



Determination of bile salt hydrolase activity as a potential biomarker for putative probiotic *Lactobacillus plantarum* strains

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INTRODUCTION

Bile acids (BAs), the major constituent of bile, are hydroxylated steroids synthesized in the liver from cholesterol, stored in the gall bladder, and released into the small intestine following food consumption. They play a major role in the emulsification and solubilization of lipids facilitating their absorption and digestion. Prior to secretion into bile, primary BAs are conjugated at their side chains with either taurine or glycine. After their release into the duodenum, conjugated bile acids are subject to chemical modifications by the gut microbiota through bacterial bile salt hydrolase (BSH) enzymes. Production of BSH enzymes has been described to have an impact on host physiological processes such as lipid metabolism, gastrointestinal (GI) homeostasis or circadian rhythms. As conjugated bile acids are known to be toxic to bacteria, the enzyme also provides a mechanism to enhance survival of commensal bacteria in the GI tract. The BSH activity, considered a conserved microbial adaptation, is distributed across the major bacterial divisions in the GI tract, and homologs of *bsh* genes have been found in some strains belonging to the species *Lactobacillus plantarum*.

This study aims to quantify the ability of food-borne *Lb. plantarum* strains to deconjugate bile acids through BSH activity. The presence and the expression of bacterial *bsh* genes were evaluated by qRT-PCR. Additionally, BSH activity was also analyzed by the ninhydrin assay, a colorimetric test to determine the amount of amino acids released from individual bile acids through BSH deconjugation.

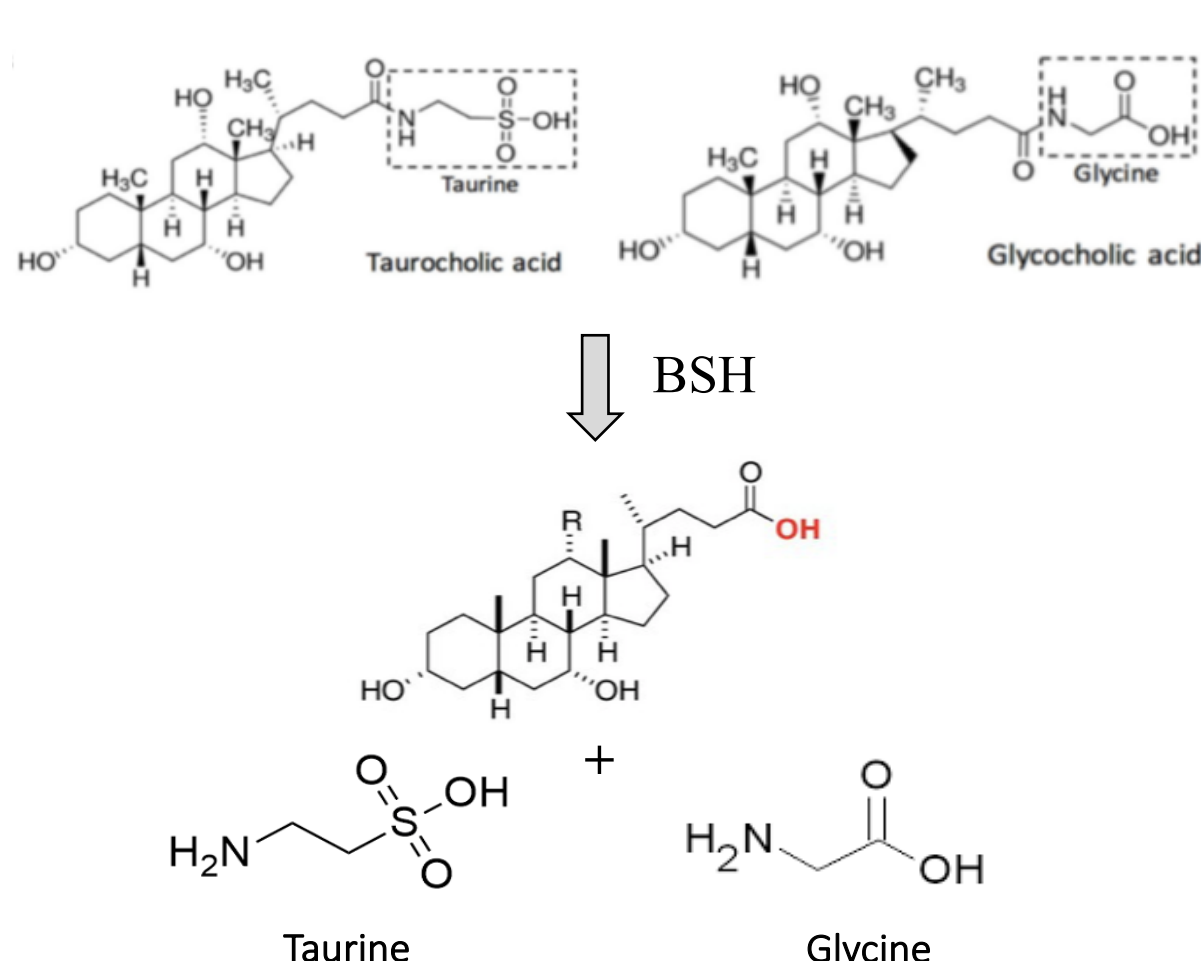
STRAINS

Lb. plantarum strains used in the study have been previously characterized for their potential probiotic properties (1, 2).

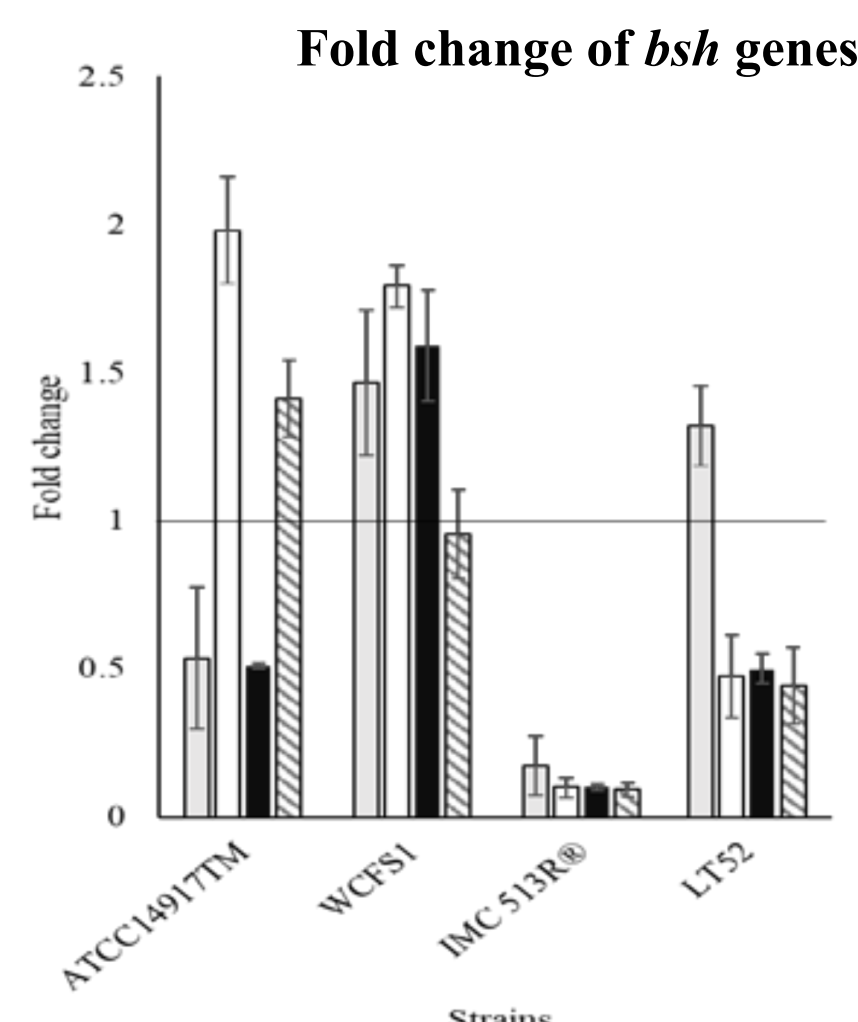
Table 1. *Lactobacillus plantarum* strains and origin

<i>Lb. plantarum</i> strains	Origin
IMC 513	Synbiotec s.r.l.
C904	Table olives
LT52	Raw-milk cheese

BSH ACTIVITY



bsh GENE EXPRESSION



Bacteria were inoculated in MRS containing bile extract porcine 0.5% (w/v) for 90 min.

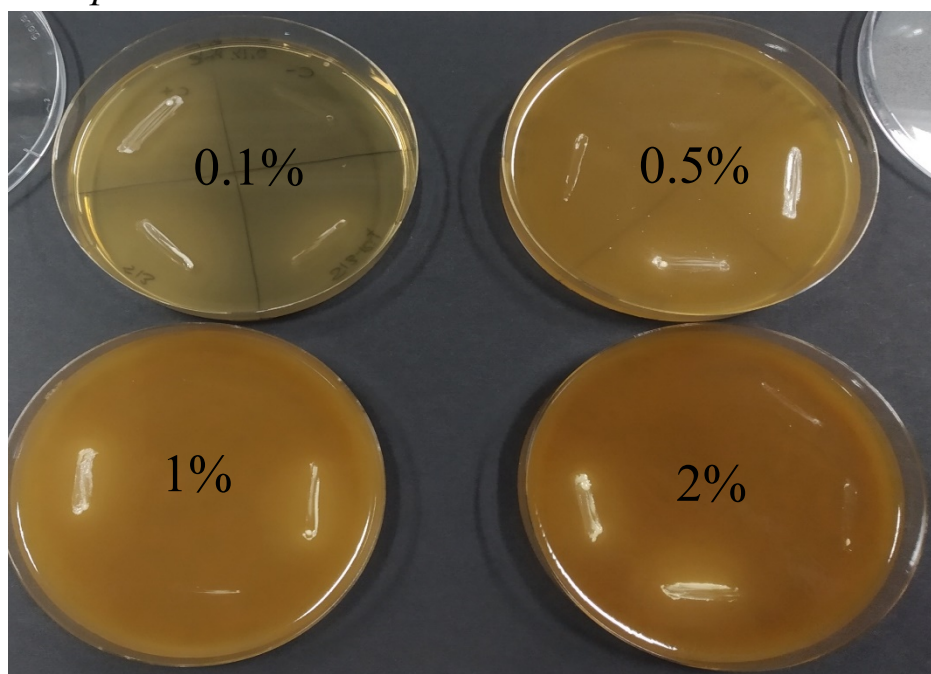
Bile Salt Hydrolase (BSH) function was evaluated through the expression of four genes: *bsh1*, *bsh2*, *bsh3* and *bsh4*.

Results are expressed as mean of relative fold change \pm SD (3 independent replicates), with bacteria grown in LB as reference. Data were normalized to the relative expression value of the housekeeping genes *ldhD* and *gyrA* in each respective sample

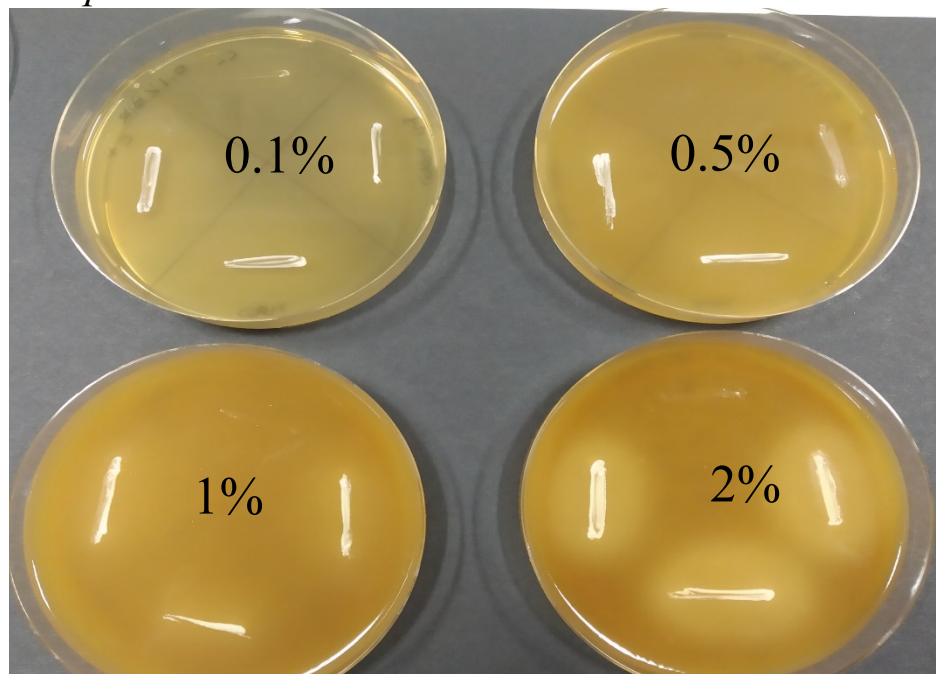
QUALITATIVE DETERMINATION OF BILE SALTS HYDROLASES ACTIVITY AND TOLERANCE

The ability of *Lb. plantarum* strains to detoxify bile salt by producing BSH enzyme activity was evaluated streaking on MRS plates containing different concentrations of bile.

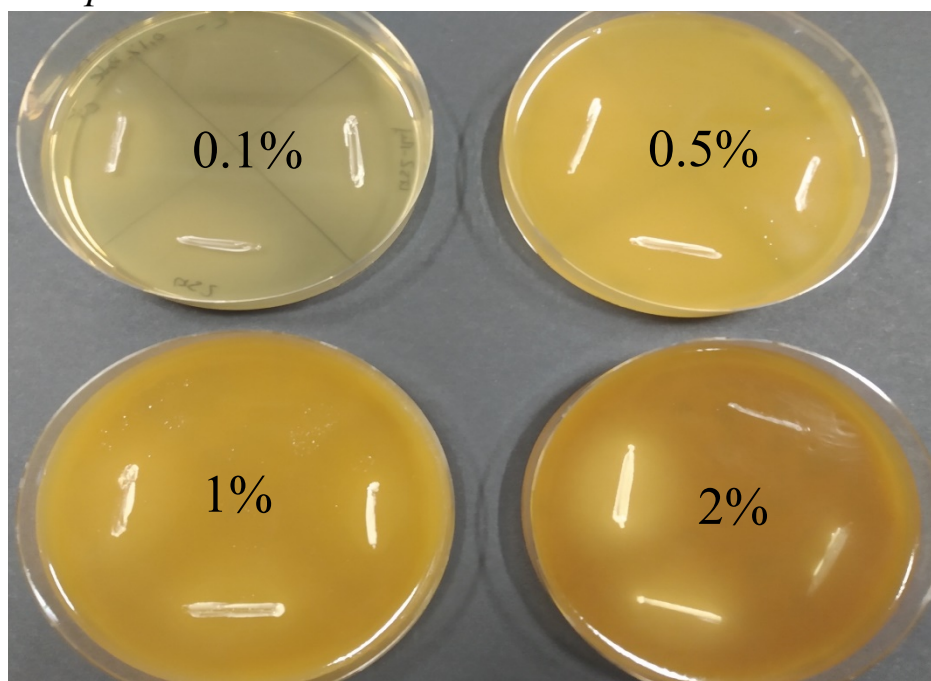
Lb. plantarum IMC 513



Lb. plantarum C904



Lb. plantarum LT52

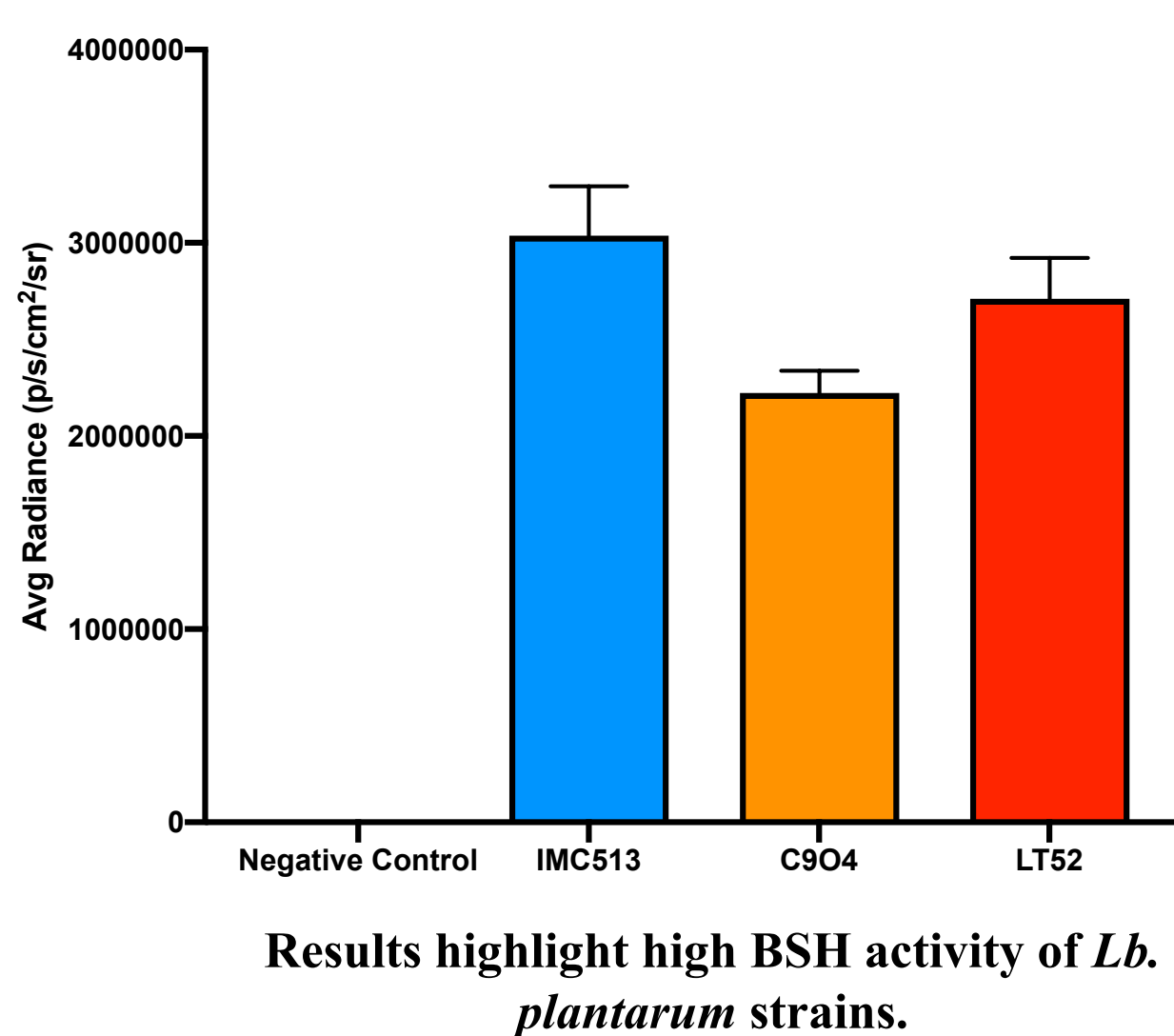


Precipitation zone surrounding colonies indicated the bile salt hydrolase activity of bacteria.

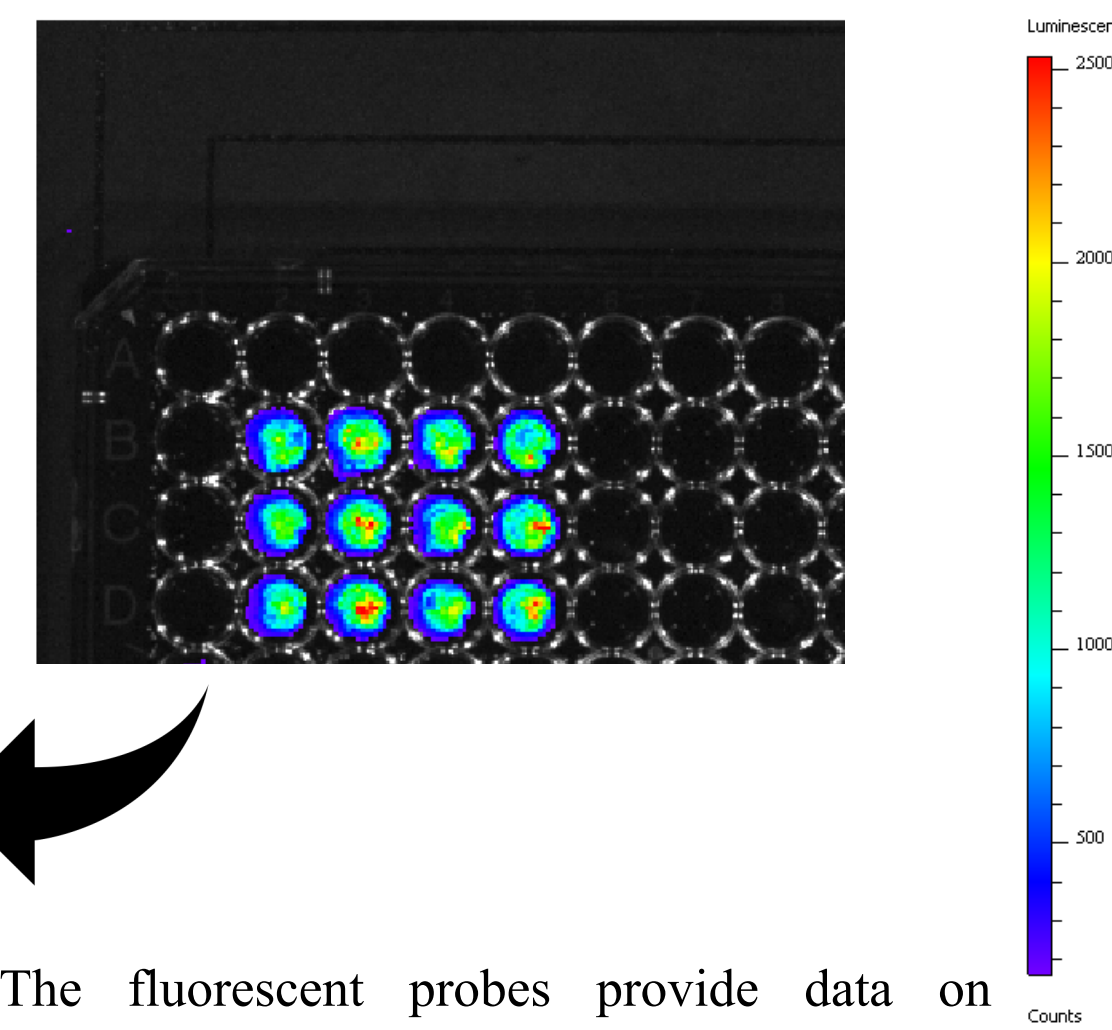
Food associated bacteria are able to survive under bile stress even better than the one isolated from gastrointestinal sources.

ENZYMATIC DETECTION PROBE ASSAY

Cell-free extracts (CFEs) of the bacterial strains were prepared by cell lysis through sonication. The protein concentrations of all CFEs were adjusted to 0.25mg/ml. The CFEs were mixed with a solution of enzymatic detection probe and the cell-free extracts. The CFEs were also mixed with a solution of aminoluciferin in mercaptoethanol to serve as a positive inducer of luminescence. Reaction was incubated in black-walled 96 well plate containing 4T1-RedLuc cells, naturally expressing luciferase. After incubation with the cells, the plates were examined for emitted luminescence using an IVIS-100 imaging system (XENOGEN Imaging Technologies) with the Living Image software (PerkinElmer). Bioluminescence intensity (BLI) was quantified using region of interest (ROI) analysis and the average signal expressed as the total number of photons emitted per second per cm² per steradian (p/sec/cm²/sr).



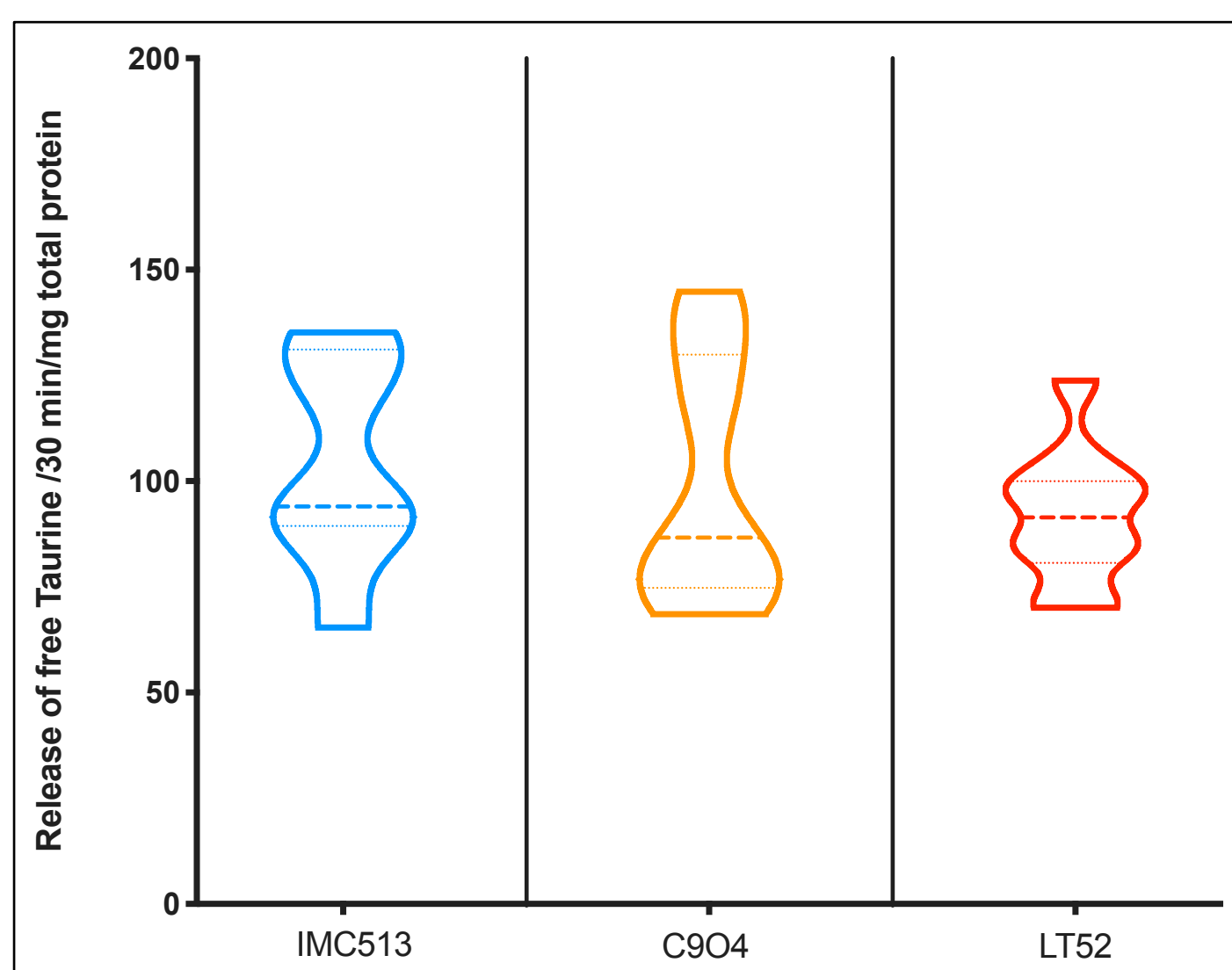
Results highlight high BSH activity of *Lb. plantarum* strains.



The fluorescent probes provide data on enzymatic activity which facilitates high throughput screening of BSH activity from bacterial culture.

TAURINE CONTENT

Lb. plantarum strains LT52, C904 and IMC513 were also examined for their ability to deconjugate bile *in vitro* using the ninhydrin assay for free taurine. Protein concentrations were measured with the Bradford assay and BSA (Sigma) was used as the standard.



CONCLUDING REMARKS

- Overall behaviour of food-associated microbes is similar to that of probiotics or gut associated microbes.
- All strains evaluated are able to grow and survive under bile stress conditions.
- All food-borne *Lb. plantarum* strains has a strain-dependent BSH activity similar to human isolates even though they are no-gut associated.
- The *Lb. plantarum*'s genome may express up to four *bsh* genes to produce bile salts hydrolases, suggesting a relationship between the BSH activity and bacterial survival under GI stress (as bile).

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REFERENCES

1. Garcia-Gonzalez N, Prete R, Battista N and Corsetti A (2018) *Front. Microbiol.* 9:2392
2. Prete R, Tofalo R, Federici E, Ciarrocchi A, Cenci G and Corsetti A (2017) *Front. Microbiol.* 8:2349



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