J. Dairy Sci. 100:1–6
https://doi.org/10.3168/jds.2016-11720

Short communication: Improving the activity of bile salt hydrolases in *Lactobacillus casei* based on in silico molecular docking and heterologous expression

Zhi-Qiang Xiong,* Qiao-Hui Wang,* Ling-Hui Kong,* Xin Song,* Guang-Qiang Wang,* Yong-Jun Xia,* Hui Zhang,* Yong Sun,† and Lian-Zhong Ai*[1]
*Shanghai Engineering Research Center of Food Microbiology, School of Medical Instruments and Food Engineering, University of Shanghai for Science and Technology, Shanghai 200093, China
†Yangzhou Sanhesimei Pickles Co. Ltd., Yangzhou 225000, China

ABSTRACT

Bile salt hydrolase (BSH) plays an essential role in the cholesterol-removing effect of lactic acid bacteria, which hydrolyze conjugated bile salts to amino acid and deconjugated bile salts. However, *Lactobacillus casei* lacks the *bsh* gene, which may make it highly sensitive to bile salt stress. We wanted to improve the BSH activity of *L. casei* for various food-industry applications (e.g., milk fermentation). Plate assay testing indicated that *Lactobacillus plantarum* AR113 has the highest BSH activity. We cloned and sequenced 4 *bsh* genes from the genome of *L. plantarum* AR113. Structure modeling and molecular docking of BSH indicated that BSH1 and BSH3 could react efficiently with bile salts, so we selected BSH1 and BSH3 for heterologous expression in *L. casei*. Compared with single expression of BSH1 or BSH3, co-expression of both protein sequences showed the highest hydrolysis activity by HPLC analysis. Our results suggested that heterologous expression of BSH in *L. casei* can significantly improve host activity against bile salts, and in silico molecular docking could be an efficient method of rapid screening for BSH with high activity.
Key words: bile salt hydrolase, *Lactobacillus casei*, molecular docking, heterologous expression

Short Communication

Lactobacillus casei has been widely applied in the food industry as an acid-producing starter culture for milk fermentation and as an adjunct culture to intensify and accelerate flavor development in ripening cheese. It is also a probiotic organism with public health benefits, such as decreasing incidence of gastrointestinal diseases caused by pathogenic bacteria, modulating the immune system, and reducing the risk of bladder cancer. However, *L. casei* faces a number of stresses from industrial processes and during gastrointestinal transit, including bile salts stress.

In nature, probiotics have evolved bile salt hydrolase (**BSH**, EC 3.5.1.2) to deal with bile salt stress. An important enzyme for the removal of cholesterol, BSH catalyzes the release of free amino acids from both conjugated and deconjugated bile salts. However, *L. casei* lacks the *bsh* gene and may be highly sensitive to bile salt stress. To improve the ability of *L. casei* to degrade bile salts, we cloned *bsh* genes from *L. plantarum* and introduced into *L. casei*.

The lactic acid bacteria used in this study (Supplementary Table S1; http://dx.doi.org/10.3168/jds.2016-11720) were cultured under anaerobic conditions at 37°C in de Man, Rogosa, Sharpe (**MRS**) medium using an anaerobic system (Whitley DG250 workstation; Don Whitley Scientific Limited, Shipley, UK) with a continuous flow of nitrogen gas. The qualitative BSH activity of lactic acid bacteria was determined by plate assay (Dashkevicz and Feighner, 1989; Jayashree et al., 2014). We grew 10 lactic acid bacteria strains (LC2W, AR302, AR342, JPP2, WCFS1, AR113, AR171, AR300, AR184, and AR328) on MRS agar plates supplemented with 0.37 g/L $CaCl_2$ and 5 m*M* bile salts containing glycocholate (**Gc**), glycodeoxycholate (**Gdc**), and glycochenodeoxycholate (**Gcdc**). The plates were incubated at 37°C for 48 h. Bile acid precipitates around the colonies (opaque halo) or the formation of opaque granular white colonies with a silvery shine were considered to be BSH activity (Jayashree et al., 2014). Strains LC2W, AR302, and AR342, which belonged to *L. casei*, could not grow on the MRS medium supplemented with bile salts, indicating that *L. casei* has low or no BSH activity. Compared with the control (no bile salts in the medium; Figure 1A), strain AR113, which belonged to *Lactobacillus plantarum,* showed higher BSH activity

Received July 27, 2016.
Accepted September 2, 2016.
[1]Corresponding author: ailianzhong@hotmail.com

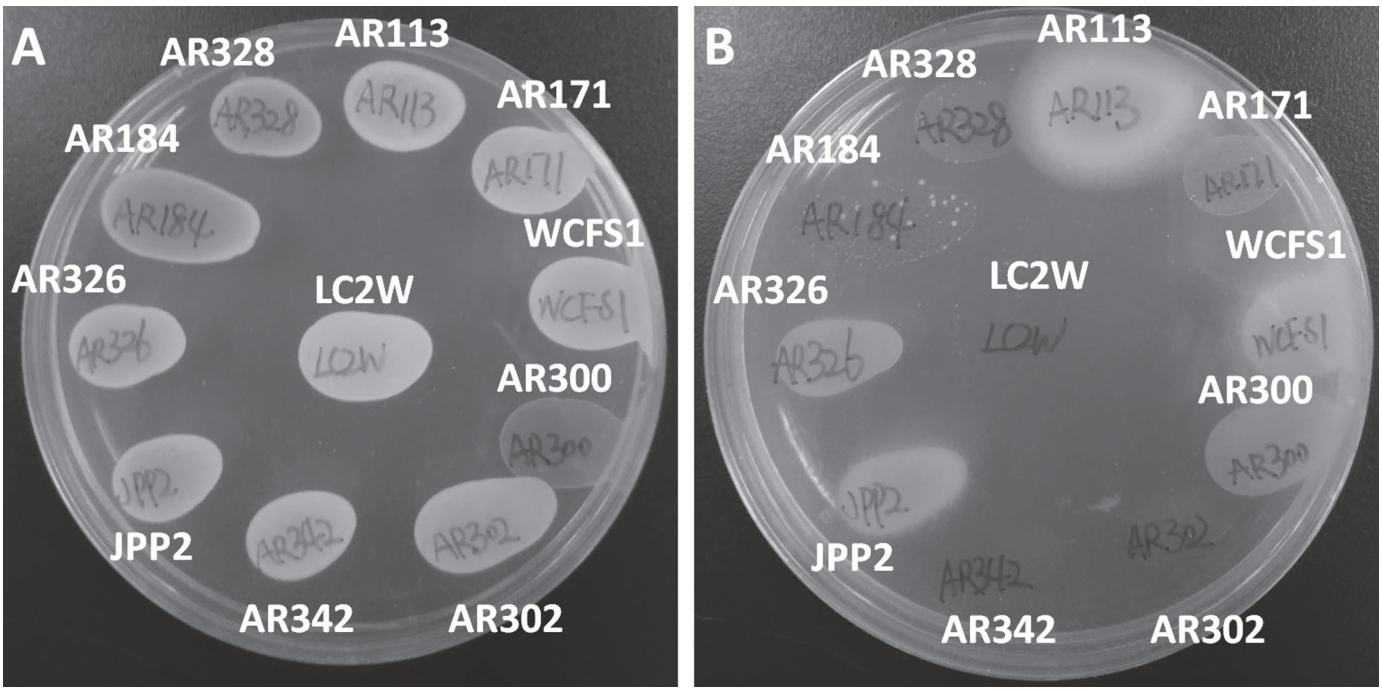

Figure 1. The detection of bile salt hydrolase (BSH) activity using a plate assay. Ten lactic acid bacteria strains (LC2W, AR302, AR342, JPP2, WCFS1, AR113, AR171, AR300, AR184, and AR328) were grown on de Man, Rogosa, and Sharpe (MRS) agar plates with 0.37 g/L $CaCl_2$ (A) or 0.37 g/L $CaCl_2$ and 5 m*M* bile salt (B) at 37°C using an anaerobic system with a continuous flow of nitrogen gas. Bile acid precipitates around the colonies (opaque halo) or the formation of opaque granular white colonies with silvery shine were considered to indicate BSH activity. Color version available online.

based on the size of the opaque halo around the colony (Figure 1B). For this reason, we selected strain AR113 for cloning of *bsh* genes.

According to a genomic analysis, *L. plantarum* has 4 *bsh* genes with different DNA sequences (Gu et al., 2014). The 4 *bsh* genes (designated *bsh1*, *bsh2*, *bsh3*, and *bsh4*) were amplified via PCR from the DNA of *L. plantarum* AR113, using primers BSH1-F/BSH1-R, BSH2-F/BSH2-R, BSH3-F/BSH3-R, and BSH4-F/BSH4-R (Supplementary Table S2; http://dx.doi.org/10.3168/jds.2016-11720), respectively. The primers were designed based on the published DNA sequence of *bsh* genes from *L. plantarum* WCFS1 (NC_004567.2). We inserted the PCR products (Supplementary Figure S1; http://dx.doi.org/10.3168/jds.2016-11720) into lactic acid bacteria shuttle vector pMG36e, containing a constitutive promoter P_{32} (Biswas et al., 2008) and using the ClonExpress 1-step cloning kit (Vazyme Biotech, Nanjing, China) to construct plasmids pWQH01 to pWQH04. Plasmids were transformed into *Escherichia coli* TOP10 and then sequenced by Sangon Biotech (Shanghai, China). The results of sequencing showed that *bsh1–4* contained single open reading frames of 975, 1,017, 987, and 954 nucleotides, respectively (Supplementary Text S1; http://dx.doi.org/10.3168/jds.2016-11720). We found that *bsh1–4*, all encoding for BSH, were similar to *bsh1–4* from *L. plantarum* WCFS1 and *L. plantarum* JPP2, with high identities (98–99%) using National Center for Biotechnology Information (**NCBI**) Blastx analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The deduced proteins BSH1–4 (Supplementary Text S2; http://dx.doi.org/10.3168/jds.2016-11720) had theoretical molecular weights of 37.06, 37.54, 36.17, and 35.64 kDa and isoelectric points of 5.13, 5.93, 5.20, and 8.30, respectively, using ExPASy analysis (http://web.expasy.org/compute_pi).

We used the protein sequences of BSH1–4 to construct a phylogenetic tree using a neighbor-joining algorithm with bootstrap replication of 1,000 in MEGA6 software (Tamura et al., 2013). Phylogenetic tree analysis also showed that the BSH of *L. plantarum* AR113 had the highest sequence identities with the BSH of 27I,N0RuTSDu *L. plantarum* WCFS1 and *L. plantarum* JPP2 (Figure 2A). We found that BSH1–4 showed low sequence identities with each other, and had longer genetic distances to each other, but were highly conserved in evolution. We found similar results for the BSH from *L. plantarum* CGMCC 8198 (Gu et al., 2014). Furthermore, BSH2 and BSH3 were more homologous among strains than BSH1 and BSH4, implying that evolutionary BSH1 and BSH4 may appear earlier than BSH2 and BSH3 (Figure 2A). We performed domain analysis