Metabolomic and transcriptional study of plant-derived lactic acid bacteria enhancing bioactivities of medicinal plant extracts

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Introduction:

Fermentation is a valuable biotechnology that can be exploited to develop novel plant-based foods with improved health promoting properties. Lactic acid bacteria (LAB) species like *Lactobacillus (L.) plantarum*, *L. pentosus*, *Pediococcus (P.)*, etc., derived from different plant sources mainly dominate spontaneous plant fermentation as they inherit many ecological niche specific metabolic enzymes. Bioactive compounds like glycosides, antioxidants, phenolic compounds, and dietary fibers, abundantly present in medicinal plants, allow species and strain-specific LAB to follow various metabolic routes. Plant fermentation process by LAB involves the decomposition and/or bioconversion of complex phytochemicals into bioavailable and bioactive compounds via action of microbial enzymes like glycosyl hydrolase, phenolic acid decarboxylase, reductase and esterase that concentrates functional microbial metabolites with beneficial consequences for human health.

In this study, the author has evaluated the bioactivities of the aqueous extracts of three medicinal herbs Paeonia Radix Alba (dried root of *Paeonia lactiflora* Pall), Mentha (*Mentha arvensis* Linné var. *piperascens* Malinvaud) and Gardenia fructus (the fruit of *Gardenia jasminoides* J. Ellis) after fermentation with plant-derived lactic acid bacteria strains like *L. brevis* 174A, *L. plantarum* SN13T and *P. pentosaceus* LP28. The bioactive metabolites released in each extract after fermentation were also determined. The whole genome sequence of *L. plantarum* SN13T was used to identify the putative genes encoding the associated enzymes of these metabolic pathways. Finally, the transcription profile of these genes were compared in each medicinal extract.

Results:

1. Increased bioactivities of medicinal plant extracts after LAB fermentation:

The fermentation of Paeonia extract with strains like *L. brevis* 174A and *L. plantarum* SN13T were found to significantly reduce the production of lipopolysaccharide (LPS) induced inflammatory mediators like nitric oxide (NO), intracellular reactive oxygen species (ROS) and cytokines IL-6 and TNF-α and their gene expressions in RAW 264.7 cells. In case of Mentha extract, it was found that fermentation with the strain SN13T could effectively increase its bioactivity against the LPS stimulated cells, but the strain LP28 could not. The strain SN13T was also found to increase the potential of Gardenia fructus extract to inhibit the inflammatory mediators and their gene expressions (Fig 1).

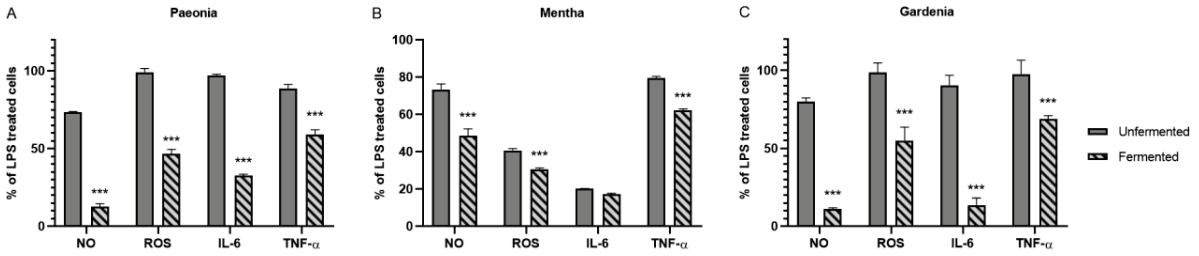

Fig. 1. Increased bioactivities of (A) Paeonia Radix; (B) Mentha; and (C) Gardenia fructus extracts after 24 hours fermentation with *L. plantarum* SN13T to reduce LPS-induced NO, ROS, IL-6 and TNF-α, expressed as percentage (%) of LPS-only treated cells. Data represent mean value and errors bars represent standard deviation. *** $p < 0.001$ vs unfermented extracts.

2. Identification of bioactive metabolites and associated metabolic pathways:

HPLC analysis revealed that the fermentation of Paeonia extract by SN13T and 174A led to metabolism of gallic acid into pyrogallol. In Mentha extract, dihydrocaffeic acid was produced by SN13T from rosmarinic via caffeic acid. Meanwhile, the fermentation of Gardenia extract by SN13T metabolized geniposide to produce genipin. The author speculated that the metabolic pathways in these extracts were mediated via decarboxylase; esterase and reductase; and β-glucosidase, respectively (Fig. 2).

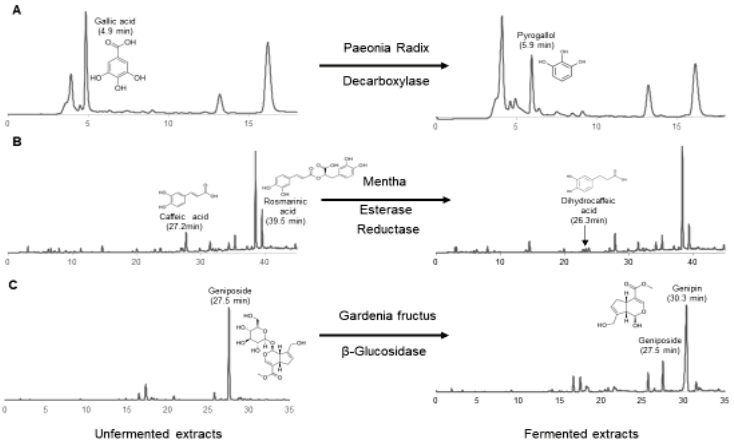

Fig. 2. HPLC chromatograms of unfermented and fermented extracts of (A) Paeonia Radix; (B) Mentha; and (C) Gardenia fructus with *L. plantarum* SN13T.

3. Identification of SN13T genes encoding metabolic pathways related to each medicinal extract:

In the whole genome sequence of *L. plantarum* SN13T (GenBank accession no. AP019815.1), genes encoding putative metabolic enzymes: decarboxylase; β-glucosidases; esterase; and reductase; that might be involved in the fermentation of Paeonia; Gardenia; and Mentha extracts were identified as *ubiD*; *pbg9* and *bglH3*; *ceh*; *hcrA* and *hcrB*; respectively (Fig. 3).

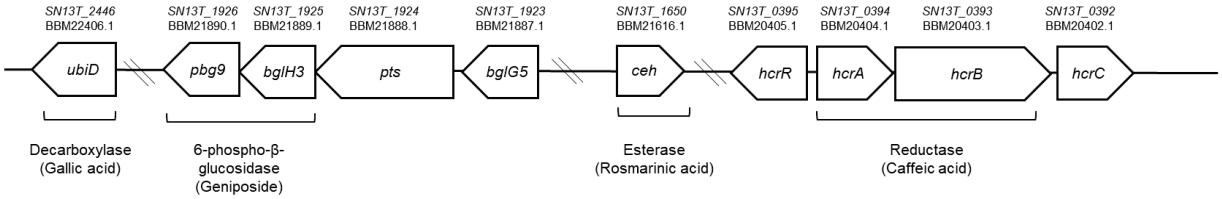

Fig. 3. Genes encoding putative decarboxylase (*ubiD*); β-glucosidases (*pbg9* and *bglH3*); esterase(*ceh*); and reductase (*hcrA* and *hcrB*) arranged in respective gene organizations. Gene loci and protein accession no. are given above each gene.

4. Transcriptional analysis of SN13T genes encoding metabolic pathways:

The SN13T genes *ubiD*; *pbg9*; and *hcrB* encoding putative metabolic enzymes decarboxylase; β-glucosidase; and reductase were specifically overexpressed in Paeonia; Gardenia; and Mentha extracts respectively. Moreover, transcription of these genes in each extract was time-dependent (Fig. 3), which also

correlated with the emergence of metabolites in the fermented extract and their subsequent bioactivities, i.e., during early hours of fermentation in Paeonia extract, while only after 8 hours in Mentha and Gardenia extracts.

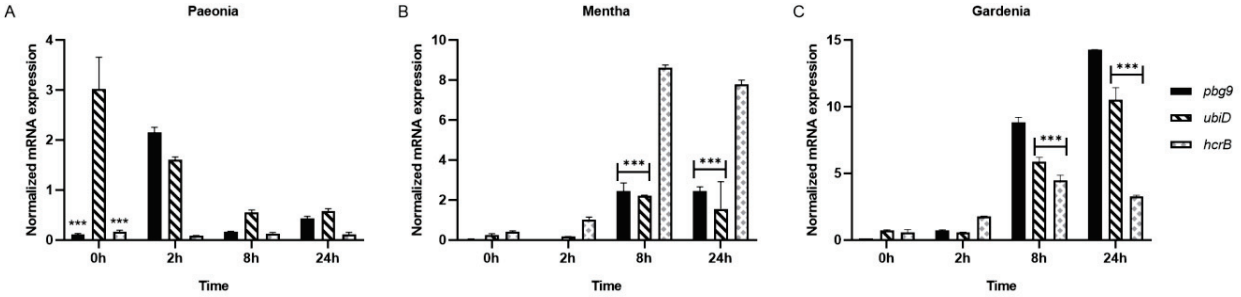

Fig.4. Transcriptional analysis of SN13T genes *ubiD*; *pbg9*; and *hcrB* encoding putative metabolic enzymes decarboxylase; β-glucosidases; and reductase in (A) Paeonia; (B) Mentha; and (C) Gardenia extracts during 0h, 2h, 8h and 24h of fermentation. mRNA expressions were normalized to *ldh* as housekeeping gene. Data represent mean and error bars represent standard deviation. *** $p < 0.001$ vs *ubiD* in (A); *** $p < 0.001$ vs *hcrB* in (B); and *** $p < 0.001$ vs *pbg9* in (C).

Discussion:

In this study, plant-derived *Lactobacillus* strains SN13T and 174A, when grown in medicinal plant extracts Paeonia, Mentha and Gardenia extract, potentiated their bioactivities to suppress LPS induced inflammatory mediators like NO, ROS, IL-6 and TNF-α along with their gene expressions in RAW 264.7 cells. In a previous study, plant-derived LAB strains *L. plantarum* MSC-C2 and *P. pentosaceus* K40, could produce anti-bacterial and anti-biofilm metabolite, 3-phenyllactic acid, when fermented in medicinal plant extracts *Paeonia lactiflora* Pall and *Carthamus tinctorius*. The strain SN13T was also previously reported to produce IL-8 inhibiting molecules like catechol and seco-tanapartholide C, when grown in another medicinal herb extract, *Artemisia princeps* Pampinini. In this study, gallic acid metabolite, pyrogallol; rosmarinic acid metabolite, dihydrocaffeic acid; and geniposide aglycone, genipin; were produced in fermented Paeonia, Mentha and Gardenia extracts respectively. Thus, separate routes were undertaken in each medicinal plant extract to produce bioactive metabolites during LAB fermentation. Putative genes encoding enzymes mediating these pathways, i.e., decarboxylase, esterase and reductases were identified in SN13T genome, which were relatively homologous to those previously identified in *L. plantarum* WCFS1, while similar 6-phospho-β-glucosidases were reported in *L. plantarum* ATCC 8014. However, transcriptional analysis of such genes in media like medicinal plant extracts had not been reported yet. Collectively, this study emphasize that medicinal plant extracts can be conveniently exploited as fermentation media for plant-derived LAB to develop functional food with enhanced bioactive properties.

List of publications:

Shakya, S., Danshiitsoodol, N., Sugimoto, S., Noda, M., & Sugiyama, M. (2021). Anti-oxidant and anti-inflammatory substance generated newly in Paeoniae Radix Alba extract fermented with plant-derived *Lactobacillus brevis* 174A. *Antioxidants*, *10*(7), 1071.

Shakya, S., Danshiitsoodol, N., Noda, M., Inoue, Y., & Sugiyama, M. (2022). 3-Phenyllactic acid generated in medicinal plant extracts fermented with plant-derived lactic acid bacteria inhibits the biofilm synthesis of *Aggregatibacter actinomycetemcomitans*. *Frontiers in Microbiology*, *13*.

Shakya, S., Danshiitsoodol, N., Noda, M., & Sugiyama, M. (2023). Role of phenolic acid metabolism in enhancing bioactivity of Mentha extract fermented with plant-derived *Lactobacillus plantarum* SN13T. *Probiotics and Antimicrobial Proteins*, 1-13.

Submitted to be published:

Shakya S, Danshiitsoodol N, Noda M, Sugiyama M. Transcriptional profiling of geniposide bioconversion into genipin during Gardenia Fructus extract fermentation by *Lactobacillus plantarum* SN13T