Screening of *Bacillus* Strains from Sun Vinegar for Efficient Production of Flavonoid and Phenol

Changqing Zhao¹ · Xingxiu Zhao¹ · Jing Zhang¹ · Wei Zou¹ · Yi Zhang¹ · Li Li¹ · Jun Liu¹

Received: 22 November 2015 / Accepted: 20 May 2016 / Published online: 27 May 2016
© Association of Microbiologists of India 2016

Abstract The aim of this study was to screen out a *bacillus* from Sichuan sun vinegar grains capable of producing a high total amount of flavonoids and total phenols. Forty-eight strains were initially screened out from Sichuan sun vinegar grains. The sodium nitrite-aluminum nitrate-sodium hydroxide colorimetry method was used to determine the total flavonoids, and the Folin-phenol method was used to determine the total phenols. Based on the results of total flavonoids and total phenol producing capacities of the strains, one strain was singled out for use in this study. This strain, which was named YB-19, is capable of producing a total amount of 96.55 µg/mL flavonoids and 86.69 µg/mL phenols. YB-19 was identified by its strain morphology, physiological and biochemical characteristics. DNA sequencing was performed on YB-19. The results of the DNA sequencing showed that the sequence similarity between strain YB-19 and *Bacillus toyonensis* strain BCT-7112 was 99 %, and the two strains were housed on the same branch in the phylogenetic tree. Combined with the results of the strain morphology and the observed physiological and biochemical characteristics, YB-19 was identified as *B. toyonensis*. The screened strain YB-19 can be applied to the traditional brewing process of sun vinegar to improve vinegar quality and to enrich the microbial resources for vinegar fermentation.

Keywords Sun vinegar · Flavonoids · Phenolics · *Bacillus*

Introduction

Traditional vinegar has a long history that dates back several thousand years. The most well-known traditional vinegars include balsamic vinegar from Italy, rice vinegar from Japan, and wine vinegar from Spain, etc. [1]. Similarly, in China, the vinegar industry has a very long history, and the brewing process is considered to be the quintessence of ancient human wisdom. South Sichuan sun vinegar, one of China's four most famous vinegars, uses bran and rice as the main raw materials, supplemented with a variety of herbal medicines such as the drug koji. The ingredients undergo a process of cooking, fermentation, and aging in the sun. The resulting vinegar is sour, mellow, sweet, and refreshing with a long aftertaste and high preservability. This vinegar has also been shown to enhance digestion, improve physical strength, boost immune function, lower blood pressure, promote blood production. It also has known anti-aging effects. As a disease preventing and health food seasoning, it is very popular [2].

In the brewing process of south Sichuan sun vinegar, microbial metabolism has a great impact on the characteristics of the fermented product. It has been reported that *Bacillus* is widely distributed during the acetic acid fermentation stage, and when combined with the bacteria in the acetic acid, it promotes the production of the vinegar's active substances [3–5]. It has also been reported that large amounts of flavonoids and polyphenols are present in vinegar, which are both known as important factors for functional health [3–5]. In the researchers' previous work, preliminary results were obtained in the study of functional

Electronic supplementary material The online version of this article (doi:10.1007/s12088-016-0602-8) contains supplementary material, which is available to authorized users.

✉ Changqing Zhao
zhaocq2010@163.com

¹ College of Bioengineering, Sichuan University of Science and Engineering, Zigong 643000, China

bacteria in vinegar. *Bacillus amyloliquefaciens* was screened out from Sichuan bran vinegar grains, which can produce a high amount of total flavonoids and total phenols [3]. After optimization of the fermentation medium, it was found that the total flavonoid yield could reach 229.8 µg/mL and the total phenol yield could reach 241.9 µg/mL [4]. The fermentation characteristics of the strain were also studied, and it was found that under optimal conditions, the total phenol yield could reach 263.3 µg/mL and the total flavonoid yield could reach 293.2 µg/mL [5].

Thus far, there has been no report on the functional bacteria found in sun vinegar. It is therefore important to undertake screening for bacillus south Sichuan sun vinegar grains, which can promote the production of functional health factors such as flavonoids and phenols.

In this study, the bacilli were isolated from the sun vinegar grains and then further cultured. The bacillus, which can produce high total flavonoids and total phenols in the fermentation medium, was screened out according to the total flavonoids and total phenol producing capacity.

Materials and Methods

Media Preparation

Enrichment medium: 3 g soluble starch, 10 g peptone, 3 g yeast extract, 1.5 g potassium dihydrogen phosphate, 2 g dipotassium phosphate, 0.1 g magnesium sulfate, 1L ddH₂O, pH 7.2, autoclaved at 121 °C for 20 min [4].

Nutrient agar: 10 g peptone, 3 g beef extract, 5 g sodium chloride, 15–20 g agar, 1L ddH₂O, pH 7.2, autoclaved at 121 °C for 20 min [4].

Fermentation medium: 1 g peptone, 1 g glucose, 5 g soybean meal, 3 g bran, 0.05 g ammonium sulfate, 0.01 g manganese sulfate, 0.10 g magnesium sulfate, 0.05 g dipotassium phosphate, 1L ddH₂O, pH 7.2, autoclaved at 121 °C for 20 min [5].

LB medium: 10 g tryptone, 5 g yeast extract, 10 g sodium chloride, pH 7.2, autoclaved at 121 °C for 20 min [5].

Initial Screening for *Bacillus*

A sample of 4 g sun vinegar grains was added into 100 mL ddH₂O and shaken for 5 min. A filtrate of 2 mL was transferred into 100 mL enrichment medium and cultured in a 37 °C incubator at 150 r/min for 24 h. At the end of the enrichment, the culture was heated in a 90 °C water bath for 5 min to kill all cells and enrich the endospores. The enriched endospores were diluted in individual samples into twenty gradients, ranging from 10⁻¹ to 10⁻²⁰. After dilution, nutrient agar plates were coated with the different

concentrations of endospores, inverted, and cultured in a 37 °C incubator for 24 h. Single colonies with different morphologies were selected from the plates and examined microscopically after being lawn grown. These single colonies were streaked onto new nutrient agar plates and cultured in a 37 °C incubator, and cell growth was monitored every 6 h. Upon observation of each isolated colony, the observed colony was inoculated to a nutrient agar slant tube, numbered, and then stored in a 4 °C refrigerator.

The Fermentation of *Bacillus*

Two loops of the *Bacillus* stored in each slant tube after initial screening were inoculated into a 100 fermentation medium with an initial number of colonies of 1–3 × 10⁷ CFU/mL and cultured in a 37 °C incubator at 150 r/min for 24 h. Then, the total flavonoids and total phenolic contents within the medium were determined. Every trial in the experiments was repeated three times.

Determination of Total Flavonoids in Fermentation Medium

In this study, the sodium nitrite-aluminum nitrate-sodium hydroxide colorimetry method was used to determine the total flavonoid content of the samples. In this method, the 3-hydroxy, 4-hydroxy, 5-hydroxy, 4-carbonyl or o-positions of phenolic and hydroxyl groups in flavonoids may have a complex reaction with the aluminum salt to form a red complex under alkaline conditions.

Plot of Rutin Standard Curve

A sample of 0.010 g rutin standard with constant weight was dissolved in 100 mL 95 % ethanol at 105 °C to form the 0.10 mg/mL rutin standard solution. A series of rutin standard solutions of 0.00, 0.20, 0.40, 0.60, 0.80, 1.00, 1.20, 1.40, and 1.60 mL were transferred into 10 mL colorimetric tubes and numbered. Then, 95 % ethanol was added to a total volume of 2 mL. The samples were then shaken well. Then, 0.5 mL 4 % NaNO₂ was added into each tube and they were left to stand still for 6 min. Then, 0.5 mL 10 % Al(NO₃)₃ was added, and the samples were left to stand still for another 6 min. Afterwards, 3 mL 4 % NaOH was added to each tube, and the samples were again left to stand still for 10 min. Finally, the absorbance values were measured at 510 nm.

Determination of Total Flavonoids

A sample of 20 mL fermentation medium was transferred into a round-bottomed flask, then 20 mL 95 % ethanol was added and left to sit in a 65 °C constant temperature water bath for 2 h for reflux extraction. A sample of 2 mL reflux

extract was transferred into a 10 mL colorimetric tube, and 0.5 mL 4 % NaNO_2 was added and left to stand still for 6 min. Then, 0.5 mL 10 % $\text{Al}(\text{NO}_3)_3$ was added, and the sample stood still for another 6 min. Afterwards, 3 mL 4 % NaOH was added and left to stand still for 10 min. Finally, the absorbance was measured at 510 nm [4].

Determination of Total Phenols in Fermentation Medium

In this study, the Folin-phenol method was used to determine the total phenol content. In an alkaline solution, the Folin-phenol reagent can oxidize polyphenols quantitatively, while the Folin-phenol itself is reduced to a blue compound. The degree of color change is proportional to the number of phenol groups.

Plot of Gallic Acid Standard Curve

A sample of 0.010 g gallic acid standard with constant weight was dissolved in 100 mL ultra-pure water at 105 °C to form the 0.10 mg/mL gallic acid standard solution. A series of gallic acid standard solutions of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 0.9 mL were transferred into 10 mL colorimetric tubes and numbered. Then, ultra-pure water was added to each tube until a total volume of 1 mL was reached. Further, 2 mL Folin-phenol reagent was added into each tube and left to stand still for 3 min. Then, 2 mL 10 % Na_2CO_3 was added, and the sample stood still for 1 h. Finally, the absorbance values were measured at 700 nm.

Determination of Total Phenols

A sample of 4 mL fermentation medium was transferred into a 5 mL centrifuge tube and centrifuged at 8000 r/min for 10 min. Then, 1 mL supernatant was transferred into a colorimetric tube, 2 mL Folin-phenol reagent was added, and it was left to stand still for 3 min. Then, 2 mL 10 % Na_2CO_3 was added, and the sample stood still for 1 h. The absorbance values were measured at 700 nm [4, 6].

Morphological Observation

The screened strain was examined for colony morphology, cell morphology, Gram staining and spore staining.

Physiological and Biochemical Characteristics of the Strain

According to the flavonoids and phenol producing capacity, the target strain was physiologically and biochemically

tested. Tests performed included the methyl red test, Voges–Proskauer (V–P) test, indole test, citrate utilization, salt tolerance test, starch hydrolysis, catalase test, and carbohydrate fermentation test [7].

DNA Extraction and Sequencing of Target Strain

The principle of the cetyl trimethyl ammonium bromide (CTAB) method is that CTAB can form a complex with proteins and polysaccharides in high ionic strength solution, but cannot precipitate nucleic acids. Combined with the enzymatic method and the SDS method, the CTAB method was used to extract DNA and remove proteins, polysaccharides, phenols, etc., using an organic solvent. Finally, isopropanol was used to isolate nucleic acids.

The procedure was as follows: two loops of strain were inoculated into LB medium and cultured in a 37 °C shaker at 150 r/min for 12 h. Afterwards, the cells were collected in 2 mL centrifuge tubes, and 500 μL CTAB solution and 50 μL lysozyme (100 mg/mL) were added. The resulting sample was cultured overnight in a 37 °C shaker at 225 r/min. Then, 250 μL 20 % SDS and 25 μL protease (20 mg/mL) were added into the tube and kept in a 65 °C water bath for 1 h. During this bath, the sample was shaken and mixed well once every 15 min. After the water bath, the tube was frozen in a –80 °C freezer for 15 min, then kept in a 65 °C water bath for 15 min. After the frozen-water bath was repeated three times, an equal volume of tris phenol–chloroform–isoamyl alcohol (25:24:1) was added and left to stand still for 10 min after vortex. The tube was then centrifuged at 12,000 r/min for 15 min, and the supernatant was collected. This process was repeated until no white precipitate appeared. Then, an equal volume of chloroform–isoamyl alcohol (24:1) was added and left to stand still for 10 min after vortex. The tube was then centrifuged at 12,000 r/min for 15 min. 0.6 volume of pre-cooled isopropanol was subsequently added to the supernatant and kept in a –20 °C freezer for 1 h to allow for DNA precipitation. The tube was then centrifuged at 12,000 r/min for 15 min to collect the precipitant. The DNA was rinsed with 1 mL ethanol (70 %) and centrifuged again at 4 °C, 12,000 r/min for 15 min and this process was repeated once. Finally, after it was completely dried on a clean bench, 50 μL of sterile distilled water was added into the tube and kept in a 65 °C water bath for 1 h. The extracted DNA was sent to Shanghai JieLi Biotechnology Co., Ltd. for sequencing. The sequencing result was queried in the NCBI database (<http://www.ncbi.nlm.nih.gov/>). The MEGA6.0 (neighbor-joining method) was used to construct the 15S rRNA phylogenetic tree [8].

Results and Discussion

Initial Screening for *Bacillus* from Vinegar Grains

In total, 48 *Bacillus* strains were obtained from the initial screening. These were numbered 1–48.

Determination of Total Flavonoids in Fermentation Medium

The total flavonoids ($\mu\text{g/mL}$) within the medium for each strain after 24 h fermentation at 37 °C, 120 r/min, according to the rutin standard curve, are shown in Table 1. As Table 1 indicates, strain 19, 21, 30, and 48 produced relatively high total flavonoids, with values of 96.55, 87.33, 85.32, and 92.78 $\mu\text{g/mL}$, respectively. Among these, strain 19 had the highest value (96.55 $\mu\text{g/mL}$). In previous work by the researchers, the fermentation medium and parameters were optimized, and the *B. amyloliquefaciens* was obtained from bran vinegar grains, which can produce total flavonoids at levels up to 293.2 $\mu\text{g/mL}$ [4, 5]. The total amount of flavonoids produced by strain 19 in the present study was significantly lower than the results found with *B.*

amyloliquefaciens. However, it is believed that the ability of strain 19 to produce flavonoids may be enhanced after future optimization of the fermentation medium and parameters.

Determination of Total Phenols in Fermentation Medium

The total phenols ($\mu\text{g/mL}$) within the medium for each strain after 24 h fermentation at 37 °C, 120 r/min, according to the gallic acid standard curve, are shown in Table 2. As shown in Table 2, strains 19, 21, 22, and 39 produced relatively high total phenols, with values of 86.69, 73.65, 75.83, and 85.45 $\mu\text{g/mL}$, respectively. Among these, strain 19 had the highest value and strain 39 had the second highest value. Because strain 39 had a relatively lower total flavonoid yield (57.64 $\mu\text{g/mL}$) than strain 19, it was not considered as a target strain.

The *B. amyloliquefaciens* obtained from previous optimization experiments is capable of producing total phenols in quantities as high as 263.3 $\mu\text{g/mL}$, but it only yielded 49.7 $\mu\text{g/mL}$ total phenols before optimization [3–5]. The total amount of phenols produced by strain 19 in this study

Table 1 The total flavonoids for each strain after 24 h fermentation

Strain (#)	Total flavonoids ($\mu\text{g/mL}$)	Strain (#)	Total flavonoids ($\mu\text{g/mL}$)
1	21.43	25	58.22
2	25.34	26	64.38
3	27.56	27	45.56
4	23.47	28	44.36
5	28.51	29	39.69
6	21.45	30	85.32
7	28.34	31	63.26
8	24.12	32	23.36
9	23.11	33	37.88
10	27.40	34	48.56
11	26.34	35	42.97
12	30.25	36	29.34
13	21.66	37	57.85
14	25.49	38	61.93
15	26.73	39	57.64
16	28.55	40	38.54
17	25.26	41	67.35
18	53.75	42	37.62
19	96.55	43	36.44
20	31.67	44	27.58
21	87.33	45	30.25
22	26.57	46	69.11
23	38.59	47	38.52
24	46.76	48	92.78

Table 2 The total phenols for each strain after 24 h fermentation

Strain (#)	Total phenols ($\mu\text{g/mL}$)	Strain (#)	Total phenols ($\mu\text{g/mL}$)
1	42.37	25	56.99
2	46.18	26	67.34
3	52.22	27	43.66
4	47.72	28	48.91
5	51.28	29	51.23
6	43.15	30	37.50
7	53.17	31	35.62
8	44.68	32	63.89
9	47.15	33	51.68
10	47.31	34	59.37
11	43.38	35	47.55
12	53.73	36	41.85
13	44.65	37	57.50
14	45.51	38	49.28
15	42.67	39	85.45
16	50.63	40	62.67
17	38.48	41	66.34
18	49.87	42	55.20
19	86.69	43	42.88
20	62.33	44	52.56
21	73.65	45	47.22
22	75.83	46	50.30
23	38.26	47	46.31
24	53.82	48	56.37

was even higher than those produced by the *B. amyloliquefaciens* before optimization. Therefore, this *Bacillus* strain is valuable for future research.

According to the total flavonoids and total phenol producing capacities of the 48 strains from the initial screening, the strain that produced the most total flavonoids and total phenols was selected as the target strain. This strain was named YB-19.

Colony Morphology Result

The colony of strain YB-19 in the nutrient agar medium had a large colony with a milky white color, and an opaque, rough surface with no remarkable smell (Supplementary Fig. 1).

Gram Staining and Spores Staining Results

When viewed under microscopy (1000×) after Gram staining, strain YB-19 showed rod-shaped bacteria with flat ends. These bacteria had a length of about 1.0–1.5 µm, and were either short-chained or long-chained. The blue–violet color indicated a result of Gram-positive. When viewed under microscopy (1000×) after spore staining, the spores of strain YB-19 were complete with an oval shape and peacock green color (Supplementary Figs. 2, 3).

Physiological and Biochemical Characteristics

As shown in Table 3, the methyl red test for strain YB-19 was positive, indicating that the strain can utilize dextrose to produce acid in the process of the glycolytic metabolism. The V–P test result was also positive, indicating that YB-19 could not break down dextrose to produce acetylmethyl carbinol. The indole test result was negative, indicating that YB-19 did not contain tryptophan enzymes and could therefore decompose tryptophan in protein. The citrate utilization test result was positive, indicating that YB-19 can utilize citrate as a carbon source. The salt tolerance test result was positive, indicating that YB-19 can tolerate 5 % sodium chloride. The starch hydrolysis test result was

positive, indicating the ability of YB-19 to hydrolyze starch. The catalase test result was positive, indicating that YB-19 can catalyze and decompose $\text{H}_2\text{O}_2\text{--H}_2\text{O}$ and O_2 . The carbohydrate fermentation test result was positive, indicating that YB-19 can utilize glucose, sucrose, and arabinose to produce acid. Combined with the Gram staining and spore staining results, and according to the “Berger Bacteria Identification Manual” and the “Identification Manual of Common Bacterial System”, YB-19 was preliminarily identified as *Bacillus*.

DNA Sequencing of Target Strain

The obtained DNA sequence of YB-19 was queried in the NCBI database. The results showed that the strains with high homology all belonged to the *Bacillus* group. The phylogenetic tree (Supplementary Fig. 4) for related strains was constructed according to strain similarities. The YB-19 strain and *Bacillus toyonensis* strain BCT-7112 are shown clustered onto a single branch in Supplementary Fig. 4, signifying that they are each other’s closest relatives. In light of the colony and strain morphology results, the Gram and spore staining results, and the observed physiological and biochemical characteristics, it can be concluded that strain YB-19 is consistent with *B. toyonensis*. Thus, strain YB-19 was identified as *B. toyonensis* [9–12].

The *B. toyonensis* strain BCT-7112 strain was discovered in 1966 in Japan. It was first identified as a variant of *Bacillus cereus*. Since 1975, it has been added to animal feed as a probiotic. The European Commission first authorized its use in pig feed in 1994, and subsequently it was approved for use in poultry, cattle and rabbit feed. According to Guillermo Jiménez et al. although the strain was originally thought to be a variant of *B. cereus*, the difference between its genome and the genome of the *B. cereus* standard strain is large enough (ANI value of less than 92 %) that the strain can be considered a different species, which was named *B. toyonensis* [12].

In the field of food science research, Zheng et al. recently found another *B. toyonensis* strain BCT-7112 from the old pit mud used for Luzhou-flavor liquor. It was used to study the survival of aerobic bacillus in the extreme environment of the mud pit, and the impact of environmental changes to the mud pit on liquor quality during fermentation [10]. Another study found a similar strain to be an important microbial resource in the probiotics research of Yibin sprouts [13].

In the field of environmental science research, Zhang Y’s study on the contamination of diethylhexyl phthalate (DEHP) showed that the *B. toyonensis* strain BCT-7112 was highly tolerant to DEHP contamination [14]. A study conducted by Bian RX on the production of N_2O in mineralization garbage bioreactor landfill indicated that

Table 3 Experimental results of physiological and biochemical identification for strain YB-19

Test	Result	Test	Result
Methyl red test	+	Starch hydrolysis	+
V–P test	+	Catalase test	+
Indole test	–	Glucose	+
Citrate utilization	+	Arabinose	+
Sodium chloride (5 %)	+	Sucrose	+

“+”: positive result; “–”: negative result

B. toyonensis strain BCT-7112 presented as an intermittent aeration semi-aerobic reactor and may be the main source of N₂O emissions [15]. Additionally, Zhang LQ's group treated the strain as a nematode resistant *Bacillus* that may be used for the prevention and control of root-knot nematodes in the future [9].

Bacillus toyonensis has been studied as an important microbial resource for wine and sprouts. The strain has also contributed to environmental science research. However, there has not yet been any study of the role of *B. toyonensis* in vinegar. Hence, further study of this strain and its optimization and improvement is important.

Conclusion

After an enrichment culture, a total of 48 strains were initially screened out from the sun vinegar grains. According to the total flavonoids and total phenol producing capacity, the strain that can produce the most total flavonoids and phenols was selected as the target strain and named YB-19. Based on the results of strain morphology, observed physiological and biochemical characteristics, and DNA sequencing, strain YB-19 was identified as *B. toyonensis*. This strain can produce a total of 96.55 µg/mL flavonoids and a total of 86.69 µg/mL phenols. The targeted *B. toyonensis* can be applied to the traditional brewing process of sun vinegar to increase the number of flavonoids and polyphenols in the sun vinegar, therefore improving the vinegar quality.

Acknowledgments This work was financially supported by the Liquor Making Biological Technology and Application of key laboratory of Sichuan Province (Item No. NJ2015-11), the scholarship of Luzhou Lao Jiao (Item No. 15ljzk09), and the Solid-state Fermentation Resource Utilization Key Laboratory of Sichuan Province (Item No. 2015GTJ003).

References

- Budak NH, Aykin E, Seydim AC, Greene AK, Guzel-Seydim ZB (2014) Functional properties of vinegar. *J Food Sci* 79:757–764. doi:10.1111/1750-3841.12434
- Zhu WY, Zhang C, Wei Q, Liu J, Yu DY (2012) Technical innovation in the baking fermented grains processing of Sichuan sun vinegar. *Chin Cond* 37:82–84. doi:10.3969/j.issn.1000-9973.2012.04.022
- Huang SM, Liu J, Zhu WY, Han ZS, Yuan HB, Xun AM (2013) Screening and identification of *Bacillus* in traditional bran vinegar grains. *Chin Cond* 38:18–22. doi:10.3969/j.issn.1000-9973.2013.05.005
- Yuan HB, Liu J, Han ZS, Huang ZS, Xun AM (2014) The fermentation medium optimization study of a screened *Bacillus* strain from the traditional bran vinegar grains. *Chin Cond* 39:1–5. doi:10.3969/j.issn.1000-9973.2014.05.001
- Xun AM, Liu J, Wang Y, Yuan HB, Guo MY (2014) The fermentation characteristics of a screened *Bacillus* strain from the traditional bran vinegar grains. *Chin Cond* 39:23–28. doi:10.3969/j.issn.1000-9973.2014.08.006
- Xu HY, Sun XD, Zhang PJ (2009) Folin-phenol method for the determination of total phenols in jujube juice. *Food Res Dev* 30:126–127. doi:10.3969/j.issn.1005-6521.2009.03.038
- Kumar V, Singh P, Jorquera MA, Sangwan P, Kumar P, Verma AK (2013) Isolation of phytase-producing bacteria from Himalayan soils and their effect on growth and phosphorus uptake of Indian mustard (*Brassica juncea*). *World J Microb Biot* 29:1361–1369. doi:10.1007/s11274-013-1299-z
- Guo F, Zhang T (2013) Biases during DNA extraction of activated sludge samples revealed by high throughput sequencing. *Appl Microbiol Biot* 97:4607–4616. doi:10.1007/s00253-012-4244-4
- Zhang LQ, Huang HQ, Cui Y, Zou XX, Liu M, Bao SX (2014) The screening and identification of a nematode resistant *Bacillus*. *Chinese J Trop Crops* 35:1825–1829. doi:10.3969/j.issn.1000-2561.2014.09.027
- Zheng Q, Zhang QP, He XX, Hu J, Huang XH (2014) Isolation and preliminary construct of phylogenetic tree for aerobic bacillus from the old pit mud for Luzhou-flavor Liquor. *J Agr Biotechnol* 22:1411–1417. doi:10.3969/j.issn.1674-7968.2014.11.011
- Wen CQ, Lai XT, He H, Xue M, Zhou SN (2007) Screening and identification of two probiotic bacillus for shrimp nursery. *Acta Hydrobiol Sin* 31:453–459. doi:10.3321/j.issn:1000-3207.2007.04.001
- Guillermo J, Mercedes U, Ana C, Aránzazu LL, Anicet RB, Javier T (2013) Description of *Bacillus toyonensis* sp. Nov., a novel species of the *Bacillus cereus* group, and pairwise genome comparisons of the species of the group by means of ANI calculations. *Syst Appl Microbiol* 36:383–391. doi:10.1016/j.syapm.2013.04.008
- Yin LG, Zhuang T, Ma ZB, Ling Y, Wu XY, Ling You, Zhang C (2015) The isolation and identification of acid producing bacteria for Yibin sprouts. *Chin Cond* 40:46–49. doi:10.3969/j.issn.1000-9973.2015.04.010
- Zhang Y, Tao Y, Zhang H, Wang L, Sun GQ, Sun X, Erinel KO, Feng CC, Song QX, Li M (2015) Effect of di-n-butyl phthalate on root physiology and rhizosphere microbial community of cucumber seedlings. *J Hazard Mater* 289:9–17. doi:10.1016/j.jhazmat.2015.01.071
- Bian RX, Sun YJ, Li JJ, Zhang HH (2014) Impact of nitrate continuous injection on N₂O releases from bioreactor landfill. *Environ Sci* 35:4371–4377. doi:10.13227/j.hjck.2014.11.046