

Screening of Lactic Acid Bacteria for Producing α -Galactosidase from Chinese Traditional Fermented Foods

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Abstract: Twenty eight kinds of Chinese traditional fermented foods were collected to isolate Lactic Acid Bacteria (LAB) with α -galactosidase activity, which could eliminated those α -Galactooligosaccharides (α -GOS) regarded as anti-nutritional factors widely existed in agricultural byproducts animal feeds. Three strains, including *Lactobacillus salivarius* XA1R (JX125455) and XH4B (JX125456) and *Pediococcus acidilactici* XS1B (JQ927329), could ferment well in soybean meal and other agricultural byproducts and had α -galactosidase activity 5.81-5.92 U/mL in soybean meal culture.

Keywords: Agricultural byproducts, animal feed, anti-nutritional factors, Chinese traditional foods, Lactic Acid Bacteria (LAB), probiotic, α -galactosidase

INTRODUCTION

Some kinds of agricultural byproducts, such as soybean meal (Ghazi and Galbraith, 2003) rapeseed dregs, rice wine vinasse and wheat brans are good protein sources which can be used in animal feed industry (Wang *et al.*, year). But the anti-nutritional factors, for example, the α -Galacto-Oligosaccharides (α -GOS), inhibit their application, because the α -GOS can result... because it can't be digested by the monogastric mammals (Carrera-silva *et al.*, 2006; LeBlanc *et al.*, 2005; Yamashita *et al.*, 2005). A good method to solve this problem is to hydrolyze them by α -galactosidase (EC 3.2.1.22), which can break the galacto-bonds specifically (Ghazi *et al.*, 2003). And the LAB with α -galactosidase activity will be more efficient and beneficial than use the enzyme alone.

First, many LAB were proved to be probiotic and feather some beneficial effect for their host (LeBlanc *et al.*, 2008, 2004). Second, compared with mycelial fungi which secrete the extracellular enzyme into their growth environment, some LAB with S-layer (surface layer) used to stick their enzyme on the S-layer (Beveridge *et al.*, 1997), which means the enzymes are naturally immobilized and can maintain their catalyst activity for a longer period than those dissociative ones. Third, some reports showed that LAB feature the ability to produce kinds of bacteriocin to inhibit the growth of pathogens (Abrams *et al.*, 2011) and they're effective whether the LAB themselves are viable or dead (Adams, 2010). Based on this theory, those LAB in animal feed should significant improve the feed's

quality just because of the high density of LABs, let alone their ability to eliminate the α -GOS.

MATERIALS AND METHODS

Samples preparation and cultures for isolation: Twenty eight kinds of Chinese traditional fermented vegetal foods, including lobster sauce, salted bamboo shoot, pickled crispifolia, cabbage and Hangzhou Meigancai etc., were collected as samples to isolate LAB with extracellular alpha-galactosidase. After incubating by sterilized water, a modified PY-Sm culture was used to acclimate and screen target LAB strains: 100 g soybean meal was boiled in 500 mL water and the filtration was added to the medium with other ingredients (Peptone 0.5%, Tryptone 0.5%, Yeast extract 1% with salts-Sodium acetate 2%, KH_2PO_4 0.6%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.6%, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 0.2%) up to the total volume of 1000 mL. The PY-Sm medium was both for broth and agar plate culture. X-Gal (5-bromo-4-chloro-indolyl-galactopyranoside) and pNPG (p-nitrophenyl- α -D-galactospyranoside) (Sigma) were both employed to screen and measure the catalyst activities. Strains with exo- α -galactosidase activity were cultured in the agar plate and solo colonies were picked carefully for purifying the target strain. After 5-7 circles, those strains with higher catalyst activities were selected for further research.

Identification of screened strain: (16s DNA) of the isolated strain was amplified and sequenced by Takara Co., Ltd (Dalian China). The colony was picked into 50

μL TaKaRaLysis Buffer for Microorganism to Direct PCR (Code No. D304) under 80°C for 15 min and then took TaKaRa 16 Sr DNA Bacterial Identification PCR Kit (Code No. D310) for amplification. The sequencing primers were 16SSEQR1, Seq Internal and 16SSEQF1. Based on the report of Takara, the strain was identified by NCBI BLAST and phylogenetic tree was made by Mega 5.05.

Agricultural material fermentation properties: Four kinds of agricultural byproducts: soybean meal, rapeseed dregs, rice wine vinasse and wheat bran were used as culture material to ferment isolated LAB strains. The material and water ratio was 1:10 without any additive and the incubation temperature was 37°C for 3 days. Then the colony amounts were measured according to Chinese National standard (Chinese Health Ministry, 2010) and then calculated by $\log_{10}^{\text{cfu/mL}}$.

α -galactosidase activity measurement: The α -galactosidase activity was measured by determining the rate of hydrolysis of p-Nitrophenyl- α -D-Galactospyranoside (pNPG) (Sigma) at 50°C for 10 min with the sodium citrate buffer (0.1 mol/L pH5.78) (Donkor *et al.*, 2007; Garro *et al.*, 1996). LAB cell sediment of 1 mL broth was re-suspended and diluted by sodium citrate buffer-10-1000 folds of culture broth, in order to make the samples' 400 nm absorption in the range 0.4-1.2 and the dilute folds would be calculated into the catalyst activity. The reaction system including: 100 μL sample, 100 μL buffer and 50 μL pNPG and 1000 μL Na_2CO_3 (0.25 mol/L) was used to cease the reaction and develop the characteristic yellow color which measured at 400 nm (Thermo Fisher Multiskan, Finland). One unit of catalyst activity (U/mL) was defined as the amount of released 1.0 μmol of pNP from the substrate pNPG/mL/min.

Statistical data analysis: Those statistical data were recorded and calculated by Microsoft 2010 Excel and other statistical analysis was completed by SPSS 16.0.

RESULTS

Sample catalyst activity measured by pNPG: Many lactic acid bacteria were regarded as probiotic because of their beneficial effects on the hosts and the strain XA1R, XH4B and XS1B with α -galactosidase activity would have more application in animal feed industry to eliminate the anti-nutritional factors in some agricultural factors such as α -GOS. The functions of LAB were easily to prove, but the safety to add them into the animal feed should be the primary premise (Reid, 2005). In this study, we choose Chinese traditional fermented foods as sources to isolate target

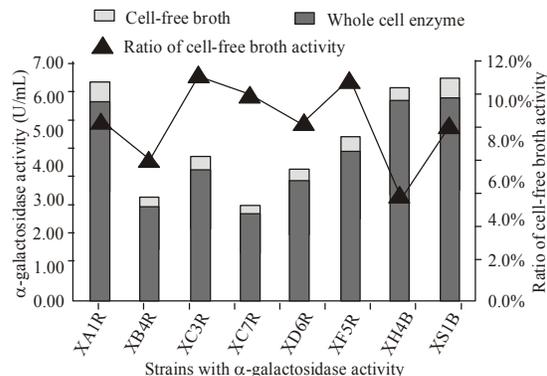


Fig. 1: α -galactosidase activity measured by pNPG of isolated strains

functional LAB, which could promise the safety of strains according to their source material.

Solo colonies in the PY-Sm agar plates were picked out and incubated in broth for 3 days and then pNPG was used to measure the α -galactosidase activity of the strains. Eight strains with relatively higher catalyst activity were showed in Fig. 1 and both the cell-free and cell sediment without any disruption treatment were taken as samples to measure their α -galactosidase activities.

The catalyst activity was mainly existed in the LAB sediment samples and the ratio of cell-free broth activity was 5.3-11.3% demonstrated by the line in Fig. 1. XA1R, XH4B and XS1B were 3 strains with higher catalyst activity compared with others.

Identification by 16 Sr DNA and phylogenetic tree analysis: A-galactosidase can hydrolyze α -GOS and the higher catalyst activity equals higher efficiency in eliminating the anti-nutritional factors in the agricultural byproducts, therefore, 3 strains (XA1R, XH4B and XS1B) out of the 8 kinds LAB mentioned above were selected to do the following assays according to their α -galactosidase activity as well as fermentation ability (Fig. 2). In order to identify their phylogenetic species, the method of 16 sr DNA sequence analysis (Takara co. Ltd., Dalian, China) and nucleic BLAST was carried out and the results showed that the strains were: *Lactobacillus salivarius* XA1R and XH4B (GenBank accession number: JX125455 and JX125456) and *Pediococcus acidilactici* XS1B (GenBank accession number: JQ927329), respectively.

Both XA1R (Fig. 2 (1)) and XH4B (Fig. 2 (2)) were identified as *Lactobacillus salivarius*, however, according to the BLAST results of NCBI, the Query coverage of XA1R was 94% and XH4B was 100% compared with *Lactobacillus salivarius* CECT 5713 (Gen Bank accession number: NC 017481.1) and *Lactobacillus salivarius* UCC118 (Gen Bank accession number: NC 007929.1). The strain XA1R was isolated from Chinese wine lees, which original material was

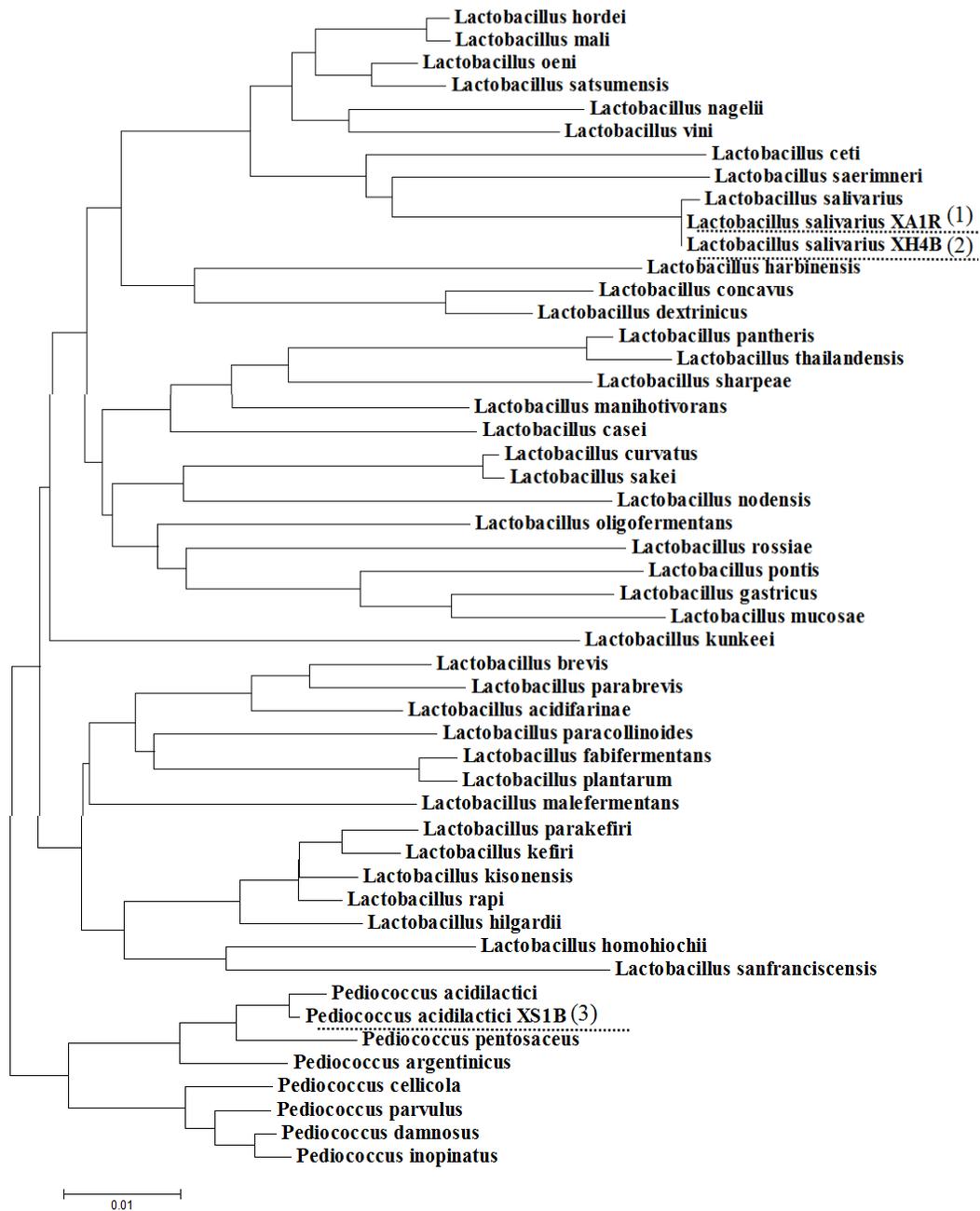


Fig. 2: Phylogenetic tree analyzed by neighbor-joining tree method of MEGA 5.05 involved all 3 strains: XA1R (1), XH4B (2) and XS1B (3)

broomcorn millet and fermented for wine before the sample was collected.

Another strain XH4B was isolated from lobster sauce, which was made of fermented soybean and traditionally cooked as a kind of spicy flavor in China.

The strains XS1B was derived from salted bamboo shoot and with the NCBI BLAST result to *Pediococcus acidilactici* RO17 (Gen Bank accession number: AF515229.1) and *Pediococcus acidilactici* strain B1104 (Gen Bank accession number: AJ 305322.1) etc., the query coverage was 100%.

Fermentation ability of natural material: The colony amounts of the LAB strains measured in the agricultural byproducts fermentation experiments (Fig. 3) showed that the soybean meal was the best culture and the average colony amounts of different strains was $10^{8.15}$ and the rapeseed dregs was worse than others with an average $10^{7.75}$. All of the 3 LAB strains could ferment well in cultures of soybean meal, rapeseed dregs, wheat bran and rice wine dregs and the LAB $\log_{10} \text{cfu}$ after 3 days incubation were 7.79-8.79. And the LAB strain

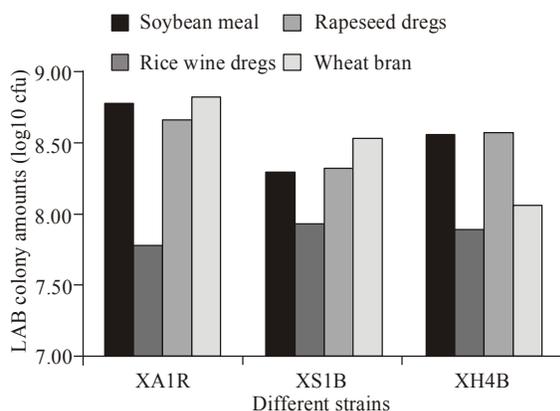


Fig. 3: Fermentation ability in the agricultural byproducts of selected LAB with α -galactosidase activity

XA1R was the best with an average colony amount $10^{8.49}$ and both the other strains XH4B and XS1B were $10^{8.22}$.

CONCLUSION

Three strains with relatively higher α -galactosidase activity were isolated and identified by 16SrDNA, which could ferment some kinds of agricultural byproducts and eliminate their anti-nutritional factors (α -GOS). Compared different samples of same strains: the whole cell enzyme, the cell-free broth, there was only 5.3-11.3% remained in the cell-free broth showed in Fig. 1. The enzyme might be an extracellular one.

According to the analysis results of 16 Sr DNA, the 3 target strains were identified as *Lactobacillus salivarius* XA1R and XH4B (GenBank accession number: JX125455 and JX125456) and *Pediococcus acidilactici* XS1B (GenBank accession number: JQ927329, Fig. 2), respectively. All of the 3 LAB strains could ferment well in cultures of soybean meal, rapeseed dregs, wheat bran and rice wine dregs and the LAB Log₁₀^{cfu} after 3 days incubation were 7.79-8.79 and they had α -galactosidase activities 5.81-5.92 U/mL in soybean meal culture.

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