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Induction of plantaricin MG under co-culture with certain lactic acid bacterial strains and identification of LuxS mediated quorum sensing system in *Lactobacillus plantarum* KLDS1.0391

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ABSTRACT

A bacteriocin named plantaricin MG was produced by *Lactobacillus plantarum* KLDS1.0391 which was isolated from "jiaoke", a traditional, naturally fermented cream from Inner Mongolia in China. Bacteriocin production was increased significantly when *L. plantarum* KLDS1.0391 co-cultured with certain lactic acid bacteria (LAB) strains. Among 76 strains belonging to the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Streptococcus*, four strains namely *Lactobacillus helveticus* KLDS1.9207, *Enterococcus faecium* KLDS4.0352, *Lactobacillus reuteri* KLDS1.0737 and *Enterococcus faecalis* KLDS4.0313 were shown to induce bacteriocin production of *L. plantarum* KLDS1.0391. Cell numbers of *L. plantarum* KLDS1.0391 were greatly enhanced when co-cultured with four bacteriocin-inducing strains. Bacteriocin production was not induced by autoclaved cultures and cell-free supernatants (CFS) of inducing strains, indicating that living cells of inducing strains might be necessary for enhancement of bacteriocin production. The presence of *plnD* and *luxS* genes was detected by polymerase chain reaction (PCR), and the existence of *plNC8HK* gene was detected by single oligonucleotide nested PCR (SON-PCR).

1. Introduction

Bacteriocins are ribosomally synthesized antimicrobial peptides produced by one bacterium that are active against similar or closely related bacteria, and as with host defence peptides (Bowdish, Davidson, & Hancock, 2005). In recent years, bacteriocins produced by LAB have been paid more attention to due to their potential use as food preservatives (Cheigh, Park, Choi, & Pyun, 2005; Cotter, Hill, & Ross, 2005; Rojo-Bezares et al., 2008), because many of these bacteria have been generally regarded as safe (GRAS) status. However, commercialized bacteriocins are limited in number, e.g. nisin and pediocin PA-1 (Anastasiadou, Papagianni, Filiousis, Ambrosiadis, & Koidis, 2008; Sobrino-López & Martín-Belloso, 2008; Turcotte, Lacroix, Kheadr, Grignon, & Fliss, 2004). Many bacteriocins are not used as biopreservatives in food due to their narrow inhibitory spectrum, narrow pH range, heat instability or relatively low yield.

Despite the above-mentioned problems, bacteriocins with broad spectrum, wider pH tolerance and heat stability have gained much interest in improving the safety of various foods. A bacteriocin named plantaricin MG produced by *Lactobacillus plantarum* KLDS1.0391 showed a broad inhibitory activity against G^+ and G^- bacteria including *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella typhimurium* (Gong, Meng, & Wang, 2010a, 2010b). Plantaricin MG was extremely heat-stable (30 min at 121 °C) and remained active after incubation at pH 2.0–10.0 (Gong et al., 2010a, 2010b). However, the bacteriocin yield of *L. plantarum* KLDS1.0391 was lower than those of certain commercial bacteriocin-producing strains, e.g. *Lactococcus lactis* AL2 used for producing nisin (Huan et al., 2003). The yield of bacteriocin produced by *L. plantarum* KLDS1.0391 is to be further enhanced.

Optimization of cultural conditions and medium composition is one strategy to increase the yield of bacteriocin, e.g. plantaricin produced by *L. plantarum* KC21 (Lim, 2010), pediocin PD-1 produced by *Pediococcus acidilactici* ITV26 (Nel, Bauer, Vandamme, & Dicks, 2001). Some studies also demonstrated that bacteriocin production of *L. plantarum* was induced in co-culture with specific G^+ bacteria (Di Cagno et al., 2010; Maldonado, Ruiz-Barba, & Jiménez-Díaz, 2004; Rojo-Bezares et al., 2008). To our knowledge, only three strains of *L. plantarum* have been described in co-culture with several G^+ bacteria, which resulted in enhancement of bacteriocin

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production, e.g. *L. plantarum* NC8 (Maldonado et al., 2004), *L. plantarum* DC400 (Di Cagno et al., 2010) and *L. plantarum* J23 (Rojo-Bezares et al., 2008). From the above studies it was concluded that some G^+ bacteria may activate specific component regulatory systems related to microbial antagonism such as the plantaricin system which is regulated through the quorum sensing (QS) pathway (Diep, Mathiesen, Eijsink, & Nes, 2009; Diep, Straume, Kios, Torres, & Nes, 2009).

QS is a bacterial intercommunication system that controls the expression of multiple genes in response to population density (Li, Han, Yang, & Zhang, 2010). QS allows population-wide synchronized production of bacteriocin as a function of cell density. The cell–cell communication phenomenon required for sensing of the cell density is mediated by secreted signaling molecules. These signal molecules accumulate in the environment as the cell density increases and activate signal transduction cascades that result in the production of bacteriocin by a particular environmental stimulus (Asanuma, Yoshii, & Hino, 2004; Quadri, 2002). Further research revealed that bacteriocin production in *L. plantarum* was regulated by a three-component regulatory system composed by a signal molecule, a histidine protein kinase (HPK) and a response regulator (RR) (Di Cagon et al., 2010). Signal molecules include acylated homoserine lactone (autoinducer-1, AI-1) (Obst, 2007; Tsai & Winans, 2010), furanosylborate-diester (autoinducer-2, AI-2) (Han & Lu, 2009) and oligopeptide (Freeman & Bassler, 1999). One class of bacterial QS signaling molecules corresponds to AI-2 synthesized through the activity of LuxS enzyme (Miller & Bassler, 2001; Xavier & Bassler, 2003). The presence of *luxS* homologs in both G^+ and G^- bacteria suggests that AI-2 is a universal language for interspecies communication (Kozlova et al., 2008). HPK and RR are well known as components of two-component regulatory systems (Klumpp & Krieglstein, 2002). Such signal molecule acts as an indicator of the cell density which is sensed by the corresponding HPK, resulting an activation of the RR, which finally activates the expression of all operons necessary for bacteriocin synthesis, transport and regulation (Kleerebezem, Quadri, Kuipers, & Vos, 1997). The constitutive bacteriocin production by *L. plantarum* NC8 on solid medium is regulated by the same QS mechanism as in broth cultures, requiring the expression of the operon encoding the three-component regulatory system *plNC8If-plNC8HK-plnD* (Ruiz-Barba, Caballero-Guerrero, Maldonado-Barragán, & Jiménez-Díaz, 2010). The bacteriocin production of *L. plantarum* WCFS1 also depends on the expression of the operon encoding the three-component regulatory system *plnABCD* (Maldonado-Barragán, Ruiz-Barba, & Jiménez-Díaz, 2009).

The aim of this study was to explain how the presence of some LAB strains affected the bacteriocin production and cell growth of *L. plantarum* KLDS1.0391. The bacteriocin production of *L. plantarum* KLDS1.0391 may be affected by co-culture with other LAB strains through LuxS mediated QS system. Hence, the second objective was to amplify the genes encoding the three-component regulatory system.

2. Materials and methods

2.1. Bacterial strains, media and growth

The plantaricin MG-producer *L. plantarum* KLDS1.0391, a strain originally isolated from "Jiaoke". The strain was maintained at −80 °C in 40% (v/v) glycerol, and grown in De Man-Rogosa-Sharp (MRS) broth at 37 °C. Seventy-six LAB strains determined as inducers of plantaricin MG production by *L. plantarum* KLDS1.0391 belonged to *Lactobacillus*, *Lactococcus*, *Leuconostoc* and *Streptococcus* were investigated. Strains were grown in MRS broth at 37 °C. *Bacillus subtilis* ATCC6051 was used as an indicator strain for detection of antimicrobial activity, and grown in beef extract-peptone broth at 37 °C. All strains were stored at −80 °C in 40% (v/v) glycerol, and propagated twice in their corresponding broth medium before use.

2.2. Measurement of antibacterial activity

Agar well diffusion assay (AWDA) was used to detect antimicrobial activity as described by Schillinger and Lücke (1989), with some modifications. Ten milliliters of 1.2% (w/v) agar were poured into a sterile plate and dried. Molten soft beef extract-peptone agar (0.7%, w/v) was first inoculated with 0.6% indicator strain (10^7 cfu/ml), *B. subtilis* ATCC6051 was used as the indicator strain. Six milliliters of the inoculated soft agar were poured onto the surface of the agar plate. After solidification, 6 mm wells were bored in each plate, and 50 μl of the tenfold concentrated cell-free supernatants (CFS) was placed into each well. Plates were held at room temperature for 3 h to allow the bacteriocin completely diffused, and these plates were incubated for 12 h at 37 °C, the diameter of inhibition zone was measured by a vernier caliper. Inhibition diameter is expressed as means ± standard deviation (SD) of mm ($n = 3$).

2.3. Determination of bacteriocin-inducing strains

Production of plantaricin MG by *L. plantarum* KLDS1.0391 in co-culture with LAB strains was determined as described below (Fig. 1): fresh MRS broth was inoculated with 1% of an overnight culture of *L. plantarum* KLDS1.0391 (approximately 10^9 CFU/ml) plus 0.5% of an overnight culture of co-culture strain (approximately 10^8 CFU/ml) to determine induction effect, the mixed cultures were incubated at 30 °C for 6 h, centrifuged at 12000x*g* for 15 min at 4 °C. The supernatants were adjusted to pH 7.0 with 5 M NaOH, treated with hydrogen peroxidase to exclude the antimicrobial effect of hydrogen peroxide, and filtered through 0.22 μm filter (Millipore Corporation, USA). Fifty microliters of the tenfold concentrated CFS was used to detect antimicrobial activity. The

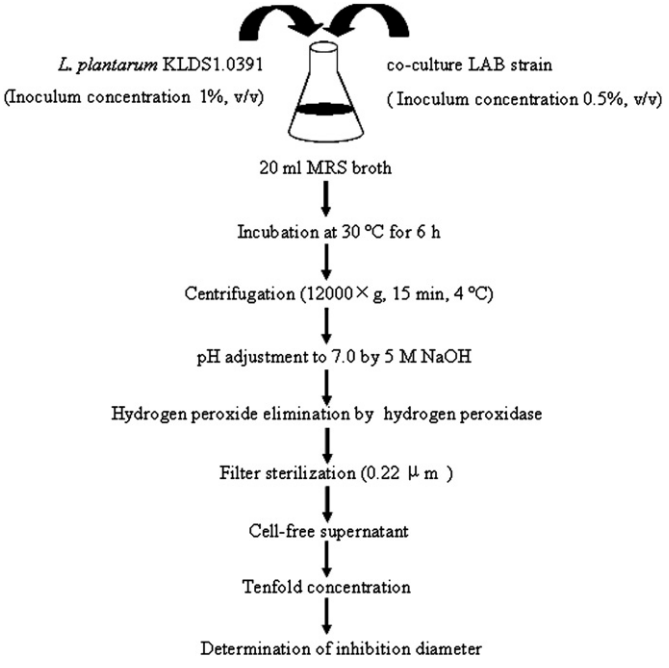

Fig. 1. Protocol for determination of induction of plantaricin MG production by *L. plantarum* KLDS1.0391 in co-culture with LAB strains.