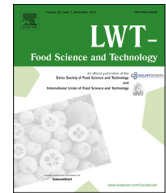
LWT - Food Science and Technology 57 (2014) 230–235

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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



Inhibition of biofilm formation of *Pseudomonas aeruginosa* by an acylated homoserine lactones-containing culture extract

Qiu Qin Zhang, Ke Ping Ye, Hu Hu Wang, Hong Mei Xiao, Xing Lian Xu*, Guang Hong Zhou

Key Laboratory of Meat Processing and Quality Control, College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China

ARTICLE INFO

Article history:
Received 27 June 2013
Received in revised form
10 December 2013
Accepted 13 December 2013

Keywords:
Acylated homoserine lactones (AHLs)
Chicken
Biofilm
Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

ABSTRACT

The objective of this study was to examine the effect of an acylated homoserine lactones-containing culture extract (AHL-CCE) on bacterial growth, enzyme activity and biofilm formation of *Pseudomonas aeruginosa*. Chicken breast muscle broth in which *P. aeruginosa* had been grown for 24 h at 20 °C was extracted with acidified ethyl acetate, the solvent was evaporated and the residue was dissolved in water and heated at 100 °C for 15 min. Thin-layer chromatography combined with *Agrobacterium tumefaciens* KYC55-based bioassays indicated the presence of C_4-, C_6-, C_8- and C_{12}-homoserine lactones in the extract. The addition of AHL-CCE to a culture of *P. aeruginosa* caused the bacteria to reach their highest count after 36 h whereas cultures without the extract required 72 h to reach their peak counts, but the maximum cell count was higher for the extract-free culture. No biofilm was detected when *P. aeruginosa* was cultured in chicken broth at 10 °C when the extract was present. Addition of extract reduced proteolysis of actin, troponin-T and tropomyosin by *P. aeruginosa*, but had no effect on lipase activity. Therefore, AHL-CCE can be used to reduce the bacterial growth time and inhibit biofilm formation; thus, benefiting food safety and quality.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

In the natural environment, microorganisms can adhere to or attach to surfaces and form biofilms (Srey, Jahid, & Ha, 2012) where they become important sources of microbial contamination (Hentzer et al., 2002; Silagyi, Kim, Lo, & Wei, 2009). Approaches for prevention, removal and killing of biofilms are therefore sought to improve food hygiene and safety (Hentzer et al., 2002; Wang et al., 2013). Biofilm development is a stepwise process where surface attachment is the first step in its formation (Srey et al., 2012). Therefore, as a means of preventing the spread of bacteria from food working surfaces, it is necessary to develop methods to inhibit biofilm formation when microorganisms first move on to and attach to surfaces.

Quorum sensing (QS) has been found in a diverse group of bacteria which communicate with each other using signaling molecules (Venturi, 2006; Whiteley, Lee, & Greenberg, 1999). Many gram negative food-associated bacteria produce acylated homoserine lactones (AHLs) as signal molecules (Wagner, Frelinger, Barth, & Iglewski, 2006; Zhu, Thuruthyil, & Willcox, 2002). AHL-based QS is a global regulation system which controls biofilm formation (De Kievit, Gillis, Marx, Brown, & Iglewski, 2001; Hentzer et al., 2002), growth (Chavant, Martinie, Meylheuc, Bellon-Fontaine, & Hebraud, 2002) and certain enzyme activities (Liu, Gray, & Griffiths, 2006). So far, the majority of studies on the role of AHLs have been based on genetic approaches (De Kievit et al., 2001; Pesci, Pearson, Seed, & Iglewski, 1997; Venturi, 2006) in which AHL synthase knock-out mutants were used to evaluate the potential phenotypes regulated by the signaling compounds (Whiteley et al., 1999). AHL-producing microorganisms are able to synthesize AHLs when they reach a sufficiently high population density (Medina-Martínez, Uyttendaele, Demolder, & Debevere, 2006; Pesci et al., 1997; Venturi, 2006; Zhang et al., 2013), and for many AHL QS-regulated genes, a quorum concentration of AHL signal molecules is necessary (Diggle, Winzer, Lazdunski, Williams, & Cámara, 2002; Schuster, Lostroh, Ogi, & Greenberg, 2003; Wagner et al., 2006). However, it is not well understood what happens when low numbers of microorganisms, that perhaps themselves do not produce AHLs, encounter AHLs in the environment.

Pseudomonas aeruginosa, a pathogenic and spoilage bacterium commonly found in the environment and food processing facilities (Hentzer et al., 2002; Huang, Han, Zhang, & Leadbetter, 2003; Zhu et al., 2002), has a great tendency to form biofilms (Hentzer et al., 2002; Whiteley et al., 1999). Studies have shown that cell-free

* Corresponding author. Tel./fax: +86 25 84395939.
E-mail address: xlxu@njau.edu.cn (X.L. Xu).

0023-6438/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.lwt.2013.12.022

cultures of *Pseudomonas* can affect bacterial growth (Dourou, Ammor, Skandamis, & Nychas, 2011; Nychas et al., 2009). Since the supernatants contain both auto-inducer 1 and auto-inducer 2 (Nychas et al., 2009), AHL extracted from *P. aeruginosa* may affect bacterial growth and biofilm formation. Therefore, the present study evaluated growth, enzyme activity and biofilm formation of *P. aeruginosa* in chicken breast muscle broth in the presence of AHLs-containing culture extract (AHL-CCE).

2. Material and methods

2.1. Bacterial strains and culture conditions

Three bacterial strains were used and routinely grown in Luria–Bertani (LB) broth (1% peptone, 0.5% yeast extract, 0.5% NaCl). *P. aeruginosa*, a strain isolated from commercial broilers and produced AHLs, was chosen as the test organism (Zhang et al., 2013). *Agrobacterium tumefaciens* strain KYC55, carrying the plasmid pJZ372, pJZ384 and pJZ410, was used for the detection of AHLs in thin layer chromatographic (TLC) assays. This strain was grown in medium supplemented with 2 μg/ml tetracycline, 100 μg/ml spectinomycin and 100 μg/ml gentamicin (Zhu, Chai, Zhong, Li, & Winans, 2003). *A. tumefaciens* strain R10 with plasmid pCF218 was used for an AHL-negative mutant and grown in medium supplemented with 2 μg/ml tetracycline (Fuqua & Winans, 1996).

2.2. Preparation of chicken breast muscle broth

Four hundred grams of chicken breast muscle meat was mixed with 4 L of sterile water, homogenized for 30 s and centrifuged at 5000× *g* for 15 min at 4 °C. The supernatant was sterilized by filtration through a 0.22-μm membrane and standardized to $OD_{280} = 1.47 \pm 0.071$ mg protein/ml using a Nanodrop ND-2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

2.3. Extraction and identification of AHLs

Chicken breast muscle broth was inoculated with *P. aeruginosa* at a starting level of 4.7 log CFU/ml and incubated at 20 °C for 24 h. AHLs were extracted according to the method described by Bruhn et al. (2004). Briefly, 2 L of chicken breast muscle broth was stirred for 20 min with an equal volume of ethyl acetate (acidified with 0.5% formic acid) (three times), then centrifuged at 5000× *g* for 15 min. The supernatant was filtered through a 0.22-μm membrane. The filtrate was then evaporated under nitrogen flow to dryness and finally dissolved in 1 ml sterile water.

The culture extract was heated in a water bath at 100 °C for 5 min then its components were profiled by TLC (Ravn, Christensen, Molin, Givskov, & Gram, 2001). In brief, 4 μl AHL-CCE and 1.5 μl standards were applied to a TLC plate (TLC aluminum sheet; 20 × 20 cm; RP-18 F254 S plate; catalog no. 1.05559; Merck, Darmstadt, Germany). The plates were developed in 10 ml 60:40 (v/v) methanol-millipore water for 90 min. After air drying, 100 ml of LB broth containing 2% agar, 60 μg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and 10 ml of an *A. tumefaciens* KYC55 overnight culture was spread over the TLC plate. The plate was incubated overnight at 28 °C in a sterilized closed plastic container. AHLs were visualized as blue spots. N-oxohexanoyl-l-homoserine lactone (3-oxo-C_6-HSL; catalog no. K3007), N-hexanoyl-dl-homoserine lactone (C_6-HSL; catalog no. 09926), N-octanoyl-dl-homoserine lactone (C_8-HSL; catalog no. 10940) and N-dodecanoyl-dl-homoserine lactone (C_{12}-HSL; catalog no. 17247) were purchased from Sigma Co. (St. Louis, MO., USA) and used as reference standards.

2.4. Preparation of samples

The effect of AHL-CCE on *P. aeruginosa* was tested by adding 3 μl AHL-CCE and 2 μl sterile water, or 5 μl AHL-CCE to 10 ml chicken breast muscle broth inoculated with *P. aeruginosa* (as previously prepared above). All samples were incubated in glass tubes at 10 °C. Tubes were shaken gently (50 rpm/min) and sealed with an oxygen permeable film (14,483 $cm^3/(m^2$ 24h atm)).

2.5. Microbiol analysis and enzyme assays

P. aeruginosa was examined by plating on Pseudomonas agar base (catalog no. CM0559; Oxoid Inc., Nepean, Ontario, Canada). For the enzyme assay, the culture was standardized to $OD_{600} = 0.4$ and the culture filtrates were collected by centrifugation at 5000× *g* for 15 min. The supernatant was used as a source of crude enzyme. Lipase and protease activities were examined with bacterial lipase (catalog no. 15020; Genmed Scientifics, USA) and bacterial protease assay kits (catalog no. 15021; Genmed Scientifics, USA), respectively. Assays were carried out according to the manufacturer's instructions. The absorbance was measured at 412 nm compared with the blank. One unit (IU) of protease or lipase activity was defined as the amount of enzyme required to liberate 1 μmol of tryptophan or dimercaptopropanol tributyrate equivalent per min at 37 °C. The values of enzyme activity were normalized to IU by dividing by the OD_{600} of the bacterial culture.

2.6. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out in a Mini-Protean II system (Bio-Rad, Hercules, CA) (Huang, Huang, Xu, & Zhou, 2009). Samples were subjected to electrophoresis in a 5% stacking gel and a 12.5% separating gel with 0.1% SDS at 220 V for approximately 2 h. The gel was stained with 0.1% (w/v) Coomassie brilliant blue for 12 h and then destained for 2 h.

2.7. Biofilm assay

Biofilm-forming capability of *P. aeruginosa* was measured in 96-well polystyrene plates using a crystal violet binding assay (Head & Yu, 2004). Samples (100 μl) were dispensed into wells of 96-well plates and incubated at 10 °C. Biofilm formation was determined every 12 h. Wells of the 96-well plates were rinsed three times with deionized water, stained with 125 μl of 0.25% crystal violet for 30 min at room temperature, then removed from the staining solution and rinsed three times with deionized water. After drying, the crystal violet that bound to the biofilm was solubilized with 125 μl of 95% ethanol for 30 min. The absorbance was measured at 595 nm.

2.8. Statistical analysis

All experiments were repeated at least three times. The data was analyzed by one-way analysis of variance (ANOVA). Differences were compared using Duncan's multiple range test, and effects were considered significant at $P < 0.05$.

3. Results

The culture extract samples were heated at 100 °C to inactivate enzymes and other chemical components. Previous studies have shown that after heating, AHLs were the major components and they remained intact (Hornby et al., 2001; Wang et al., 2013). The TLC profile showed that *P. aeruginosa* produced four AHL

Download English Version:

https://daneshyari.com/en/article/6403766

Download Persian Version:

https://daneshyari.com/article/6403766

Daneshyari.com