Microbiological Research 169 (2014) 759–767

Contents lists available at ScienceDirect

Microbiological Research

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

Synergistic combination of marine oligosaccharides and azithromycin against *Pseudomonas aeruginosa*

Xiaojia He[a,b], Huey-min Hwang[b], Winfred G. Aker[b], Peng Wang[a], Yunfeng Lin[c], Xiaolu Jiang[a,*], Xiaoyu He[d]

[a] College of Food Science and Engineering, Ocean University of China, Qingdao 266003, People's Republic of China
[b] Department of Biology, Jackson State University, Jackson, MS 39217, United States
[c] Department of Chemistry and Biochemistry, Jackson State University, Jackson, MS 39217, United States
[d] College of Food Science and Technology, Huazhong Agriculture University, Wuhan 430070, People's Republic of China

ARTICLE INFO

Article history:
Received 21 October 2013
Received in revised form 14 January 2014
Accepted 15 January 2014
Available online 25 January 2014

Keywords:
Alginate-derived oligosaccharide
Chito-oligosaccharides
Pseudomonas aeruginosa
Quorum sensing
Antibiotics

ABSTRACT

In this paper we describe how utilization of low molecular weight alginate-derived oligosaccharide (ADO) and chito-oligosaccharide (COS) in conjunction with antibiotics, could more effectively inhibit the growth of wild-type and resistant *Pseudomonas aeruginosa*. Inhibition is effected by modulating the bacteria's quorum sensing (QS) system, thus regulating biofilm formation and reducing resistance to antibiotic treatment. This can be demonstrated by using conventional MIC screening. COS showed synergistic effects with azithromycin, whereas ADO indicated additive effects against wild-type *P. aeruginosa*. Using electrospray-ionization mass spectroscopy (ESI-MS), matrix-assisted laser desorption/ionization-time of flightmass spectroscopy (MALDI-TOF-MS) and nuclear magnetic resonance (NMR), the chemical structure of ADO and of COS was characterized. The wild-type and resistant strains were identified by 16S rRNA sequence analysis. This report demonstrates the feasibility of attenuating the tolerance of *P. aeruginosa* to azithromycin by using specific marine oligosaccharides.

© 2014 Elsevier GmbH. All rights reserved.

1. Introduction

Increasing health consciousness of individuals and the rapid emergence of antibiotic-resistant pathogens have heightened the demand for the development of new antibiotics. It has become increasingly apparent that a new strategy is needed to cope with the challenges of diagnosing and combatting the escalating infectious diseases.

Recently, combination treatments of antibiotics and functional marine oligosaccharides have gained in popularity due to the increase in health promoting benefits. Among those oligosaccharides, alginate-derived oligosaccharides (ADO) and chito-oligosaccharides (COS) are extensively utilized in foods and medicines owing to their physiological benefits. ADO is a product of alginate degradation catalyzed by the enzyme alginate lyase (Boyd and Turvey 1978). COS is the degradation product of chitosan after enzymatic and acidic hydrolysis. In general, the molecular weights of ADO and COS are less than 10 kDa; thus they are classified as low molecular weight carbohydrate. Being polyanionic and polycationic respectively, ADO and COS are water-soluble oligosaccharides. These unique properties enable them to interact with biomacromolecules, which allow exploitation to benefit humans. Concern about their safety has been well addressed. Chitosan is "generally recognized as safe (GRAS)" by Norway, Iceland, and Belgium, as documented by US FDA GRAS Notice 000073 (FDA 2001), 000397 (FDA 2005), and 000170 (FDA 2011), respectively. Alginate salts, including sodium alginate and potassium alginate, are recognized as safe products by the U.S. Food and Drug Administration (FDA 2012).

The biocompatibility and biodegradability properties of these oligosaccharides, along with their biological properties such as immunity-enhancing and antitumor effects (Iwamoto et al. 2005; Park et al. 2011), anti-UVR and antioxidative activities (He et al. 2013), and antimicrobial activities (Dutta et al. 2012; Khan et al. 2012), make them promising candidates for various industrial and clinical applications. Among these benefits, the antimicrobial activity is considered to be one of the most important properties, which contributes to their potential applications. Šimůnek and colleagues reported that the growth rate of seven selected non-pathogenic anaerobic bacterial strains decreased in the presence of COS (Šimůnek et al. 2012). The supplementation of COS with ADO was also found to be effective against *Salmonella* colonization (Yan et al. 2011). Regarding antimicrobial activities, the ability to eradicate biofilms and inhibit quorum sensing (QS) presents a novel opportunity to control infectious bacteria (Hentzer et al. 2003).

* Corresponding author. Tel.: +86 0532 82032290; fax: +86 0532 82032389.
E-mail addresses: jiangxl@ouc.edu.cn, xiaojia.alexandrio.he@gmail.com (X. Jiang).

0944-5013/$ – see front matter © 2014 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.micres.2014.01.001

Microbial communication *via* QS has been recognized to be important in the production of virulence factors, antibiotic sensitivity, and biofilm formation. It has been reported that ADO is a novel and safe antibiofilm therapy against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* biofilms (Powell et al. 2013). In addition, COS was also able to perturb *Streptococcus mutans* by modulating biofilm formation and its persistence (An et al. 2009; Chen et al. 2011).

Although the exact mechanisms of antimicrobial activity of ADO and COS remain to be elucidated, some reports have indicated that structurally the negatively/positively charged functional groups within them interact with the positively/negatively charged cell membrane that leads to the leakage of proteinaceous and other intracellular constituents of the microorganisms. Furthermore, their synergistic interactions with antibiotics also account for their medicinal benefits. Khan et al. (2012) demonstrated that ADO increased the efficacy of conventional antibiotics of several classes against multi-drug-resistant strains, including *Pseudomonas*, *Acinetobacter*, and *Burkholderia* spp. On the other hand, Tin et al. (2009, 2010) reported that COS-antibiotics could be used as a combination treatment against *P. aeruginosa* and *Staphylococcus aureus*. A combination of one-fourth minimum inhibition concentration (MIC) of the three antibiotics (sulfamethoxazole, tetracycline and ceftriazone) with one-eighth MIC of COS synergistically acted against *S. aureus* (Tin et al. 2010).

In this study, we describe the use of COS- and ADO-azithromycin as therapeutic strategies against the Gram-negative bacterium *P. aeruginosa*. To our knowledge, this is the first attempt to investigate the mechanism of antimicrobial activity of the above-mentioned oligosaccharides in interfering with bacterial QS systems.

2. Materials and methods

2.1. Materials

Alginate and chitosan were purchased from Hua-hai Pharmaceutical industry (Qingdao, China), and Qingdao Hecreat Bio-tech Company Ltd. (Qingdao, China), respectively. Azithromycin (AZM), cefotaxime (CTX), oxytetracycline (OTC), and ampicillin (AMP) were obtained from Conba Bio-pharm. Co., Ltd. (Jinhua, Zhejiang, China), National Institutes for Food and Drug Control (Beijing, China), Qilu Pharmaceutical Co., Ltd. (Jinan, Shandong, China), and North China Pharmaceutical Group Co. (Shijiazhuang, Hebei, China), respectively. All other chemicals are commercial products with best grade.

2.2. Synthesis and characterization of oligosaccharides

ADO and COS were generated from alginate extracted from alginate (M/G, 2.28; molecular weight (MW), 300 kDa) and chitosan (degree of acetylation (DA), ~65%; MW, 250 kDa), respectively, in accordance with the publication by He et al. (2013). Purified oligosaccharides were analyzed based on electrospray-ionization mass spectroscopy (ESI-MS, TSQ LC/MS/MS, Finnegan Company) and matrix-assisted laser desorption/ionization-time of flight mass spectroscopy (MALDI-TOF-MS, Bruker Daltonics Co. Ltd., Bremen, Germany) analysis. ADO was further characterized by hydrogen-1 nuclear magnetic resonance (H NMR). ^{1}H–^{1}H COSY, HMQC, and HMBC were performed on a JEOL ECP 600 MHz spectrometer at 298 K. Acetone was used as the internal standard (δ_H = 2.10 ppm, δ_C = 31.45 ppm and 216 ppm).

2.3. Bacterial strains

Cultures of wild-type *P. aeruginosa* were isolated from a specimen of brown algae from the South China Sea (GenBank database under accession numbers KC959478). Cultivation and enrichment of the wild type strain were carried out with nutrient broth/agar (pH 7.2) medium at 32 °C. Resistant strains (GenBank database under accession numbers KC959479) were obtained and purified in conjunction with the major classes of antibiotics (β-lactams-AMP, macrolides-AZM, cephalosporin-CTX, tetracyclines-OTC). The employed concentrations were ten fold higher than their respective MICs. The resistant strain was identified as multi-drug resistant strain, showing resistance to AZM, CTX, OTC and AMP. Both wild-type and resistant strains were identified *via* 16S rRNA sequencing and cloning (see Supplemental material).

2.4. MIC determination

P. aeruginosa was incubated in nutrient broth (NB) at 37 °C for 24 h. The cultured suspension was immediately diluted to 1×10^8 CFU mL^{-1}. Bacteria were then incubated with antibiotics and/or oligosaccharides with a final density of 5×10^5 CFU mL^{-1} at 32 °C for 20 h. MICs were determined by the two-fold broth microdilution method according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines (CLSI 1997) combined with the protocols published by Espinel-Ingroff et al. (1991) and Sewell et al. (1994). Additionally, 2 mg mL^{-1} aliquots of oligosaccharides were used to test the synergistic effect of antibiotics in combination with them. The stock solutions were 1.22 mg mL^{-1}, 1.07 mg mL^{-1}, 1.24 mg mL^{-1}, and 1.13 mg mL^{-1}, for AZM, OTC, AMP, and CTX, respectively, and were stored at −20 °C. The fractional inhibitory concentration (FIC) was calculated as follows (Tin et al. 2010; Chung et al. 2011):

FIC of oligosaccharides (FIC_a) = concentration of oligosaccharides in combination/MIC of compound **A** alone;
FIC of antibiotics (FIC_b) = MIC of antibiotics in combination/MIC of antibiotics **B** alone.
The sum of fractional inhibitory concentration (FIC_s) indices of two compounds in the combination was calculated as follows:
$FIC_a + FIC_b = FIC_s$
Synergism = $FIC_s \leq 0.5$; antagonist = $FIC_s \geq 4$; additive = $FIC_s > 0.5$ and ≤ 1; indifference = $1 < FIC_s < 4$.

2.5. Effects on antimicrobial activity

The antimicrobial activities of oligosaccharides and antibiotics were tested against *P. aeruginosa*, using inocula of 1×10^8 CFU mL^{-1}. ADO, COS, plus AZM were combined in NB medium with respective final concentrations of 2 mg mL^{-1}, 2 mg mL^{-1}, and 16 μg mL^{-1} in a final volume of 100 mL. The ADO (2 mg mL^{-1}) or COS (2 mg mL^{-1}) with AZM (16 μg mL^{-1}) were combined in NB medium. Moreover, to compare the effects of ADO and COS, the solution without AZM was added to NB medium. Each bacterium sample (wild-type and resistant strains) was incubated at 37 °C for 48 h after which CFUs were counted.

2.6. Effects on biofilm susceptibility

Colony biofilms were prepared by growing bacteria on flat-bottomed polystyrene 96-well mircoplates (Solarbio, Beijing, China) with NB medium as described by Peeters et al. (2008) with modification. Briefly, 100 μL aliquots of treated cell culture were added into 96-wells at 32 °C and incubated without shaking for 48 h. Then, the wells were washed with sterile distilled water and dried. After which, supernatants were removed. Biofilm staining was achieved by first adding 150 μL to the wells. After 20 min, the excess crystal violet was removed by washing the plates under running distilled water. Finally, 200 μL aliquots of acetic acid (30%, v/v) were used to release the bounded crystal violet. The absorbance

Download English Version:

https://daneshyari.com/en/article/2092405

Download Persian Version:

https://daneshyari.com/article/2092405

Daneshyari.com