Process Biochemistry 49 (2014) 1699–1707

Contents lists available at ScienceDirect

Process Biochemistry

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

Identification and biochemical characteristics of lipopeptides from *Bacillus mojavensis* A21

Hanen Ben Ayed[a], Noomen Hmidet[a,*], Max Béchet[b], Marlène Chollet[b], Gabrielle Chataigné[b], Valérie Leclère[b], Philippe Jacques[b], Moncef Nasri[a]

[a] Laboratoire de Génie Enzymatique et de Microbiologie, Université de Sfax, Ecole Nationale d'Ingénieurs de Sfax, B.P. 1173-3038 Sfax, Tunisia
[b] ProBioGEM – EA1026, Polytech'Lille/IUTA, Université Lille-Nord de France, F-59655 Villeneuve d'Ascq, France

ARTICLE INFO

Article history:
Received 25 March 2014
Received in revised form 30 June 2014
Accepted 3 July 2014
Available online 11 July 2014

Keywords:
NRPS
PCR
MALDI-TOF-MS
Fengycin
Surfactin
Pumilacidin

ABSTRACT

This study reports the potential of a marine bacterium, *Bacillus mojavensis* A21, to produce lipopeptide biosurfactants. The crude lipopeptide mixture was found to be very effective in reducing surface tension to 31 mN m^{-1}. PCR experiments using degenerate primers revealed the presence of nonribosomal peptide synthetases genes implied in the biosyntheses of fengycin and surfactin. Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS) performed on whole cells of *B. mojavensis* A21 confirmed the presence of lipopeptides identified as members of surfactin and fengycin families. Further, a detailed analysis performed by MALDI-TOF-TOF revealed the presence of pumilacidin compounds. The crude lipopeptide mixture was tested for its inhibitory activity against Gram-positive and Gram-negative bacteria, and fungal strains. It was found to display significant antimicrobial activity. Strain A21 lipopeptide mixture was insensitive to proteolytic enzymes, stable between pH 3.0 and 11.0, and resistant to high temperature. Production of lipopeptides is a characteristic of several *Bacillus* species, but to our knowledge this is the first report involving identification of pumilacidin, surfactin and fengycin isoforms in a *B. mojavensis* strain.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

The genus *Bacillus* is known to produce a broad spectrum of biologically active molecules with great potential for medical and biotechnological applications. Among these molecules, biosurfactants have received great attention in different fields, including phytosanitary sector, medicine, cosmetics, food and feed additives, bioremediation, etc., [1]. Structurally, biosurfactants are amphiphilic molecules and comprise various different chemical structures, such as glycolipids, lipopeptides, polysaccharide–protein complexes, phospholipids, fatty acids and neutral lipids [2]. Compared to chemical surfactants, biosurfactants have several advantages, including low toxicity, high biodegradability under natural conditions, ecological acceptability and effectiveness at extreme temperatures and pH values [3].

Lipopeptides are among the most commonly isolated and characterized biosurfactants. They have received great attention due to their medical, food and biotechnological applications. Further, they were found to remove efficiently petroleum hydrocarbons and heavy metals from contaminated soils [4]. The lipopeptides produced by numerous *Bacillus* spp. are classified into three families depending on their amino acids sequence: surfactins, iturins and fengycins [5] and are considered as safe. These advantages make lipopeptides potential alternatives to chemically synthesized surfactants. From another side, the fast progress of biotechnology has accelerated the research and development of new and more effective lipopeptides.

These molecules are synthesized by multimodular enzymes complexes known as nonribosomal peptides synthetases (NRPSs) [5,6]. Lipopeptides contain hydrophilic peptides, which differ in amino acid composition and sequence (seven to ten amino acids) linked to a hydrophobic fatty acid with different chain lengths and isomeries [5].

Among the *Bacillus* species, *Bacillus subtilis* is best known for the production of lipopeptides, mainly surfactin and fengycin [7]. Surfactin is one of the most effective biosurfactants and shows several pharmacological activities including the antimicrobial, antiviral, antitumoral and antifibrinolytic ones. It is a cyclic lipoheptapeptide which contains a β-hydroxy-fatty acid with a chain length of 13–15 carbon atoms [8]. Several variants of surfactin have been described such as lichenysin from *Bacillus licheniformis* or pumilacidin from *Bacillus pumilus*. In addition to surfactin, fengycin is

* Corresponding author. Tel.: +216 22 76 31 14; fax: +216 74 275 595.
E-mail address: hmidet_noomen@yahoo.fr (N. Hmidet).

http://dx.doi.org/10.1016/j.procbio.2014.07.001
1359-5113/© 2014 Elsevier Ltd. All rights reserved.

another best-known lipopeptide, which exhibits several biological activities, including activity against filamentous fungi [9]. Fengycin is a cyclic lipodecapeptide containing a β-hydroxy-fatty acid with a length of 15–18 carbon atoms [9]. Two variants of fengycins (A and B) have been already described in the literature. They differ by the amino acid residue in position 6. Few studies reported the production of lipopeptides by *Bacillus mojavensis* strains [10,11].

In this study, *B. mojavensis* A21 isolated on the basis of its proteolytic activity [12] was also found to produce high amounts of biosurfactants (based on their ability to reduce surface tension from 71 to 31 mN m^{-1}) [13]. The biosurfactant mixture showed high physico-chemical properties in terms of the surface activities and emulsification index. Further, the mixture was found to remove diesel more effectively than synthetic surfactants. Therefore, this study reports on (i) the detection of NRPS genes by PCR and (ii) structural characterization of lipopeptides by MALDI-TOF-MS. In addition, the stability of the produced lipopeptides under extreme conditions was investigated in order to estimate their potential applications.

2. Materials and methods

2.1. Bacterial strain and lipopeptides production

The microorganism used in this study was isolated in our laboratory from marine water in Sfax, Tunisia. It was identified as *B. mojavensis* A21 based on its biochemical and physiological characteristics, and on the 16S rRNA gene sequence analysis. It was assigned the accession number EU366229 [12].

The strain *B. mojavensis* A21 was inoculated in 250 mL Erlenmeyer flask containing 25 mL Luria–Bertani (LB) broth medium (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl) and cultivated at 37 °C for 24 h under agitation at 200 rpm. For lipopeptides production, culture was conducted in 1 L Erlenmeyer flask containing 100 mL of Landy medium [14] consisting of: glucose, 20 g/L; L-glutamic acid, 5 g/L; yeast extract, 1 g/L; K_2HPO_4, 1 g/L; $MgSO_4{\cdot}7H_2O$, 0.5 g/L; KCl, 0.5 g/L; $CuSO_4$, 1.6 mg/L; $Fe_2(SO_4)_3$, 1.2 mg/L and $MnSO_4$, 0.4 mg/L. The medium was complemented by 100 mM MOPS and the initial pH was adjusted to 7.0 with 3 M KOH. Culture was carried out for 72 h at 30 °C under shaking at 160 rpm. After fermentation, the culture broth was centrifuged at 13,000 × *g* for 30 min at 4 °C, and the supernatant containing the crude lipopeptide was collected. Lipopeptide molecules were partially purified from the cell-free supernatant by different steps of ultrafiltration/diafiltration. The crude lipopeptide mixture obtained was evaluated for its antimicrobial activity and its stability against extreme conditions. All experiments were carried out in triplicates.

Lipopeptide production was also analyzed by measurement of surface tension during the growth of the strain. The surface tension of the cell-free supernatant was determined according to the Du Noüy ring method in a TDI tensiometer (Lauda, Königshofen, Germany) as described by Leclère et al. [15]. The values obtained are the mean of three measurements.

Glucose concentration was analyzed in the filtered samples by HPLC Spectra SYSTEM P1000 XR supplied by Thermoelectron Corporation (Thermo Fisher Scientific Inc., Waltham, MA, USA) using a Fast Fruit Juice column (150 × 7.8 mm, Waters Corp., Milford, MA, USA). The flow rate is set at 0.8 ml/min and the column was maintained at 55 °C. Elution was achieved under 0.05% H_3PO_4. Detection was carried out by using a refractometer Spectra System RI-150.

2.2. Extraction and quantification of lipopeptides

Lipopeptides were extracted from culture supernatant by solid phase extraction using C18 Maxi clean cartridges (Extract – Clean SPE 500 mg, Grace Davison-Alltech, Deerfield, IL, USA) according to Guez et al. [16]. The supernatant was applied to a C_{18} cartridge, which retained lipopeptides. The cartridge was rinsed with 8 mL of bi-distilled water and lipopeptides were then eluted with 8 mL of 100% methanol. After evaporation of the solvent, the crude lipopeptide mixture was dissolved in 200 μL methanol.

Lipopeptides were analyzed and quantified by reversed-phase high-performance liquid chromatography (600 s, Waters Corp.) using a C_{18} column (5 μm, 250 mm × 4.6 mm, 218 TP, VYDAC, Hesperia, CA, USA).

2.3. Detection of NRPS genes by PCR and DNA sequencing

Two pairs of degenerate primers (Af2-F/Tf1-R and As1-F/Ts2-R), designed using the alignment of adenylation or thiolation domains that compose lipopeptide synthetases, were used for the detection by PCR of the NRPS genes (Table 1) [17]. DNA was extracted from overnight culture of A21 strain using the Wizard Genomic DNA Purification Kit and protocol (Promega Corp., Madison, USA).

The PCR thermal cycle program included an initial denaturation step at 94 °C for 3 min, followed by 30 cycles, with denaturation at 94 °C for 1 min, annealing at 43 °C for 30 s, with As1-F/Ts2-R, and at 45 °C with Af2-F/Tf1-R primers, and extension at 72 °C for 45 s. Final extension was performed at 72 °C for 10 min. The Taq polymerase used was "Master Mix" (Thermo Scientific Fermentas, Illkirch, France) with a final primer concentration of 1.2 μM. PCR products were analyzed by 0.7% (w/v) agarose gel electrophoresis.

PCR products were purified with QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) and then cloned into pGEM-T Easy Vector (Promega Corp.). Recombinant plasmids were introduced into *Escherichia coli* JM109 cells by heat shock, according to the manufacturer's protocol (Promega). Transformants were selected on Luria–Bertani (LB) solid medium supplemented with ampicillin, IPTG (isopropyl-β-D-thiogalactopyranoside) and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) at final concentrations of 100 μg/mL, 200 μg/mL and 20 μg/mL, respectively. White colonies were picked and then cultivated in LB medium supplemented with 100 μg/mL ampicillin for 24 h at 37 °C. Plasmids were isolated from the transformed cells using the QIAprep Spin Miniprep kit (Qiagen, Germany). Cloned PCR products were sequenced using the universal primers pUC-M13-R/F (Eurofins MWG Operon, Ebersberg, Germany). DNA sequences were analyzed

Table 1
List and characteristics of primers used in this study.

Primer names	Sequence of primers[a]	HyC[b]	Expected fragment size (bp)	Identified nonribosomal lipopeptides	References
Af2-F	GAATAYMTCGGMCGTMTKGA	34	443, 452,455	Fengycins	[17]
Tf1-R	GCTTTWADKGAATSBCCGCC	72			
As1-F	CGCGGMTACCGVATYGAGC	12	419, 422, 425, 431	Surfactins	[17]
Ts2-R	ATBCCTTTBTWDGAATGTCCGCC	36			

bp, base pair.
[a] Using IUPAC DNA code: Y = C or T, M = A or C, K = G or T, W = A or T, D = G, A or T, S = G or C, B = G, T or C, R = A or G.
[b] Coefficient of hybridization (HyC) calculated as described by Tapi et al. [17].

Download English Version:

https://daneshyari.com/en/article/34502

Download Persian Version:

https://daneshyari.com/article/34502

Daneshyari.com