International Journal of Food Microbiology 225 (2016) 38–42

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Short communication

Autoinducer-2 properties of kimchi are associated with lactic acid bacteria involved in its fermentation

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ARTICLE INFO

Article history:
Received 15 December 2015
Received in revised form 3 March 2016
Accepted 6 March 2016
Available online 7 March 2016

Keywords:
Fermented food
Autoinducer-2
Quorum sensing
Kimchi
Lactic acid bacteria (LAB)

ABSTRACT

Bacteria use the cell density-dependent quorum signalling system to regulate particular gene expressions. In food microbiology, signalling is well known for its relation to (foodborne) pathogenicity, food spoilage, and biofilm formation. Quorum quenching and inhibition are thus being considered as a feasible approach in food preservation and safety. In the case of the *luxS*-mediated universal quorum sensing using autoinducer-2 (AI-2), however, it could be a different issue. Several studies have reported a *luxS* AI-2 synthase homologue in numerous bacteria, comprising both pathogens and beneficial strains. A recent study has shown the AI-2 signal to restore the balance of the major phyla of the gut microbiota in antibiotic-induced dysbiosis. We measured the AI-2 activity of the lactic fermented food, kimchi, and found different AI-2 signalling intensities. In order to trace the origin of the signal production, we obtained 229 lactic acid bacterial isolates from the kimchi samples, and detected the AI-2 properties of each isolate using a modified AI-2 bioluminescence assay. Our results showed isolates of dominant species of the genera *Lactobacillus*, *Weissella* and *Leuconostoc* which either produced or inhibited the AI-2 signal. No isolate of the dominant species *Lactobacillus sakei* (75 isolates) and *Lactobacillus curvatus* (28 isolates) showed AI-2 producing activity, while AI-2 inhibition could not be detected for any of the 31 *Lactobacillus plantarum* isolates. These results suggest the AI-2 activity of kimchi to result from the interaction of the associated microbial food cultures (MFCs) during fermentation. Thus far, only sparse information is available on AI-2 signalling interaction in fermented food, however, we suggest that fermented food may be a supplier of AI-2 signalling molecules via typical MFCs.

1. Introduction

Bacterial quorum sensing is a cell density-dependent signalling system using diffusible auto-inducers (AIs) as signal molecules. The signalling system requires a specific concentration of the AIs to regulate particular gene expression as a group (Miller and Bassler, 2001). Several studies have reported on the important role of the communication system, both in bacterial interactions and in their collective behaviour (Bansal et al., 2008; Camilli and Bassler, 2006; Chen et al., 2011). Food related bacteria also have such signalling systems (Gobbetti et al., 2007), and important roles in food microbiology may include:

- expression of virulence factors in foodborne pathogenic bacteria (Smith et al., 2004),
- regulation of food spoilage activities including proteolysis, lipolysis, chitinolysis, and pectinolysis (Bai and Rai, 2011), and
- production of biofilms by diverse bacterial species in the food matrix (Jahid and Ha, 2012; Skandamis and Nychas, 2012).

Recent studies have underlined the importance of quorum signal detection, quenching and inhibition as key issues in food preservation and safety (Cloak et al., 2002; Rasch et al., 2005; Truchado et al., 2012).

Cell signalling has been suggested as one of the various mechanisms of microbial interactions in food fermentations (Ivey et al., 2013). Yet, the principle of *luxS*-mediated quorum signalling using autoinducer-2 (AI-2) has not been a major focus of investigations thus far. The *luxS* AI-2 synthase gene homologue is found in a wide range of both Gram-positive and Gram-negative bacteria (Pereira et al., 2013), with 17% of the phylum *Bacteroidetes* and 83% of the *Firmicutes* predicted to have the homologue according to the KEGG database (Thompson et al., 2015). This implies that many of the non-pathogenic and even beneficial bacteria also use this 'universal' and 'common' signalling system to regulate their own behaviour. According to recent studies, *Lactobacillus* spp. use AI-2 signalling to respond to environmental stresses (Moslehi-Jenabian et al., 2009; Yeo et al., 2015) and to regulate growth and metabolism (Lebeer et al., 2007a, b, 2008). In the case of bifidobacteria, their AI-2 activity correlated with

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http://dx.doi.org/10.1016/j.ijfoodmicro.2016.03.007
0168-1605/

biofilm formation and gut colonisation (Christiaen et al., 2014; Sun et al., 2014). Furthermore, Thompson et al. (2015) suggested that AI-2 activity can serve to restore the balance of gut microbiota following antibiotic-induced dysbiosis.

Fermented foods are well known for their benefits to human gastrointestinal and metabolic health (Ouwehand and Röytiö, 2015). Their typical microbial populations, also termed "microbial food cultures" (MFCs), are directly and/or indirectly associated with these beneficial effects (Bourdichon et al., 2012). We could expect a dynamic AI-2 signalling from MFCs in non-heat treated fermented foods, and also that the microbial interactions should affect the AI-2 signalling status. However, at present our understanding of AI-2 quorum signalling in fermented foods is clearly deficient, and available reports and data are still scarce.

In this study, we selected samples of kimchi, a well known Korean fermented vegetable food prepared by different recipes and ingredients (Lee et al., 2011), and containing a range of diverse of lactic acid bacteria (LAB) including representatives of the genera *Weissella*, *Leuconostoc* and *Lactobacillus* as dominant MFCs (Jung et al., 2011; Park et al., 2012). Typically, kimchi preparation does not involve heat treatment, thereby allowing viable bacterial interaction by MFCs during the whole process, even up to consumption. We studied the AI-2 activity of different kimchi samples and profiled the AI-2 properties of dominant LAB isolates from these samples using the AI-2 bioluminescence assay.

2. Materials and methods

2.1. Bacterial preparation

2.1.1. Strains

Lactic acid bacterial isolates were isolated from thirty-six kimchi samples obtained from Korean traditional markets. After incubation at 37 °C for 24 h, 229 representative colonies were selected as dominant MFCs based on dilution levels of $>10^6$ from at least three MRS (de Man, Rogosa and Sharp) agar (Difco Co., USA) plates, and identified by 16S rRNA sequencing. From thirteen kimchi samples selected for AI-2 detection 102 isolates were obtained representing seven different LAB species. *Vibrio harveyi* strains BB152 (ATCC BAA-1119) and BB170 (ATCC BAA-1117) were used for the AI-2 bioluminescence assay, and were obtained from the ATCC (American Type Culture Collection, Manassas, USA).

2.1.2. Cultivation

All tested isolates in this study were inoculated in MRS broth and sub-cultured twice at 37 °C before use. For the AI-2 bioluminescence assay, *V. harveyi* strains BB152 and BB170 were grown at 30 °C with an orbital shaker of 125 rpm either in Marine broth 2216 (Difco Co., USA) or Autoinducer Bioassay (AB) medium (Greenberg et al., 1979). All strains and isolates were stored at −80 °C in cultured broth with 10% of glycerol.

2.2. AI-2 bioluminescence assay

2.2.1. Sample preparation

Thirteen kimchi samples were tested for the presence of AI-2. Each sample was mixed 1:1 (*w*/*v*) in distilled water, and 10 mL of each suspension was collected and centrifuged at 12,000 *g* for 10 min, then adjusted pH to 7.0. The samples were drawn through a sterile syringe filter of 0.2 μm pore size (Minisart®). For the LAB, we used the AL medium (Autoinducer Bioassay medium for lactic acid bacteria) based on separate MRS ingredients (obtained from Sigma-Aldrich, St. Louis, MO), but with four components (glucose, ammonium citrate, sodium acetate and di-potassium phosphate) omitted, and diluted in distilled water to comprise 0.1% of the original concentration of MRS. The test isolates were inoculated at a level of 1% into 5 mL MRS broth and cultured at 37 °C for 16 h and then centrifuged at 12,000 *g* for 10 min. The supernatants were removed and the sediments washed three times using the AL medium. After re-suspension in 1 mL of the AL medium, samples were incubated for 60 min. Then the cultures were centrifuged at 12,000 *g* for 10 min and filtered through a sterile syringe micro-filter of 0.2 μm pore size (Minisart®).

2.2.2. AI-2 detection

The AI-2 bioluminescence assay suggested by Surette and Bassler (1998) was modified. *V. harveyi* BB170 as a reporter strain was grown with aeration (125 rpm) for 16 h and diluted in the AB medium to reach about 50,000 initial RLU (Relative light units), and transferred into a 96-well micro-titre white plate (Whatman 7701-3350). Prepared samples were also added to the plate at a final concentration of 10% (*v*/*v*). The plate was incubated at 30 °C with aeration (125 rpm) for 2 h, and bioluminescence was measured as RLU every 30 min during 2–4 h in a Glomax® 96 Microplate Luminometer (Promega, USA). Either distilled water or AL medium was used as basal control of the minimum luminescence (Lum_{min}) to measure the RLU of samples. When the Lum_{min} of the sample was significantly lower than that of the control and the enhancement phase was delayed over 1 h, we defined the AI-2 property of the sample as inhibition.

3. Results

3.1. AI-2 properties of kimchi and its MFCs

The tested kimchi samples showed AI-2 signalling activities with intensities over a wide range between 50 and 100,000 RLU (Fig. 1). Samples 1, 4, 5, 6, 10, 11, and 12 (black bars) showed weak AI-2 activities below 1000 RLU, while significantly stronger activities of at least 10,000 RLU were measured for samples 2, 3, 7, 8, 9, and 13 (white bars). Among the latter, the strongest AI-2 activities above 100,000 RLU could be determined for samples 7, 8 and 9. The product profiles and dominant MFCs of each kimchi sample are presented in Table 1 and Fig. 2. Against expectations, there was no correlation between the kimchi type and AI-2 activity. Still, we tested the possible impact of capsaicin, a putatively bioactive ingredient typical of kimchi, on the AI-2 reporter strain *V. harveyi* BB170. However, capsaicin did not affect AI-2 expression of BB170 at a concentration of 1 mg/mL (data not shown), a level exceeding concentrations typical of kimchi. Even among samples of the same type of kimchi the measured intensity of AI-2 activity differed, suggesting that the ingredients of kimchi did not affect the AI-2 signal production noticeably.

The results of bacterial identification by 16S rRNA sequencing showed the dominant MFCs to be represented by the genera *Lactobacillus*, *Leuconostoc* and *Weissella*, thus confirming former reports on

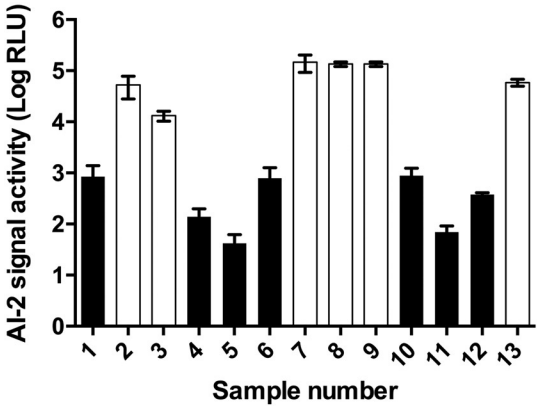

Fig. 1. AI-2 signalling activity of different kimchi samples determined by using the optimised AI-2 bioluminescence assay for LAB. For more information on the samples, see Table 1. The data represent the means and standard errors.