose	+	+	–	–	+	+	W	–
D-lactose	W	N	–	–	–	–	+	–
D-maltose	+	+	–	–	–	N	+	–
D-mannitol	W	+	–	–	–	–	–	–
D-mannose	W	+	–	–	–	–	–	–
D-melibiose	W	–	–	–	+	–	+	–
D-raffinose	W	N	–	–	+	–	–	–
D-sorbitol	W	–	–	–	–	N	–	–
D-trehalose	W	+	–	–	–	N	–	–
L-rhamnose	W	N	–	–	–	–	–	–
myo-inositol	W	+	–	–	–	N	–	–
Starch	–	–	–	–	–	–	–	–
Sucrose	–	+	–	–	+	–	–	–
Predominant menaquinone	MK-8, MK-7	MK-8, MK-7	MK-8, MK-7	MK-8,7	MK-7,MK-8	MK-8	MK-8, MK-7, MK-6	MK-8, MK-7
GC content (mol%)	44.5	44.6	46	45.9	39	34.8	47	48.3

Note: Strains: 1, Y74^T (data from this study); 2, *Planococcus halotolerans* SCU63^T (Gan, Zhang, Zhang, et al., 2018); 3, *Planomicrobium okeanoikoites* IFO 12536^T (carbon source utilization and enzyme activity test data were from this study, other data were from Nakagawa, 1996); 4, *Planomicrobium flavidum* ISL-41^T (Jung, 2009); 5, *Planococcus maitriensis* S1^T (Alam et al., 2003); 6, *Planomicrobium chinense* DX3-12^T (Dai et al., 2005); 7, *Planomicrobium koreense* JG07^T (Yoon et al., 2001); 8, *Planococcus salinarum* DSM 23820^T (Yoon et al., 2010).

C, cocci; SR, short rods; R, rods; +, positive; –, negative; W, weak positive; N, not determined.

^aThe range of the growth temperatures was not reported.

^bThe lower limit was the minimum value that the strain could grow in, and the upper limit was the maximum value of the optimal growth range.

^cThe optimal growth range.

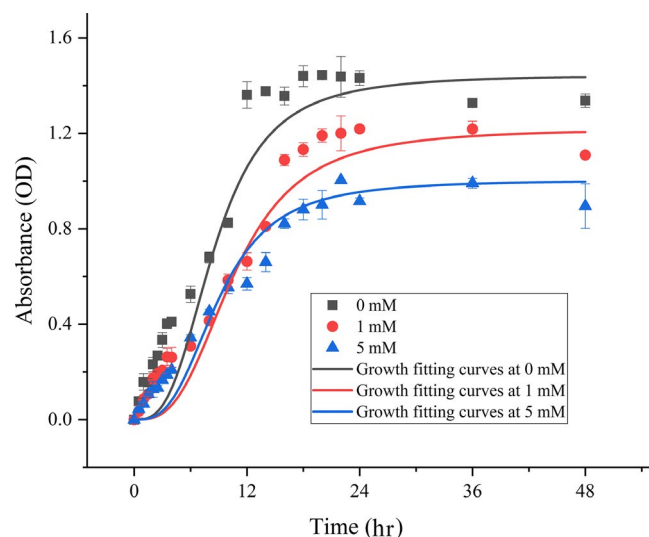
such as *Lactobacillus plantarum* DM5, *Lactobacillus plantarum* NRRL B-4496, and *Lactobacillus acidophilus* NRRL B-4495 (Das & Goyal, 2015). Simultaneously, the radical-scavenging ability of DPPH of strain Y74^T was higher than *Lactobacillus plantarum* NRRL B-4496

but slightly lower than *Lactobacillus acidophilus* NRRL B-4495 (Das & Goyal, 2015; Kaizu, Sasaki, Nakajima, & Suzuki, 1993). This indicates that the antioxidant activity of strain Y74^T had reached a practical level. The species of *Planococcus* genus were always isolated from

TABLE 3 Cellular fatty acid composition of strain Y74^T

Fatty acid	Y74 ^T
C _{16:0}	1.04
C _{18:0}	1.14
iso-C _{14:0}	13.90
iso-C _{15:0}	3.54
iso-C _{16:0}	9.62
iso-C _{17:0}	2.77
anteiso-C _{15:0}	28.81
anteiso-C _{17:0}	2.81
C _{16:1} ω11c	3.02
C _{17:0} 10-methyl	1.03
C _{16:1} ω7c alcohol	20.88
iso-C _{17:1} ω10c	3.05
Sum In Feature 4	4.37

Note: The amount of fatty acid was omitted when <1%. Sum In Feature 4: iso-C_{17:1} I and/or anteiso-C_{17:1} B.

**FIGURE 4** The growth curves of strain Y74^T cultivated on LB medium with 0, 1, and 5 mM H₂O₂

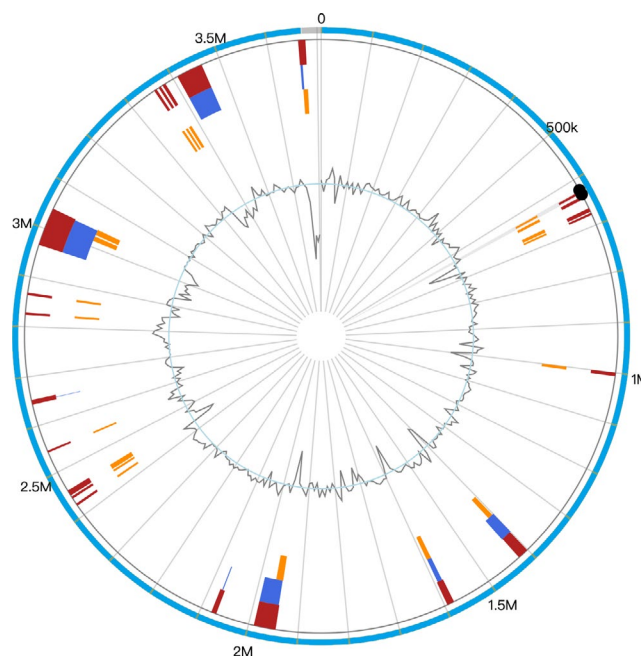
some extreme environments, such as the Arctic permafrost or the Antarctic (Mykytczuk et al., 2012; Reddy et al., 2002). Therefore, these strains have strong stress-resistant abilities generally. Previous research found a new antioxidant in *Planococcus* (Shindo & Misawa, 2014). The species of *Planococcus* genus had the potential for antioxidant applications, but few studies were emphasized on this property.

3.5 | Genome properties

The draft genome of strain Y74^T was 3,672,033 bp (Table 4). The G + C content of the DNA of Y74^T was 44.5 mol%. A total of 3,831 genes were detected in strain Y74^T, 3,668 of which were

TABLE 4 Summary of *Planococcus antioxidans* Y74^T

Feature	Genome	
	Value	% of total
Size (bp)	3,672,033	100
Coding region (bp)	3,150,934	85.81
Total genes	3,831	100
RNA genes	106	2.77
tRNA	67	1.75
5S rRNA	10	0.26
16S rRNA	7	0.18
23S rRNA	12	0.31
Other RNA genes	10	0.26
Protein-coding genes	3,668	95.75
Protein-coding genes with function prediction	2,913	76.04
Protein-coding genes with enzymes	967	25.24
Protein-coding genes coding signal peptides	178	4.65
Protein-coding genes coding transmembrane proteins	987	25.76
Protein-coding genes with COGs	3,006	78.47

**FIGURE 5** Characteristics and position of the predicted genomic islands found in the draft genome sequence of strain Y74^T. The genomic islands show that several horizontal gene transfer events have occurred in strain Y74^T. The red in the circles represents the prediction from integrating three different methods (IslandPath-DIMOB, SIGI-HMM, and IslandPick); the orange represents the prediction result using IslandPath-DIMOB; the dark blue represents the prediction result using SIGI-HMM

protein-coding genes. There were 967 protein-coding genes containing enzymes. The number of protein-coding genes with a function prediction was 2,913. The number of protein-coding genes with COGs was 3,006, which accounted for 78.47% of all genes. The genome of strain Y74^T contained 106 RNA genes, including 67 tRNA genes, 10 5S rRNA genes, 7 16S rRNA genes, and 12 23S rRNA genes (Table 4). The copy number of the rRNA operons in prokaryotic organisms is generally thought to be related to growth rates (Klappenbach, Dunbar, & Schmidt, 2000). It could be suggested that strain Y74^T could grow rapidly at lower temperatures. Multiple copy numbers of key genes could increase the radiation resistance of the bacteria as well (Slade & Radman, 2011). There are six peroxidase genes in the genome of Y74^T (Table A1), including two glutathione peroxidases, one catalase family peroxidase, one heme-dependent peroxidase, one thioredoxin-dependent thiol peroxidase, and one thiol peroxidase. Peroxidases are enzymes that catalyze the oxidation of substrates by hydrogen peroxide as an electron acceptor (Welinder, 1992). This property may explain why the strain can grow in a medium containing hydrogen peroxide. Genes with antioxidant abilities, DNA-protecting protein (DprA), and superoxide dismutase were also found in the genome of Y74^T.

In addition to the core proteins and other orthologs presenting in the organism, some nonortholog proteins were found in the strain of Y74^T. Therefore, the gene cluster of strain Y74^T was analyzed by the method of Bertelli et al. (2017). The results showed that multiple horizontal gene transfer events were found in the Y74^T genome. There were 28 gene islands be found in the genome, which contained 395 genes ranging from 4,000 to 700,000 bp, including 162 unclear functional genes of them were annotated as hypothetical proteins; 16 genes were predicted to be recombinase and phage-associated proteins (Figure 5, Table A2). Based on the gene function analysis on gene island, most of the genes were involved in metabolism, signal transduction, and DNA repair.

4 | CONCLUSIONS

According to an analysis of phenotypic, phylogenetic, and chemotaxonomic characteristics, strain Y74^T was determined to be a new member within the genus *Planococcus*. Therefore, it was named as *Planococcus antioxidans* sp. nov. Y74^T. Strain Y74^T was found to have potent antioxidant activity via its hydrogen peroxide tolerance and its DPPH radical-scavenging activity.

4.1 | Description of *Planococcus antioxidans* sp. nov

Planococcus antioxidans (an.ti.o'xi.dans. gr. pref. anti, against; N.L. v. oxidare, to oxidize; N.L. part. adj. antioxidans, referring to the characteristic of this strain).

The colonies on LB agar (Oxoid) are circular, smooth, and white. The cells are aerobic, Gram-stain-positive, non-spore-forming, and occur as cocci, short rods, or rods (0.8–1.1 × 0.8–3.4 μm). Growth occurs

between 4 and 42°C (optimum temperature 28°C), pH values of 6–8.5 and 0%–7% (w/v) NaCl. Strain Y74^T utilizes D-fructose, D-galactose, D-glucose, D-lactose, or D-maltose as sole carbon sources, weakly utilizes D-cellobiose, D-lactose, D-mannitol, D-mannose, D-melibiose, D-raffinose, L-rhamnose, D-sorbitol, D-trehalose, or myo-inositol, and does not utilize starch or sucrose. Strain Y74^T shows positively for catalase and for gelatin hydrolysis, and negativity for oxidase, nitrate reduction, methyl red, and the Voges–Proskauer tests. In assays with the API ZYM system, strain Y74^T is weakly positive for α-glucosidase, cystine arylamidase, esterase lipase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, and valine arylamidase, and negative for α-chymotrypsin, α-fucosidase, α-galactosidase, α-mannosidase, acid phosphatase, alkaline phosphatase, β-galactosidase, β-glucosidase, β-glucuronidase, esterase, lipase, N-acetyl-β-glucosaminidase, and trypsin. The cell wall contains ribose. The peptidoglycan type was L-Lys-D-Glu. The dominant quinones are MK-8 and MK-7. The polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and an unknown phospholipid. The majority of the fatty acid is anteiso-C_{15:0} (28.8%), followed by C_{16:1} ω7c alcohol (20.9%) and iso-C_{14:0} (13.4%).

The type strain, Y74^T (=JCM 32826^T = CICC24461^T), was isolated from the sandy soil in the town of Huatugou, Qinghai province, China. The G + C content of the DNA of strain Y74^T is 44.5 mol%.

ACKNOWLEDGMENTS

The research was funded by the Bureau of International Cooperation, Chinese Academy of Sciences (131B62KYSB20160014), and the National Natural Science Foundation of China (No. 41801045, 31570498), CAS "Light of West China" Program.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTION

Binglin Zhang: Conceptualization-Equal, Software-Equal, Writing-review & editing-Equal; Ruiqi Yang: Investigation-Equal, Resources-Equal; Gaosen Zhang: Methodology-Equal, Resources-Equal; Yang Liu: Formal analysis-Equal; Dongming Zhang: Formal analysis-Equal, Software-Equal; Wei Zhang: Methodology-Equal; Tuo Chen: Conceptualization-Equal, Writing-review & editing-Equal; Guangxiu Liu: Conceptualization-Equal, Writing-review & editing-Equal.

ETHICS STATEMENT

None required.

DATA AVAILABILITY STATEMENT

All data are provided in full in the results section of this paper apart from the DNA sequences. The 16S rRNA gene sequence of strain Y74^T is available in the GenBank database under accession number KU601236. The genome of strain Y74^T is deposited in GenBank with accession number RCWH00000000. The type strain Y74^T is deposited in the Japan Collection of Microorganisms (https://www.jcm.riken.jp/cgi-bin/jcm/jcm_number?JCM=32826) and the China

Center of Industrial Culture Collection (<http://sales.china-cicc.org/category.php?id=1&sh=jd&keywords=24461>).

ORCID

Binglin Zhang  <https://orcid.org/0000-0002-8466-9494>

Ruiqi Yang  <https://orcid.org/0000-0003-3188-2547>

REFERENCES

- Alam, S. I., Singh, L., Dube, S., Reddy, G. S. N., & Shivaji, S. (2003). Psychrophilic *Planococcus maitriensis* sp. nov from Antarctica. *Systematic and Applied Microbiology*, 26, 505–510. <https://doi.org/10.1078/072320203770865792>
- Berdy, J. (2005). Bioactive microbial metabolites. *Journal of Antibiotics*, 58, 1–26.
- Bertelli, C., Laird, M. R., Williams, K. P., Simon Fraser University Research Computing Group, Lau, B. Y., Hoad, G. H., ... Brinkman, F. S. (2017). IslandViewer 4: Expanded prediction of genomic islands for larger-scale datasets. *Nucleic Acids Research*, 45(W1), W30–W35. <https://doi.org/10.1093/nar/gkx343>
- Bonda, D. J., Wang, X. L., Perry, G., Nunomura, A., Tabaton, M., Zhu, X. W., & Smith, M. A. (2010). Oxidative stress in Alzheimer disease: A possibility for prevention. *Neuropharmacology*, 59, 290–294. <https://doi.org/10.1016/j.neuropharm.2010.04.005>
- Brettin, T., Davis, J. J., Disz, T., Edwards, R. A., Gerdes, S., Olsen, G. J., ... Xia, F. (2015). RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Scientific Reports*, 5, 8365. <https://doi.org/10.1038/srep08365>
- Chen, I. M. A., Chu, K., Palaniappan, K., Pillay, M., Ratner, A., Huang, J. H., ... Kyrpides, N. C. (2019). IMG/M vol 5.0: An integrated data management and comparative analysis system for microbial genomes and microbiomes. *Nucleic Acids Research*, 47, D666–D677. <https://doi.org/10.1093/nar/gky901>
- Choi, J. H., Im, W. T., Liu, Q. M., Yoo, J. S., Shin, J. H., Rhee, S. K., & Roh, D. H. (2007). *Planococcus donghaensis* sp. nov., a starch-degrading bacterium isolated from the East Sea, South Korea. *International Journal of Systematic and Evolutionary Microbiology*, 57, 2645–2650. <https://doi.org/10.1099/ijs.0.65036-0>
- Chun, J., Oren, A., Ventosa, A., Christensen, H., Arahall, D. R., da Costa, M. S., ... Trujillo, M. E. (2018). Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *International Journal of Systematic and Evolutionary Microbiology*, 68(1), 461–466. <https://doi.org/10.1099/ijsem.0.002516>
- Collins, M. D., Pirouz, T., Goodfellow, M., & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *Journal of General Microbiology*, 100, 221–230. <https://doi.org/10.1099/00221287-100-2-221>
- Dai, X., Wang, Y. N., Wang, B. J., Liu, S. J., & Zhou, Y. G. (2005). *Planomicrobium chinense* sp. nov., isolated from coastal sediment, and transfer of *Planococcus psychrophilus* and *Planococcus alkanoclasticus* to *Planomicrobium* as *Planomicrobium psychrophilum* comb. nov and *Planomicrobium alkanoclasticum* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, 55, 699–702. <https://doi.org/10.1099/ijs.0.63340-0>
- Das, D., & Goyal, A. (2015). Antioxidant activity and γ -aminobutyric acid (GABA) producing ability of probiotic *Lactobacillus plantarum* DM5 isolated from Marcha of Sikkim. *LWT-food Science and Technology*, 61(1), 263–268. <https://doi.org/10.1016/j.lwt.2014.11.013>
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences - a maximum-likelihood approach. *Journal of Molecular Evolution*, 17, 368–376.
- Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39, 783–791. <https://doi.org/10.1111/j.1558-5646.1985.tb00420.x>
- Fischer, R., & Maier, O. (2015). Interrelation of oxidative stress and inflammation in neurodegenerative disease: Role of TNF. *Oxidative Medicine and Cellular Longevity*, 2015, 1–18. <https://doi.org/10.1155/2015/610813>
- Gan, L. Z., Zhang, H. M., Tian, J. W., Li, X. G., Long, X. F., Zhang, Y. Q., ... Tian, Y. Q. (2018). *Planococcus salinus* sp. nov., a moderately halophilic bacterium isolated from a saline-alkali soil. *International Journal of Systematic and Evolutionary Microbiology*, 68, 589–595. <https://doi.org/10.1099/ijsem.0.002548>
- Gan, L. Z., Zhang, Y., Zhang, L. L., Li, X. G., Wang, Z. K., He, L. L., ... Tian, Y. Q. (2018). *Planococcus halotolerans* sp nov., isolated from a saline soil sample in China. *International Journal of Systematic and Evolutionary Microbiology*, 68, 3500–3505. <https://doi.org/10.1099/ijsem.0.003019>
- Gil, R., Silva, F. J., Pereto, J., & Moya, A. (2004). Determination of the core of a minimal bacterial gene set. *Microbiology Molecular Biology Reviews*, 68, 518–537. <https://doi.org/10.1128/MMBR.68.3.518-537.2004>
- Hao, M. V., Kocur, M., & Komagata, K. (1984). *Marinococcus* Gen-Nov, a new genus for motile Cocci with Meso-Diaminopimelic acid in the cell-wall - and *Marinococcus-Albus* Sp-Nov and *Marinococcus-Halophilus* (Novitsky and Kushner) Comb-Nov. *Journal of General and Applied Microbiology*, 30, 449–459. <https://doi.org/10.2323/jgam.30.449>
- Hao, M. V., & Komagata, K. (1985). A new species of *Planococcus*, *Planococcus-Kocurii* isolated from fish, frozen foods, and fish curing brine. *Journal of General and Applied Microbiology*, 31, 441–455. <https://doi.org/10.2323/jgam.31.441>
- Jukes, T. H., & Cantor, C. R. (1969). Evolution of protein molecules. *Mammalian Protein Metabolism*, 3, 21–132.
- Jung, Y. T., Kang, S. J., Oh, T. K., Yoon, J. H., & Kim, B. H. (2009). *Planomicrobium flavidum* sp. nov., isolated from a marine solar salt-tern, and transfer of *Planococcus stackebrandtii* Mayilraj et al 2005 to the genus *Planomicrobium* as *Planomicrobium stackebrandtii* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, 59, 2929–2933. <https://doi.org/10.1099/ijs.0.009191-0>
- Kaizu, H., Sasaki, M., Nakajima, H., & Suzuki, Y. (1993). Effect of antioxidative lactic acid bacteria on rats fed a diet deficient in vitamin E. *Journal of Dairy Science*, 76, 2493–2499. [https://doi.org/10.3168/jds.S0022-0302\(93\)77584-0](https://doi.org/10.3168/jds.S0022-0302(93)77584-0)
- Kämpfer, P., & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Canadian Journal of Microbiology*, 42, 989–1005. <https://doi.org/10.1139/m96-128>
- Kaur, L., Das, A. P., Acharya, M., Klenk, H. P., Sree, A., & Mayilraj, S. (2012). *Planococcus plakortidis* sp. nov., isolated from the marine sponge *Plakortis simplex* (Schulze). *International Journal of Systematic and Evolutionary Microbiology*, 62, 883–889. <https://doi.org/10.1099/ijsem.0.029967-0>
- Kim, J. H., Kang, H. J., Yu, B. J., Kim, S. C., & Lee, P. C. (2015). *Planococcus faecalis* sp. nov., a carotenoid-producing species isolated from stools of Antarctic penguins. *International Journal of Systematic and Evolutionary Microbiology*, 65, 3373–3378. <https://doi.org/10.1099/ijsem.0.000423>
- Kim, M., Oh, H. S., Park, S. C., & Chun, J. (2014). Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *International Journal of Systematic and Evolutionary Microbiology*, 64, 346–351. <https://doi.org/10.1099/ijs.0.059774-0>
- Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., ... Chun, J. (2012). Introducing EzTaxon-e: A prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *International Journal of Systematic and Evolutionary Microbiology*, 62, 716–721. <https://doi.org/10.1099/ijs.0.038075-0>

- Klappenbach, J. A., Dunbar, J. M., & Schmidt, T. M. (2000). rRNA operon copy number reflects ecological strategies of bacteria. *Applied and Environmental Microbiology*, *66*, 1328–1333. <https://doi.org/10.1128/AEM.66.4.1328-1333.2000>
- Kroppenstedt, R. M. (1982). Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger as stationary phases. *Journal of Liquid Chromatography*, *5*, 2359–2367. <https://doi.org/10.1080/01483918208067640>
- Kumar, S., Stecher, G., Li, M., Nkya, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*, 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Kurup, P. V., & Schmitt, J. A. (1973). Numerical taxonomy of Nocardia. *Canadian Journal of Microbiology*, *19*, 1035–1048. <https://doi.org/10.1139/m73-164>
- Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., ... Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, *23*, 2947–2948. <https://doi.org/10.1093/bioinformatics/btm404>
- Le, H., & Cao, Y. (2010). Lactic acid bacterial cell factories for gamma-aminobutyric acid. *Amino Acids*, *39*, 1107–1116. <https://doi.org/10.1007/s00726-010-0582-7>
- Lechevalier, M. P., & Lechevalier, H. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *International Journal of Systematic Bacteriology*, *20*, 435–443. <https://doi.org/10.1099/00207713-20-4-435>
- Lee, B. J., Kim, J. S., Kang, Y. M., Lim, J. H., Kim, Y. M., Lee, M. S., ... Je, J. Y. (2010). Antioxidant activity and γ -aminobutyric acid (GABA) content in sea tangle fermented by *Lactobacillus brevis* BJ20 isolated from traditional fermented foods. *Food Chemistry*, *122*, 271–276. <https://doi.org/10.1016/j.foodchem.2010.02.071>
- Lin, M. T., & Beal, M. F. (2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature*, *443*, 787–795. <https://doi.org/10.1038/nature05292>
- Luo, X. N., Zhang, J. L., Li, D., Xin, Y. H., Xin, D., & Fan, L. (2014). *Planomicrobium soli* sp. nov., isolated from soil. *International Journal of Systematic and Evolutionary Microbiology*, *64*, 2700–2705. <https://doi.org/10.1099/ijms.0.055426-0>
- Meier-Kolthoff, J. P., Auch, A. F., Klenk, H. P., & Goker, M. (2013). Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics*, *14*, 60. <https://doi.org/10.1186/1471-2105-14-60>
- Migula, W. (1895). *Über ein neues System der Bakterien*.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A., & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *Journal of Microbiological Methods*, *2*, 233–241. [https://doi.org/10.1016/0167-7012\(84\)90018-6](https://doi.org/10.1016/0167-7012(84)90018-6)
- Mykytczuk, N. C. S., Wilhelm, R. C., & Whyte, L. G. (2012). *Planococcus halocryophilus* sp. nov., an extreme sub-zero species from high Arctic permafrost. *International Journal of Systematic and Evolutionary Microbiology*, *62*, 1937–1944. <https://doi.org/10.1099/ijms.0.035782-0>
- Novitsky, T. J., & Kushner, D. J. (1976). *Planococcus halophilus* sp. nov., a Facultatively Halophilic coccus. *International Journal of Systematic Bacteriology*, *26*, 53–57. <https://doi.org/10.1099/00207713-26-1-53>
- Reddy, G. S. N., Prakash, J. S. S., Vairamani, M., Prabhakar, S., Matsumoto, G. I., & Shivaji, S. (2002). *Planococcus antarcticus* and *Planococcus psychrophilus* spp. nov isolated from cyanobacterial mat samples collected from ponds in Antarctica. *Extremophiles*, *6*, 253–261. <https://doi.org/10.1007/s00792-001-0250-7>
- Richter, M., Rossello-Mora, R., Glockner, F. O., & Peplies, J. (2016). JSpeciesWS: A web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics*, *32*, 929–931. <https://doi.org/10.1093/bioinformatics/btv681>
- Romano, I., Giordano, A., Lama, L., Nicolaus, B., & Gambacorta, A. (2003). *Planococcus rifietensis* sp. nov., isolated from algal mat collected from a sulfurous spring in Campania (Italy). *Systematic and Applied Microbiology*, *26*, 357–366. <https://doi.org/10.1078/072320203322497383>
- Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, *4*, 406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*.
- Schleifer, K. H., & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriological Reviews*, *36*, 407–477.
- Shindo, K., & Misawa, N. (2014). New and rare carotenoids isolated from marine bacteria and their antioxidant activities. *Marine Drugs*, *12*, 1690–1698. <https://doi.org/10.3390/md12031690>
- Slade, D., & Radman, M. (2011). Oxidative stress resistance in *Deinococcus radiodurans*. *Microbiology Molecular Biology Review*, *75*(1), 133–191. <https://doi.org/10.1128/MMBR.00015-10>
- Suresh, K., Mayilraj, S., Bhattacharya, A., & Chakrabarti, T. (2007). *Planococcus columbae* sp. nov., isolated from pigeon faeces. *International Journal of Systematic and Evolutionary Microbiology*, *57*, 1266–1271. <https://doi.org/10.1099/ijms.0.64742-0>
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, *28*(10), 2731–2739. <https://doi.org/10.1093/molbev/msr121>
- Velho-Pereira, S., Parvatkar, P., & Furtado, I. J. (2015). Evaluation of antioxidant producing potential of halophilic bacterial bionts from marine invertebrates. *Indian Journal of Pharmaceutical Sciences*, *77*, 183–189.
- Wang, W. P., Wang, L. P., & Shao, Z. Z. (2010). Diversity and abundance of oil-degrading bacteria and alkane hydroxylase (alkB) genes in the subtropical seawater of Xiamen Island. *Microbial Ecology*, *60*(2), 429–439. <https://doi.org/10.1007/s00248-010-9724-4>
- Wang, X., Wang, Z., Zhao, X. Q., Huang, X., Zhou, Y., & Li, W. J. (2017). *Planococcus ruber* sp. nov., isolated from a polluted farmland soil sample. *International Journal of Systematic and Evolutionary Microbiology*, *67*, 2549–2554. <https://doi.org/10.1099/ijsem.0.001960>
- Welinder, K. G. (1992). Superfamily of plant, fungal and bacterial peroxidases. *Current Opinion in Structural Biology*, *2*(3), 388–393. [https://doi.org/10.1016/0959-440X\(92\)90230-5](https://doi.org/10.1016/0959-440X(92)90230-5)
- Yoon, J. H., Kang, S. J., Lee, S. Y., Oh, K. H., & Oh, T. K. (2010). *Planococcus salinarum* sp. nov., isolated from a marine solar saltern, and emended description of the genus *Planococcus*. *International Journal of Systematic and Evolutionary Microbiology*, *60*, 754–758. <https://doi.org/10.1099/ijms.0.013136-0>
- Yoon, J. H., Lee, K. C., Kang, S. S., Kho, Y. H., Lee, E. S., Park, Y. H., & Kang, K. H. (2001). *Planomicrobium koreense* gen. nov., sp. nov., a bacterium isolated from the Korean traditional fermented seafood jeotgal, and transfer of *Planococcus okeanokoites* (Nakagawa et al 1996) and *Planococcus mcmeekii* (Junge et al 1998) to the genus *Planomicrobium*. *International Journal of Systematic and Evolutionary Microbiology*, *51*, 1511–1520. <https://doi.org/10.1099/00207713-51-4-1511>
- Yoon, J. H., Weiss, N., Kang, K. H., Oh, T. K., & Park, Y. H. (2003). *Planococcus maritimus* sp. nov., isolated from sea water of a tidal flat in Korea. *International Journal of Systematic and Evolutionary Microbiology*, *53*, 2013–2017. <https://doi.org/10.1099/ijms.0.02557-0>
- Zhang, B., Tang, S., Chen, X., Zhang, G., Zhang, W., Chen, T., ... Dyson, P. (2018). *Streptomyces qaidamensis* sp. nov., isolated from sand in the Qaidam Basin, China. *The Journal of Antibiotics*, *70*, 880–886. <https://doi.org/10.1038/s41429-018-0080-9>

- Zhang, B., Tang, S., Chen, X., Zhang, L., Zhang, G., Zhang, W., ... Dyson, P. (2016a). *Streptomyces lacrimifluminis* sp. nov., a novel actinobacterium that produces antibacterial compounds, isolated from soil. *International Journal of Systematic and Evolutionary Microbiology*, 66, 4981–4986. <https://doi.org/10.1099/ijsem.0.001456>
- Zhang, B., Tang, S., Yang, R., Chen, X., Zhang, D., Zhang, W., ... Dyson, P. (2019). *Streptomyces dangxiongensis* sp. nov., isolated from soil of Qinghai-Tibet. *Plateau. International Journal of Systematic and Evolutionary Microbiology*, 69(9), 2729–2734. <https://doi.org/10.1099/ijsem.0.003550>
- Zhang, B., Wu, X., Zhang, G., Li, S., Zhang, W., Chen, X., ... Chen, T. (2016b). The diversity and biogeography of the communities of Actinobacteria in the forelands of glaciers at a continental

scale. *Environmental Research Letters*, 11, 054012. <https://doi.org/10.1088/1748-9326/11/5/054012>

How to cite this article: Zhang B, Yang R, Zhang G, et al. Characteristics of *Planococcus antioxidans* sp. nov., an antioxidant-producing strain isolated from the desert soil in the Qinghai-Tibetan Plateau. *MicrobiologyOpen*. 2020;9:e1028. <https://doi.org/10.1002/mbo3.1028>

APPENDIX A

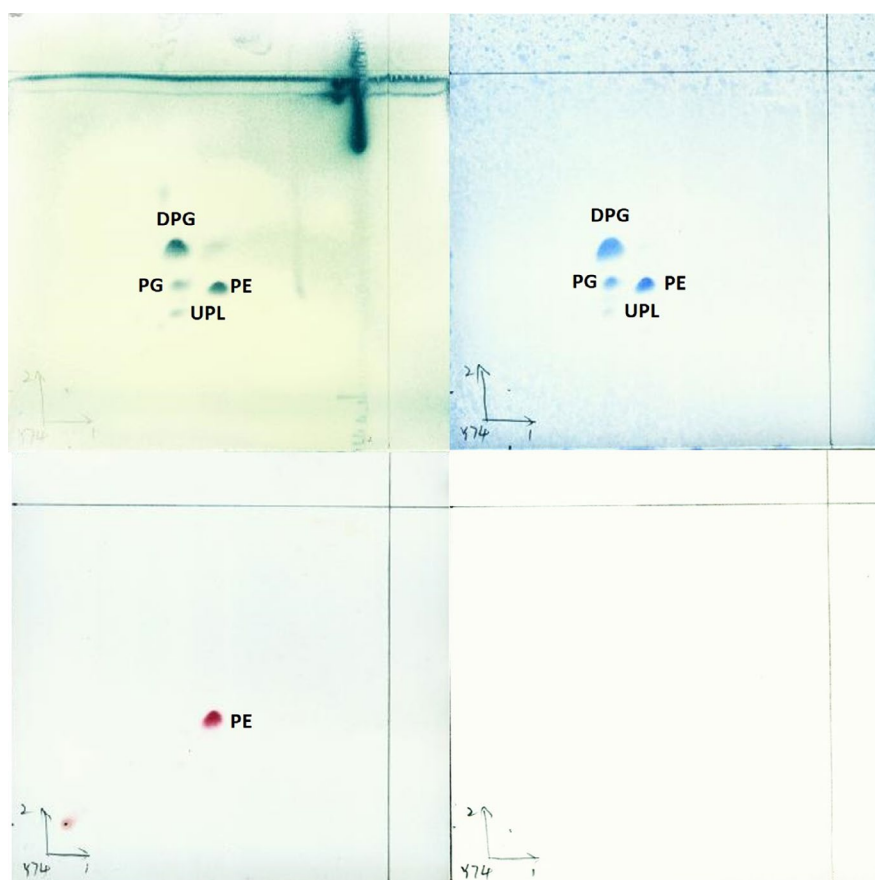


FIGURE A1 Polar lipid profiles of strain Y74^T separated by two-dimensional thin-layer chromatography and detected with molybdato-phosphoric acid (a), molybdenum blue (b), ninhydrin (c) and alpha-naphthol (d). The solvent systems used were as following: Direction 1 was chloroform/methyl alcohol/H₂O (65/25/4, by vol.), Direction 2 was chloroform/ acetic acid/methyl alcohol/H₂O (80/15/12/4, by vol.). Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; UPL, unidentified phospholipid

TABLE A1 Antioxidant related genes of strain Y74^T

Gene	Protein	Length	DNA sequence
cds_RLQ92685	DNA-protecting protein <i>DprA</i>	906	ATGGATTCATTGTTTGAACAAAGACTGATGGCATTGCATTATGTATACCCTCAACCACTCAACCGCA TAAAACGGCTGATGATTGACGATTCGAATCTTGAACATTTGGAAGCAGGCCAGCCTGGGAAATC AGCCAATTACTCGGCATAAAGCCGAAGCCGATATCACTGAAGATGCTTATAGAAAATCACT GAACAACCCCTATTCTGAACTTATGAAAAACATAAGATAATCCCCATATCTTATAACCATCCCAAT TATCCACAAAGTCTATTTCACTGTATCGACCCACCTGTAATCTTTACGCCAAAGGAAAAATAGAG TACTTGCTGAATGAAGATCGGATAGCTGTGATAGGTGCCCGTAAGGCTTCTGTTTATTCACAGAAA GCTATGGATCTTATACTTCTGTATCTTGTGACGCGGGCTTTATCGTGGTAAGCGGCTTGGCAAAA GGGGCAGATGCAATGGCTCACCGGACAGCAATCGATTGCGGCGGCAAAACGATTGCTGTTACCC GCAGCGGCTTTTGCATCCGTATCCGAAAGAGAATGATGAATGAATATTATAAGAAAGAACTC AACTCGCAATTACAGAATATCCGCCATATATGCAGCCGAAACGCTGGAATTTCCCTATGCGGAACC GCATTATAAGCGGCTTGGCAAAAGGGGTACTGGTAACGGAAGCGGAAGTGAAGCGGCACGCT CAGCAGATTGAACATGCCCTGGAACACGGCAAGGATATTTTGCGGTACCAGGGAGTATCTGTTC ACCTCTGTGACGCGGGCCGATAAACTGATTTTTGAAGGTGCAAAACCGGCTGGAATGGGCTTC AAGTGTGGAGGAATACCGTGAAATTAGGGCTTTAAATAAGTCGATAAAATGA
cds_RLQ92091	Superoxide dismutase	609	ATGGCTTATGAATTACCGAACTACCTTACGCGTATGACGCACTGGAACCACACATCGACAAAGAG ACGATGAATATCCACCACAAAAACCCATAATACTTACGTAACCAATGTTAACGCTGCCCTGGAA GGCCACGAAGATCTTCTTCAAAATCTGTAGAAGAACTGATTTCTGACTTGAACGCTGTGCCTGA AGATATTCGTACAGCTGTACGCAATAACGGCGGTGGACACGCAAAACCACTATTATTCGGCAAT ATTGACTCCAAACGGCACTGGCGCTCCATCAGGTGCACTTGCAGGAAGCAATCGACAGCAAGTTC GGCAGCTTTGACGAATTCAAAACGAAATTCGAAGCAGCCGGTAAAACACGCTTCGGTTCAGGC TGGCTTGGCTTGTGTATCTAATGGTGAATTGAAGTAACTTCAACTGCCAACAGGATTCTCCA TTGATGGACGGCAAAACGCCAATCTTGGAGTAGACGTTTGGGAGCATGCTTACTACTTGAAATA CCAGAACAAACGCCCTGACTATTTGGCTGCTTTCTGGAACGTAGTAACTGGGACGGAAGTTTC AAAACGCTATGAAGCTGCAAAATAA
cds_RLQ91554	Glutathione peroxidase	477	ATGAGTATTTATGAATTTTCGGCCAGAAAGTCCGATGGCAGCATTATCCGTTAAGTGAATACGAA GGGAAAACGATGTTGATTGTCAACTACTGTACGAAATGCGGGTTGCGTGATCAATTCGATGGACT GGAGAAGTTGTACCAGAAGTATGAAGATGACGACTTGTGCTCCTTGGCTTTCCCTCCGATCAGT TCGGCAGGAACCTTGATGGGGCGAAGAAGCGGAGGAATCCTGCCGATGACTTACGGAGTTT CGTCCCAATGCATGACCTGGTCAAAGTAAACGGAAAAAATGCCGATCCTTTTTAAATATCTAA CTGAAAACAGCAAGGGAGTCTTGGCAGCAGCATCAAGTGAATTTACAAAAATTTCTCATCAAC AAAGAGGGAAAGCTGTTGACAGGTTTTCGCCAAAAGATAAACCTGAAAAATTTGAAGAAGAA ATTAACAATACTTAACGAAGTGA
cds_RLQ90872	Catalase family peroxidase	927	ATGGCGAAGGAAAAGCTTGCAGAAACCGCAGTCAATAAAATCGAAAAGTGTTTGGGGAACATA AACTTATAGACGTGCGCATCAAGAGGAACGGGATATGAAGCCCTATTACAGCAAAACGGCGA AGGGCAGAATTTGACCGTTCGCGCATCTCCGGGAAGGGACGACCAAAAGCTGTGGTGCATT TTCCGACAGTTCTCCAGATCCATTTTGGACGGATAATTTATACCCGGTAAAAGGAATGGCTGTGC AATTCCAGTTGCCGGATGGCCAAGTATGATGAACAGTGTGCGGTAACCTCCCGATATCTTTTC GCGCACTCCGGAAGTGTTTACGGAATGCTGGATATCGCGAAATCGTTTAAAAAGGGCAAGCC CCGGCTCGGGATCTCATCAATTTGTTATCAAAATATCCCGAAAGCCGAGCAGCAATCCGCATC ATCCGAAAATGCAGAGTCCGGCTAGTTTCGCGACCCGGTCTTACCATTCCATTACGCATTTTA CCTGTTAACGGTACTGGCAGCGGTACCCGTAATTCAGTGGCATCCGGAAGCGGGCGT GGAGTCGTTGAATCCGGTGGAGGCTGCGTCACTGAAAAAAGGAGATTTGAGGAAGAGTTG AAGAACGCGTCTTGAGCGGAGAGACGGCTTTCCGTCTGATGGCAGTATTGGGGATGCGGAT GACCCTGTAGATGACCCGACGAAAGACTGGGCTAAAGATAGAAAAGAAAGTGAATTTGGGGCG CCTGGTGTGAAAGAACAGACTGACGAAGCGGAAGGGTGTGTATGATCCAACCATCCTGG CAGAAGGCGTGAATGACGAGATGATCCATCCTGCATTTCCGCAACCCGGCATATGCTATT TCTTATATGCGGAGAGAAGGGGAGAAGCAAAAAGAAAGTTGA
cds_RLQ90525	Heme-dependent peroxidase	750	ATGAATGAAGCAGCAATCACTTTAGACGGCTGGTACGCTCCACGATTTCCGTTTCGATGGA CTGGGTATCATGAAAAATGCTTGAAGACGAAGAACGCCAATTCGCAATCGCAATATCAGGC ATTCATGGACAAAAGTAAACAGGCCGATGAAAATAAAACCGGTGCACACGATTTGATTCAAATTA TTGGCCAAAAGCTGACTTGTATGCTGATGCTATTGCGCGAAACTATGGACGAATTCGCTGAACTT GAAACGGAATACAATAAGCTGACATTTGGTCGCTTACACGGTTCGACTTACTCTTACGTATCTGTA GTGGAATTTCCAATATCTTGCAGGTAATCAGAAGAAGATCCATACCAGAACCCGCATGTCCG CGCGCTCTGTATCCGGAGCTTACGCTTTCGAGTACATCTGCTTACCCGATGGACAAGCGCCG CGACGGCAACGACAACTGGTACATGCTGCCGATGGACGAGCGCAAAGATTTGATGCTGTACACG GCAAAATCGGCCGAGCTACGCAAGCAAGTAAACAGATCATTTCCGGCTCTGTCGGCTTTGAT GATTACGAATGGGGCTAACCTTGTTCGAGATGACGTTTTCGAGTTTAAAAAAGTATGCTATG AAATGCGTTTTGACGAAGTACGCGCGGTTACGCTGAATTCGGTTCGTTTACGTCGGCACTCG CCTTGATAAAGAAAGAAATCGTTAAGTTTTTGAAGTTTAA

(Continues)

TABLE A1 (Continued)

Gene	Protein	Length	DNA sequence
cds_RLQ82443	Thioredoxin-dependent thiol peroxidase	477	TTGACAACATTAGAAGGTTTGCATGCACCGGATTTACATTGAAAAATGAAAACGGCGAAACAGT TTCTTTGGAGGATTTTGCCGGCAAAAAATACGTAGTGCTTTATTTTACCCGAAAGATATGACA CCGGGCTGCACTACACAGGCTGCGATTTCCGGGATGCAGAGAAGGATTTTCCGAATTGGGA GCAGTCATTCTTGGCGTTAGCGCAGACTCTGAAAAACAGCACAGTAAATTTATCAGCAAACACGG TTTGCCATTCTTTATTGGTTGACGAAGATCATAAAGTTTCTGAGGCATACGGTGTGTGGGTGG AGAAGAAGATGTACGGAAAAGAATTTATGGGGATTGAACGCTCTACATTTTAAATCGACCCAACC GGGACTGTCGTAAAAGAATGGCGAAAAGTCAAAGTGAAGACCATATCCAGGAAGTCCTTG AAACGGTCAAAGAGCTCAGCCAAGCATAG
cds_RLQ81587	Glutathione peroxidase	477	ATGTTTTCGGTTTATGATTACAAAGTTAAAAATTTGCAGGGAGAAATGGAATCACTTGAAAAGTTAAAGG GAATGCGCTGGTAATAGTCAATACAGCAAGCAAATGTGGATTGACTCCTCAATTTGAAGACCTTCAA AACTCTATGAAAAGTATCCAGTAAAGATTTCAAATCCTCGGTTTTCCAAGTTCTCAATTTAATAATCAG GAATTTGAAAATCAGGAAGAAACGATGGAATTCGCCAGATGAATTATGGTGAACATTTCCATGTTTG CAAAAACAGATGTCAAAGGAGCAGATGCAGCTCCACTATTTACCTATCTGACTTCAAAGCATGAAAATCT AGAGGCTGAAGAAATTGCATGGAACTTTGCGAAGTTTTAATAGATAAAGAAGGACATGCATTAATA GATATCCCCCTCGATCTTCGCCACTGGAATTTGAAGAAGACCTGAAGACTATTCTATAA
cds_RLQ91872	Thiol peroxidase	507	ATGGTACAAGTTACATTTTCATGAAAATCCTGTTACCTTACCAAACAAGAAGTCAAAGTTGGAGACC AAATCCCGAATTTACAGTACTTGACAATGACTTGAACCCTGTAACCTGCACAGGATACAGCAGGTAA AGTCAGATTATTCACAGTGTACCATCCTTGGATACAAGTGTGTTGTTTCAGATCAGGCGAAACGTTTCAG CGAAGAAGCTTCATCAATGGGAGACGAAGTTGCAATCTACTATGTAACCTGCGATTTGCCTTTCCGCAC AAAAGCGTTGGGTTGAAGTTAATGAAGCCAAAAATCTCACTACTCTTTCCGATCACCCTGATCTTTCTT TCGGTGAAGCGTTCCGAGTAACGATGCAGGAACCTGCGTTTGCTGGCCCGTTCCATATTTGTAGTGGAT GAAAACGATAAAGTGACTTATGTGGAATATGTTCTGAGGGAACGAATCATCCGAATTACGATAAA CGGATTGAAGCAGTAAAAGAACTGACAAAATAA

TABLE A2 Characteristics of the genomic islands found in the genome of strain Y74^T

GI	Length	Total no. of gene	hypothetical proteins	Integrase/ phage	Predicted function
1	4,681	6	2	0	Metabolism/ Transcription factors
2	6,367	8	2	0	
3	7,602	6	3	0	General function prediction only/ Metabolism
4	4,374	3	2	0	Metabolism
5	5,080	6	4	0	
6	6,976	5	3	0	Replication and repair
7	21,818	19	7	1	Genetic information processing /Transcription factors/Cell processing/ Nucleotide metabolism
8	12,222	10	6	1	Transcription factors/DNA repair
9	14,623	20	14	0	Secretion system/DNA repair
10	11,232	14	11	0	
11	16,787	17	4	2	Transporters/Carbohydrate metabolism/ Transferases/ Two-component system
12	42,958	29	4	2	Two-component system/ Metabolism/Transcription factors
13	10,266	7	1	0	Metabolism/DNA repair
14	4,777	7	1	0	
15	4,674	5	3	0	
16	11,402	9	4	0	
17	9,706	11	4	1	Metabolism/Transcription factors/ Transferases /Signal transduction
18	53,470	60	25	0	
19	4,656	6	0	0	Metabolism/Membrane transport
20	75,201	74	30	2	Secretion system/Metabolism/Transcription factors/ Membrane transport
21	11,374	13	10	1	Genetic information processing
22	13,041	16	6	1	Metabolism/Transcription factors
23	5,465	9	4	0	Transcription factors/Genetic information processing
24	5,113	6	1	0	Transcription factors/Transferases
25	6,724	10	3	0	
26	4,021	6	3	0	
27	6,818	8	4	2	Transcription factors
28	10,911	5	1	3	
Total	392,339	395	162	16	