Biochemical Engineering Journal 101 (2015) 18–22

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Short communication

Universal cell capture by immobilized antimicrobial peptide plantaricin

Saadet Albayrak Guralp[a,*], Ilkay Hilal Gubbuk[b], Semahat Kucukkolbasi[b], Erdogan Gulari[a]

[a] Department of Chemical Engineering, University of Michigan, Ann Arbor, MI, USA
[b] Department of Chemistry, University of Selcuk, Konya 42031, Turkey

ARTICLE INFO

Article history:
Received 8 January 2015
Received in revised form 24 April 2015
Accepted 27 April 2015
Available online 29 April 2015

Keywords:
Biomedical
Biosensors
Immobilization
Antimicrobial peptide
Cell capture
Viability

ABSTRACT

Plantaricin-423 is a short antimicrobial peptide and displays bactericidal activities against several food-borne pathogens and spoilage Gram positive bacteria. The goal of this study was to investigate the potential of using immobilized plantaricin for capturing microorganisms on glass arrays. The peptide used for immobilization consists of N-terminal domain of Plantaricin-423 with an additional cysteine (Pln-17C) to form disulfide bonds with thiol groups present on silanized slides. Our results showed that Pln-17C is able to capture all six strains that were tested with varying affinities. The cell capture occurred within five minutes of incubation and the binding level was highest for *Listeria innocua* followed by other Gram positive strains tested. Pln-17C was also able to capture *Escherichia coli* with lower affinity, but the binding was significantly lower for *Mycobacterium smegmatis* compared to other strains. In addition, we have observed that immobilized Pln-17C maintained its anti-listerial activity; however, it did not kill *E. coli* as expected. Our results demonstrate the feasibility of utilizing antimicrobial peptides in biosensors for pathogen detection and for creating antimicrobial surfaces. Moreover, in combination with other peptides, different target species from food-borne pathogens to biodefense agents can be captured on more stable, economic, and robust platforms.

1. Introduction

Antimicrobial peptides (AMPs) belong to a class of natural microbicidal molecules and present a large spectrum of activities against a broad range of organisms [1–3]. These peptides display their activity by binding to surface components of target cells and causing membrane disruption, which is driven by hydrophobicity/amphipathicity, electrostatic interactions, and peptide's secondary structure [4]. The semi-selective cell recognition and binding properties of AMPs make them potentially valuable detection agents. The utilization of these peptides as recognition elements in array formats has been exploited in numerous studies with the goal of developing robust, portable, and highly-sensitive biosensors for pathogen detection. Kulagina and co-workers reported several studies where AMPs like Magainin-1, Polymyxins B and E, Cecropin A and Melittin were immobilized in direct and sandwich binding formats for the detection of pathogenic bacteria including *Escherichia coli* O157:H7, *Salmonella typhimurium* and *Coxiella burnetti* [5–8]. In another notable study by Mannoor et al., authors developed a highly sensitive electronic biosensor by hybridizing Magainin-1 onto gold microelectrodes for the detection of *E. coli* and other bacteria and real-time monitoring of their interaction with AMPs via impedance spectroscopy [9]. More recently, a Class-IIa bacteriocin, Leucocin-A, and its C-terminal fragment immobilized on gold substrates were shown to capture primarily *Listeria monocytogenes* and to a lesser degree other Gram-positive pathogens *Staphylococcus aureus* and *Enterococcus hirae* with no observed binding to Gram-negative bacteria [10,11].

Bacteriocins are a group of ribosomally synthesized antimicrobial peptides (AMPs) mainly produced by lactic acid bacteria (LAB) [12]. Class-IIa group of bacteriocins, containing more than 20 peptides, consist of a highly conserved cationic N-terminal domain and a less conserved but more hydrophobic/amphiphilic C-terminal domain with a flexible hinge in between [13]. Mutagenesis studies indicate that the N-terminal domain is responsible for initiating the binding of the peptide to the target cell surface whereas the amphiphilic C-terminal domain penetrates into the hydrophobic core of the membrane (through specific interactions with the mannose phosphotransferase protein complex) allowing for the bacteriocin to form pores within the membrane that

* Corresponding author at: 2800 Plymouth Rd., B028-1051W, Ann Arbor, MI 48109, USA. Tel.: +1 734 764 3518; fax: +1 734 647 5432.
E-mail addresses: saadetal@umich.edu (S.A. Guralp), ihilalg@gmail.com (I.H. Gubbuk), ksemahat@gmail.com (S. Kucukkolbasi), gulari@umich.edu (E. Gulari).

http://dx.doi.org/10.1016/j.bej.2015.04.018
1369-703X/

leads to eventual cell death [14,15]. One member of this group, called Plantaricin-423 (Pln-423), is a small and heat-stable peptide with bactericidal activities against several foodborne pathogens and spoilage Gram positive bacteria including *Listeria* and *Enterococcus* species [16,17]. Our group have recently constructed and screened a library coding for more than twelve thousand Pln-423 derivatives against *Listeria innocua* to generate novel peptides with enhanced activity and discovered that one of the derivatives carrying only the N-terminal domain of the full-length peptide retained partial activity against *L. innocua* growth [18]. This finding, supporting previous predictions about the role of N-terminal domain in cell binding and AMP activity, prompted us to investigate the cell capture-capability of surface-bound Pln-423 fragment in an array format.

To facilitate peptide immobilization, the first 17 amino acids of Pln-423 was obtained with an additional cysteine residue on the C-terminal end (Pln-17C) and the covalent attachment of the peptide molecules to the –SH modified surface was achieved via the formation of disulfide bridge between thiol groups of cysteine and the surface groups. Several strains of Gram positive and Gram negative bacteria were incubated on Pln-17C-immobilized surfaces and the binding characteristics were investigated by fluorescence microscopy. The results of the present study show that Pln-17C is capable of capturing a variety of bacteria including Gram negative *E. coli* and highlight the potential of utilizing this peptide for universal cell detection in biosensors.

2. Materials and methods

2.1. Strains and chemicals

Bacterial strains used in this work were *E. coli* JE5505 (Yale Genetic Stock Center), *Bacillus coagulans* NRS-609, *E. hirae* B-1295, *M. smegmatis* B-24018 (ARS Culture Collection, Department of Agriculture), *Staphylococcus epidermidis* ATCC 12228 and *Listeria innocua* ATCC 33090. Plantaricin-17C (Pln-17C, KYYGNGVTCGKHSCSVN ***C***) was obtained from Peptide 2.0, Inc. (VA) and dissolved in 1× phosphate-buffered saline (PBS), pH 7.4, to a final stock concentration of 2.5 mg/ml and stored in aliquots at −20 °C.

2.2. Surface functionalization and characterization

For surface activation, glass slides were immersed in hot piranha solution (3:1, concentrated H_2SO_4:30% H_2O_2) for 1 h and then rinsed with deionized water and ethanol. After activation, the cleaned glass substrates were immersed in 100 mM (3-mercaptoproyl) trimethoxysilane (MPTS)-toluene solution for 6 h at room temperature to form a layer of thiol functional groups on the surface. The resulting –SH modified surfaces were washed with toluene and dried with compressed air. To characterize surface hydrophobicity, water-contact angle measurements were taken before and after silanization using a goniometer.

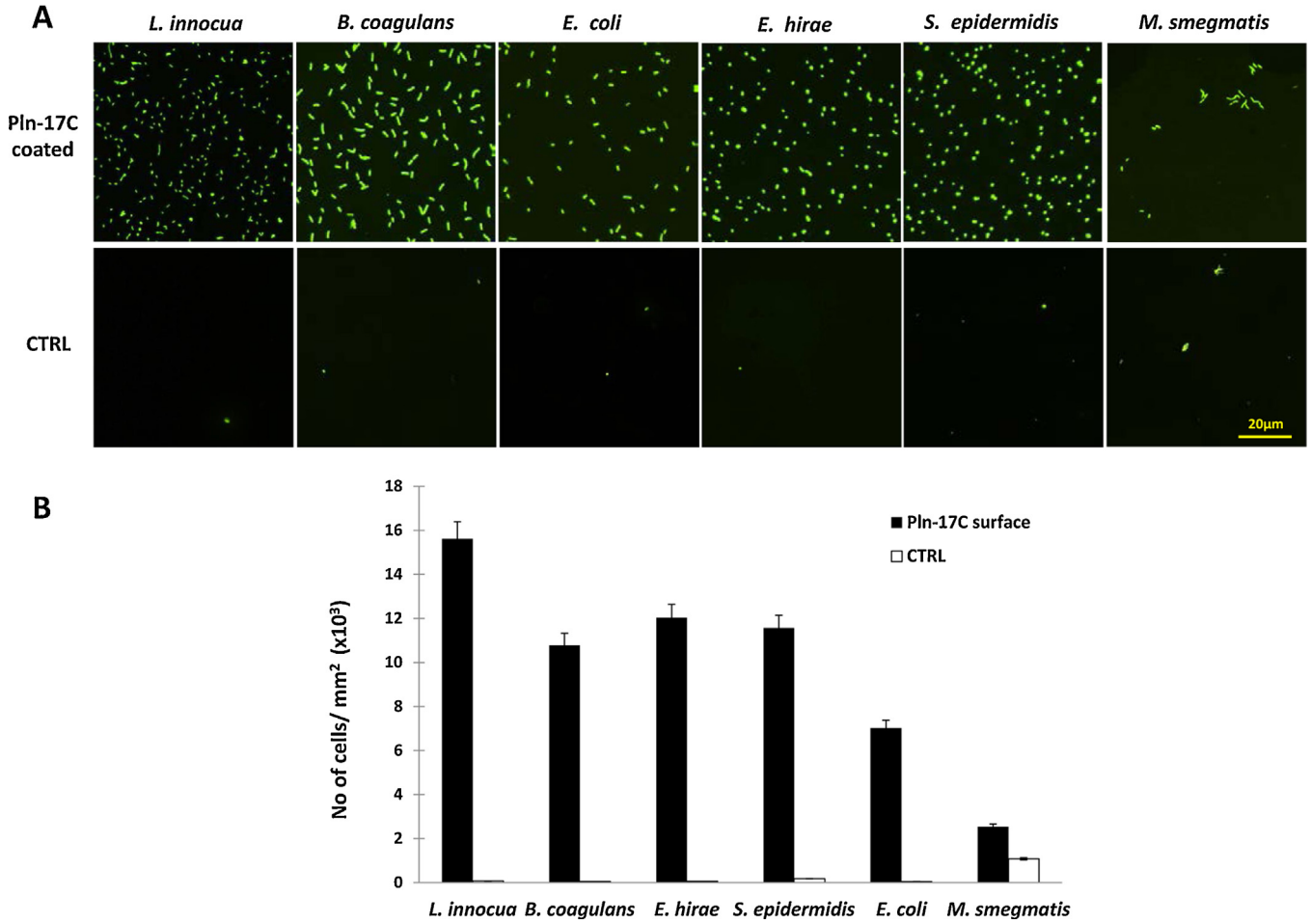

Fig. 1. Binding of bacterial cells by immobilized Pln-17C on silanized surfaces.
(A) Fluorescence microscopy images for each cell type tested. Control (CTRL) reactions were set up by incubating cells on peptide-free spots. (B) Plot shows average number of surface-bound bacteria on both Pln-17C coated and control surfaces. Experiments were performed in triplicate with 3 replicate samples in each set up.