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Screening and Identification of Lactic Acid Bacteria with D-tagatose Production Capability

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Authors' contributions

This work was carried out in collaboration among all authors. Authors XQ, QG and WL designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ZZ, JH and GZ managed the analyses of the study. Authors FNA and RC managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: D-tagatose is a natural ketohexose which can be used as a functional sweetener in foods, diary and beverages products. Isolation of new bacterial strains having the ability to produce D-tagatose is a continuously trending topic of research.
Study Design: Screening of strains with D-tagatose production by identification and determination of its ability to produce D-tagatose content.
Place and Duration of Study: School of Food and Biological Engineering, Jiangsu University between May 2018 and April 2019.
Methodology: We initially screened and identified the strains capable of producing D-tagatose through kimchi liquid, and determined the species and genetic characteristics of the strain by physiological, biochemical and molecular biological identification, and then determined the content of D-tagatose by high performance liquid chromatography. Finally calculate the ability of the strain to produce D-tagatose.

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Results: In this study, 4 strains of lactic acid bacteria (LAB) were isolated from kimchi sample. The isolates were identified as *Lactobacillus spp.* (*Lactobacillus plantarum*, *Lactobacillus fermentum* and *Lactobacillus salivarius*) on the basis of morphological, physicochemical characteristics and analysis of 16S rDNA gene sequence. Because of the novelty, strain designated as *L. salivarius* UJS 003 was considered for D-tagatose yield. Fermentation of D-tagatose was carried out using galactose as substrate for 48 hr at 37 °C, and HPLC method was used to determine the yield. The experimental results exhibited a D-tagatose yield of 3.134 g/L by *L. salivarius* UJS 003.
Conclusion: The strain UJS 003 represented as a potent D-tagatose producer and could be useful in a variety of biotechnological and industrial processes, particularly food and beverage industries.

Keywords: D-tagatose; identification; L. salivarius; strain- screening; biotransformation.

1. INTRODUCTION

The rare sugar monosaccharide D-tagatose, which has the advantage of having low calorific content, accompanied by more than 90% sweetness compared to sucrose. As D-tagatose has been classified by the US Food and Drug Administration (FDA) to be generally recognized as safe (GRAS), it is considered as a promising sweetener that can be utilized in various applications in food industries [1-3]. Due to its relative low glycemic index, it can be considered as an alternative to glucose by playing an important role in mitigating the effect on hyperglycemia, type-2 diabetes, probiotic function, and antioxidant activity [3-5].

Being ketohexose and isomer of D-galactose, the production D-tagatose via chemical isomerization through the calcium catalyst was found to be inapplicable due to the lengthy and complex purification steps as well as the environmental unfriendly wastes. Therefore, the production of D-tagatose by biological approaches is found to be more desired as safer regards to both health and environment. In recent years, many studies demonstrated the production of D-tagatose via the enzymatic catalysis by employing L-arabinose isomerase which is capable of directly converting D-galactose to D-tagatose [6-9]. However, the key determinative steps are to screen the strains with high L-arabinose enzyme activity suitable for large-scale production, to construct the enzyme expression system and to explore the appropriate conversion process. The use of lactic acid bacteria (LAB) strains in food industry is a major field as the final fermentation products are readily available for human consumption [10]. To the above context, the current study aims to screen and identify novel D-tagatose producing lactic acid bacterium strain from kimchi liquid sample.

2. MATERIALS AND METHODS

2.1 Samples, Chemical and Reagents

Kimchi liquid sample, the source of isolated strains, was obtained from in local markets in Zhenjiang city (Jiangsu, China). All chemical and reagents used are of analytical grade quality.

2.2 Enrichment and Isolation of D-tagatose Producing Bacteria

A potential D-tagatose producer was isolated by applying enrichment culture technique using De Man Rogosa and Sharpe (MRS) medium. The composition of liquid medium was as following (g/L): Glucose (4.0), Beef extract (2.0), Peptone (1.0), Yeast extract (1.0), CH_3COONa (1.0), $C_6H_{17}N_3O_7$ (0.4), Na_2HPO_4 (0.4), $MgSO_4$ (0.24), $MnSO_4$ (0.06) and $C_6H_{12}O_5$ (2.0) [1]. The pH of the medium was adjusted to 6.2 and sterilized by autoclaving at 121ºC for 20 min. 1 mL of kimchi liquid sample as inoculated in the MRS medium and incubated at 37ºC on the rotary shaker at 150 rpm for 24 hr. Following the incubation period, 3 mL of culture was transferred to fresh medium containing and re-incubated for another 24 hr. This step was repeated several times, and the growth was considered when the natural purple color of the culture medium becomes yellowish. Then, samples from appropriate multiple dilutions were inoculated on MRS agar plates containing (g/L): $CaCO_3$ (20), bromcresol purple (16) and Agar (15). After the incubation period of 24 hr, single colonies exhibiting a yellowish color were selected and serially cultured on MSR agar plates. Further, the isolated strains were stored as frozen stock cultures at −70ºC in 25% (v/v) glycerol.

2.3 Screening for D-tagatose Producers

The isolates were inoculated in MRS broth for12 hr at 37°C and activated for 2 generations. Later, 1 mL of static inoculum culture was used to access the test tube containing 30 mL fermentation medium. The fermentation broth was centrifuged for 20 min (5000 rpm, 4°C), washed 2 times with phosphate buffer, and the supernatant was discarded. Later, the bacterial cells possessing the characteristics of yellowish colouration and the $CaCO_3$-dissolving capacity were selected and inoculated on MRS agar plate at 37ºC for 48 hr. Ketoses production ability of strains were tested by using the cysteine carbazole sulfuric-acid (CCSA) method, and by measuring absorbance at 560 nm [8,11,12].

2.4 Strain Identification

2.4.1 Phenotypical characterization of isolated strains

The bacterial cell morphology was observed under microscope after Gram staining. Additionally, gluconate test, carbohydrate fermentation test, and mannitol test were conducted for physiological and biochemical identification of the strain according to Bergey's manual of systematic bacteriology.

2.4.2 Molecular identification of isolated strains

The isolates were identified by sequencing the 16S ribosomal DNA gene. For 16S rDNA sequencing, total genomic DNA was extracted from the isolate and amplified by using the universal primers 27F [5-AGAGTTTGATCCTGGCTCAG-3] and 1492R [5-GGTTACCTTGTTACGCTT-3]. The PCR conditions were as follows; an initial denaturation at 94ºC for 5 min, followed by 35 cycles of denaturing at 94ºC for 30 s, annealing at 53 ºC for 30 s, extension at 72ºC for 90 s and a final extension step at 72ºC for 10 min. The resulted PCR products were loaded on 2.0% agarose gel and analyzed under UV transilluminator after staining with ethidium bromide solution. The PCR amplified DNA was purified using Tiangel Midi Purification Kit (TIANGEN, Beijing, PR China). DNA sequencing was performed by Suzhou Hongxun Biotech Co., Ltd., (Suzhou, China). All the 16S rDNA gene sequences were cross-checked manually, edited for phylogenetic analysis and the similarities were determined by using BLASTN multiple sequence alignment.

2.5 D-tagatose Production

D-tagatose production was carried out in the fermentation medium with the following composition (g/L); Galactose (20), Peptone (5.0), Yeast extract (10), K_2HPO_4 (0.2), $MgSO_4$ (0.2), $MnSO_4$ (0.1) and NaCl (0.01). The pH of the medium was adjusted to 6.2 and sterilized by autoclaving at 121°C for 20 min. An aliquot of 2 mL pre-cultured isolate from the MRS culture broth was inoculated in the flask containing 200 mL of fermentation medium and incubated for 48 hr at 37°C. After the incubation period, the fermentation broth was centrifuged for 20 min (5000 rpm, 4°C), washed 2 times with phosphate buffer, and then finally the supernatant was discarded. D-tagatose production ability of strains was determined by High Performance liquid chromatography (HPLC) equipped with Xtimate Sugar-Ca, 5 µm, 7.8 × 30 mm (Welch, Shanghai, China) column and RID-20A refractive index detector (Shimadzu, Japan). The mobile phase was pure water running at a flowrate of 0.5 mL/min. A sample volume of 10 µL was injected to the column temperature (80ºC) [1].

3. RESULTS AND DISCUSSION

3.1 Bacterial Strains Screening

Due to diversity of microorganisms and different niches in which they inhabit, there is a need of efficient isolation and screening methods. Apart from direct isolation of strains by diluting and plating, enrichment cultures very promising for the isolation of D-tagatose producing microbes.The initial screening of all isolates obtained from kimchi solution revealed a total of 15 potential D-tagatose producing strains which were identified as Gram-positive bacteria based on the enrichment and isolation strategy employed in this study. Theses selected strains obtained were inoculated into the fermentation medium, and 4 strains with the ability of D-tagatose production was identified by the cysteine carbazole sulfuric-acid method. The strains were thereafter designated as UJS001, UJS002, UJS003 and UJS004 respectively.

3.1.1 Morphology physiology and biochemistry characteristics

The selected strains were cultured on the MRS solid medium for 24 hr at 37 °C, and the morphological characteristics of the colonies were observed. The results of morphological,

physiological and biochemical characteristics of the selected isolates designated as UJS 001, UJS 002, UJS 003 and UJS 004 are presented in Table 1. Colony shapes varied between irregular circle and circular, with white or milky white color, whereas cells were Gram-positive, rod-shaped, and capable of producing aerosis via gluconate. The morphological and biochemical characteristics of these 4 strains matched with the description about genus *Lactobacillus* which is available in the literature [13-15].

3.2 Molecular Identification of Bacterial Strain

Fig. 1 represents the 16S rDNA gel electrophoresis exhibiting four bands corresponding to each of the four screened strains. Subsequent phylogenetic analysis confirmed that the strain UJS 001, UJS 002, UJS 003 and UJS 004, belongs to genus *Lactobacillus*. The relationship of these strains with the nearest phylogenetic relatives are described in Fig. 2. According to the similarity calculations following the phylogenetic analysis, the closest relatives of UJS 001, UJS 002, UJS 003 and UJS 004, were *L. plantarum* (99 %, UJS 001/002), *L. salivarius* (100%) and *L. fermentum* (99%), respectively. Since there are many studies on *L. plantarum* and *L. fermentum* that can produce D-tagatose, the authors have selected UJS 003 for further research. The strain UJS 003 is being designated as *L. salivarius* UJS 003. The similarity between the UJS003 and the JCM 1231 of *L. salivarius* was 99%. However, *Lactobacillus spp*. are well documented for D-tagatose production except for *L. salivarius* [7,9, 12-14]. Henceforth, authors have considered to *L. salivarius* UJS 003 as a model strain to determine the D-tagatose producing capability.

Table 1. Morphological, physiological and biochemical characteristics of the isolated strains

Strain No.	Colour	Colony shape	Pellucidity	Mycelial morphology	Gluconate	Carbohydrate fermentation	Mannitol
UJS001	White	Irregular circle	Lucency	Short rod	-ve	-ve	+ve
UJS002	Milky white	Irregular circle	Translucency	Short rod	-ve	-ve	+ve
UJS003	Milky white	Circular	Lucency	Short rod	-ve	-ve	+ve
UJS004	White	Circular	Lucency	Short rod	-ve	-ve	+ve

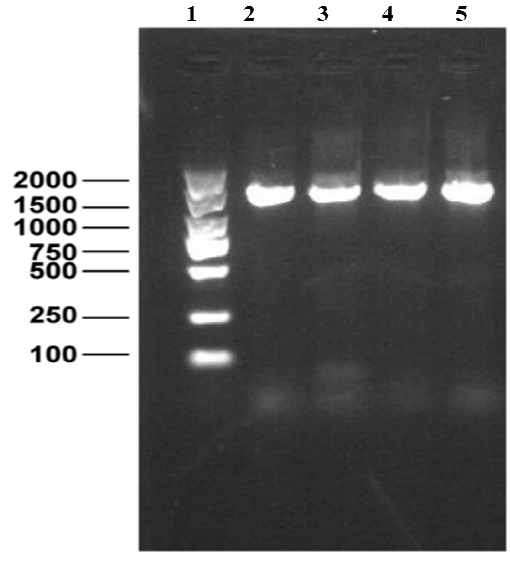

Fig. 1. Agarose gel electrophoresis showing partial rDNA sequence by genomic PCR
Lane 1: 2000 bp DNA marker, Lane 2, 3 ,4 and 5 represents the DNA isolated from UJS 001, UJS 002, UJS 003 and UJS 004, respectively

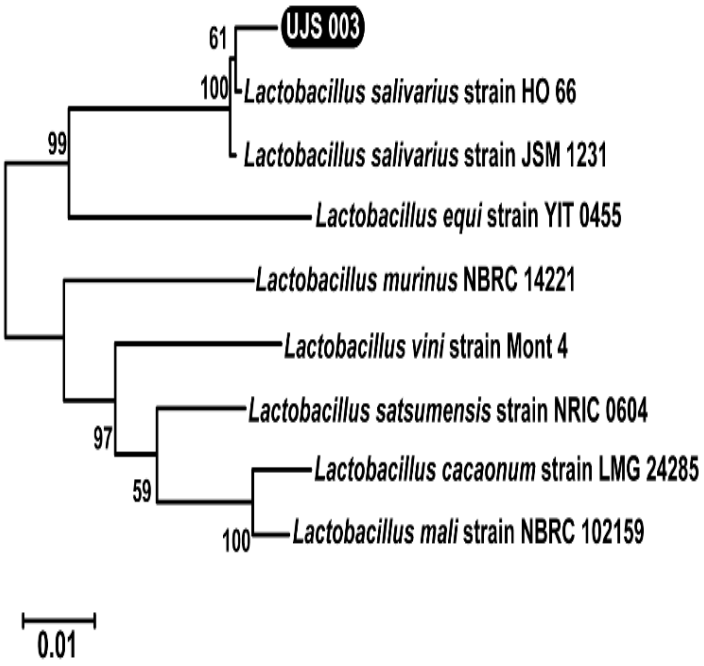

Fig. 2. Phylogenetic tree of *L. salivarius* UJS 003

3.3 Quantitative Analysis of D-tagatose

HPLC was employed for the detection and quantification of tagatose production, after analysing, the yield is 3.134 g/L. Several studies demonstrated the use of *Lactobacillus* strains for the biosynthesis of D-tagatose including *L. sakei* [7], *L. fermentum* [12], *L. reuteri* [13] *L. plantarum* [9] and *L. lactis* [14]. This study reports for the first time, the use of the probiotic bacterial strain *L. salivarius* for the production of D-tagatose [15]. However, there are currently few studies about *L. salivarius*, so we have considered study the capability of producing D-tagatose.

4. CONCLUSION

A D-tagatose producing *L. salivarius* UJS 003, isolated from kimchi sample is reported. At present, the use of enzymes to convert D-galactose into D-tagatose has a lot of reports, but there is a dearth report on the conversion of *L. salivarius*. We need to further study the transformation mechanism to improve the conversion rate. This is the first report on D-tagatose production by *L. salivarius* which may show potential application in some industries especially for food industries. And therefore, merit for further research.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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