Iranian Journal of Pharmaceutical Sciences
Spring 2011: 7(2): 117-121
ijps.sums.ac.ir

Original Article

Biosurfactant Producing Bacteria on Oily Areas of Ruminant Skin

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Abstract

Biosurfactants are surface-active compounds produced by microorganisms. In this study, we collected 60 inguinal area and ear canal samples from cows, sheep, and goats (each, 10 animals) and screened for biosurfactant-producing bacteria. We also determined the genera of culturing strains. Fifty six hemolytic bacterial strains (27, 22 and 7, from cows, sheep and goats, respectively) were isolated. Oil spreading test and bioemulsifying activities were measured for all isolates. The cows' samples had higher population of positive strains than other animals, so that 5 isolates from inguinal area and 4 from ear canal samples (16.1%) were positive for all tests. For sheep, the numbers were 6 and one (12.5%) while for goats one and two (5.3%), respectively. Totally, 19 isolates (33.9%) were positive for all examinations out of them 12 were gram positives. The microorganisms isolated in this study could well be sources of novel biosurfactants. Further investigation into the composition of the biosurfactants and phylogenetic determination of biosurfactant producing bacteria is suggested.

Keywords: Biosurfactant; Emulsification; Oil spreading; Ruminant skin.
Received: February 2, 2011; *Accepted:* April 5, 2011

1. Introduction

Biosurfactants are amphiphilic compounds produced on living surfaces, mostly on microbial cell surfaces, or excreted extra cellularly and contain hydrophobic and hydrophilic moieties that confer the ability to accumulate between fluid phases, thus reducing surface and interfacial tension at the surface and interface, respectively [1]. They are a structurally diverse group of surface-active molecules synthesized by microorganisms [2].

Rosenberg and Ron [3] suggested that biosurfactants can be divided into low-molecular-mass molecules, which efficiently lower surface and interfacial tension, and high molecular- mass polymers, which are more effective as emulsion-stabilizing agents.

Apart from their obvious role as agents that decrease surface and interfacial tension, thus promoting the formation and stabilization of emulsions, surfactants can have several other functions. They improve consistency

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Table 1. Biosurfactant-producing bacteria isolated from Cows

Isolate	Ear			IA		
Ear/IA*	$E_{24h\%}$	$E_{72h\ \%}$	$O.S._{SD}(cm)^{**}$	$E_{24h\%}$	$E_{72h\%}$	$O.S._{SD}(cm)$
Bacillus spp/Escherichia spp	56	56	4.55±0.05	40	47.8	5.75±0.75
Bacillus spp/*Bacillus* spp	40	52	3.2±03	44	52.3	5.00±0.5
Staphylococci/Providentia spp	40	48	3.55±0.25	50	63.6	5.55±0.25
Lacto*bacillus* spp/Aeromonas spp	44	48	5.25±0.65	52.3	47.8	5.75±0.15
Bacillus spp/Staphylococcus spp	45	44	6.45±1.05	42.8	45	5.65±0.35
Pasteurella spp/*Bacillus* spp	47	60	5.4±0.2	52.3	47.8	4.5±0.3
Bacillus spp/*Bacillus* spp	48	48	4.75±0.15	50	55	5.75±0.45
Lacto*bacillus* spp/Lacto*bacillus* spp	56	56	4.55±0.05	54.5	60.8	4.75±0.15
Acinetobacter spp/Acinetobacter spp	56.5	52.1	6.02 ±0.25	59	58.3	5.4±0.1
Lacto*bacillus* spp/*Bacillus* spp	52	52	4.15±0.25	45.4	43.4	4.55±0.25
Bacillus spp/Falavobacterium spp	48	48	5.4±0.2	52.1	48	5.25±0.25
Bacillus spp/Lacto*bacillus* spp	48	52	5.05±0.05	45.4	56	4.6±0.0
Staphylococcus spp./Lacto*bacillus* spp	40	48	5.7±0.1	54.5	56	4.9±0.3
Bacillus spp/-	44	44	7.05±0.25	-	-	-
Control	50	50	3.55±0.05	50	50	3.55±0.05

*IA stands for inguinal area, ** O.S. SD for oil spreading and the standard deviation

and texture of fat-based products [4]. Several biosurfactants have shown antimicrobial action against bacteria, fungi, algae and viruses [5].

There are many advantages of biosurfactants compared to their chemically synthesized counterpart. Research in this subject, will make them highly sought after biomolecules for present and future applications as fine specialty chemicals, biological control agents and new generation molecules for pharmaceutical, cosmetic and health care industries.

Although a large number of biosurfactant producers have been reported in the literature, reports regarding screening and isolation of these microorganisms from animals are scarce. The primary aim of the present study was to investigate biosurfactant producing bacteria (PBB) habitats in ear canal and inguinal areas (IA) (as oily skin areas) of ruminants.

2. Materials and methods

2.1. Sample collection

The study was carried out hrough June 2009 to December 2010 on 30, Holestein cows, native sheep and goats, (each 10) randomly selected from animals in farms of Shahrekord University.

All animals were adults and were found to be apparently healthy. Samples were collected, by inserting sterile cotton-tipped applicator sticks into the ear canal and rubbering on inguinal areas. The surfaces were thoroughly rubbed by rolling the swabs to attain effective contact. The swabs were put in separate sterile test tubes containing sterile pepton water (Merck cat. QB-65-5015), labeled and kept in a cool box and transported to the veterinary microbiology laboratory of veterinary college of Shahrekord University on the day of sampling for further processing.

For bacteriological examination, the swabs were removed from the bottles and streaked over the plates of blood agar-base (Scharlau 01-352) supplemented with 7% sheep blood. The streaking was further spread with inoculating loop to aid colony isolation. The plates were labeled and incubated aerobically at 37 °C for 24-48 h [6].

One colony was selected from those colonies that have similar morphologies and sub-cultured on blood agar plates for further analysis.

2.2. Screening methods

The first screening test for identification and isolation of BPB is hemolysis test [7]. For assaying hemolytic activity, each strain was streaked onto blood agar plates and incubated for 48 h at 37 °C. The plates were visually

Table 2. Biosurfactant-producing bacteria isolated from Sheep

Isolate	Ear			IA		
Ear/ IA*	$E_{24h\%}$	$E_{72h\%}$	O.S. $_{SD}$**(cm)	E24h%	E72h%	O.S.$_{SD}$(cm)
Lacto*bacillus* spp /Lacto*bacillus* spp	44	44	4.95±0.35	47	54.5	5.25 ±0.05
Lacto*bacillus* spp/Streptococcus spp.	40	44	5.55±0.15	47	43.5	5.800 ±0.15
Bacillus spp / *Bacillus* spp	44	45.5	3.2±0.4	48	52	3.4 ±0.2
Bacillus spp /Lacto*bacillus* spp	52	48	4.4±0.1	47.6	45.5	6.5 ±0.25
Bacillus spp / *Bacillus* spp	40	38.5	4.85±0.25	42.9	45.5	4.5 ±0.25
Bacillus spp /Lacto*bacillus* spp	44	52	4.4±0.2	50	43.3	5.5 ±0.05
Aeromonas spp/ *Bacillus* spp	48	50	3.4±0.15	47.8	45.8	3.4 ±0.25
Staphylococcus spp. / *Bacillus* spp	52	48	5.3±0.15	48.8	50	5.7 ±0.05
Escherichia spp./ *Bacillus* spp	42.3	37.1	5.5±0.2	34.8	40	6.1 ±0.2
Staphylococcus spp. /Lacto*bacillus* spp	40	46.2	3.5±0.05	40	40.7	3.6 ±0.4
Pseudomonas spp/Pseudomonas spp	44	51.9	5.2±0.1	50	60	6.2 ±0.15
Control	40	40	4.35±0.15	40	40	4.4 ±0.15

*IA stands for inguinal area, **O.S. SD for oil spreading and the standard deviation.

inspected for zones of clearing around the colonies, indicative of biosurfactant production. After gram staining, catalase and oxidase tests, identification of the isolated hemolytic positive strains were done using a standard biochemical scheme according to Balows et al [8].

Each hemolytic isolate was inoculated in tubes containing Lauria bertani broth (LB, Biomark-B699) media and incubated at 37 °C for 72 h with shaking (~50 rpm). For each set of cultures one tube of strile LB was also incubated to use as control in further analysis.

For the oil spreading technique (OS), 50 ml of distilled water was added to a large petri dish (25 cm diameter) followed by addition of 20 µl of n-Decane (Merck, UN 2247) to the surface of the water. Ten microliters of cell-free broth of LB culture (Centrifuged at 10000 rpm for 10 min.) were then added to the surface of oil [9]. The diameter of the clear zone on the oil surface was measured. The diameters of triplicate samples from the same culture of each strain were determined.

The emulsifying capacity was evaluated by an emulsification index (E24). The E24 of culture samples was determined by adding 1.5 ml of kerosene and 1.5 ml of the cell-free broth in test tube, vortexed at high speed for 2 min and allowed to stand for 24 h and 72 h. The E24 (and E72) index is given as the percentage of the height of emulsified layer divided by the total height of the liquid column (cm). The percentage of emulsification index calculated by using the following equation [10],

$$E_{24} = \frac{\text{Height of emulsion formed} \times 100}{\text{Total height of solution}}$$

For each test strain, centrifuged samples of incubated tubes of strile LB were used as control.

3. Results

After culture and incubation of 60 samples (20 from each animal species, 10 ear and 10 IA) 56 hemolytic strains (27, 22 and 7, from cows, sheep and goats, respectively) were isolated. OS and bioemulsifying activities were measured for all isolates (Tables 1-3).

The cow's samples had higher population of E24, E72 and OS positives than other animals, so that 5 isolates from IA and 4 from ear canal samples (16.1%) were positive for all tests. For sheep the numbers were 6 and one (12.5%) while for goats one and two (5.3%) respectively. Totally 19 isolates (33.9%) were positive for all examinations, out of them 12 were gram positives.

More sensitive OS test was positive for 13 IA and 12 ear canal isolates of cows (44.6%), for sheep the numbers were 7, 1 (14.3%) and for goat 2, 3 (8.9%), respectively. Totally 38 isolates (67.8%) were positive for this test (Tables 1-3).

4. Discussion

Table 3. Biosurfactant-producing bacteria isolated from goats

Isolate	Ear			IA		
Ear/ IA*	$E_{24h\%}$	$E_{72h\%}$	O.S.** $_{SD}$ (cm)	$E_{24h\%}$	$E_{72h\%}$	O.S. $_{SD}$ (cm)
Providentia spp / *Bacillus* spp	54	54	7.1 ±2.05	50	54.2	5.5 ±0.05
Bacillus spp / *Bacillus* spp	45.8	45.5	5.4 ±0.1	45.8	50	6.3 ±0.75
Lactobacillus spp/*Lactobacillus* spp	54.1	50	6.4 ±0.1	45.8	45.5	4.1 ±0.6
Staphylococcus spp. / -	45.8	47.8	4.9 ±0.4	-	-	-
Control	45	45	3.55 ±0.05	45	45	3.55 ±0.05

*IA stands for inguinal area, **O.S. SD for oil spreading and the standard deviation

Hemolytic activity appears to be a good screening criterion in the search for BPB (7). Such screening can be used to limit the number of samples. Further screening of BPB is generally carried out using monitoring parameters that estimate surface activity, such as ability to emulsify oils and dispersing or solubilization activity [11].

Comparatively high abundances of surfactant-producing bacteria were isolated from the cows and sheep (9 and 7 out of 56 isolates were positive for all tests respectively). In contrast, goats had lower surfactant producing bacteria (3 isolates).

These results suggest that probably the oily places of the skin of only some ruminants might be potential sources of surfactant-producing bacteria. However, some skin areas did not study here may contain even more surfactants produced by BPB as compared to studied areas.

Biosurfactant production by many of the isolated strains suggests that the resident bacteria could be a source of surfactants in the studied areas. A relatively biosurfactant producing *Bacillus* spp and *Lactobacillus* spp domination are represented in the isolated strains.

The function and composition of surfactants in the organisms of the examined areas has not been established. It might be suggested that the surfactants assist in the surface fat layer removal process by solubilizing hydrophobic fat layer or preventing destructive function of skin lytic substances. It may also dissolve organic matter of skin surface secreted by the different body systems or has some roles in the bacterial community formation of the skin surfaces.

Biosurfactants are often superior to commercial surfactants at solubilizing different chemicals and are more easily biodegraded [5]. Viewing biosurfactant producing bacteria in tables 1-3, the genera isolated from the studied areas, are well documented to be present in different oily environments as BPB [5, 11].

The microorganisms isolated in this study could well be sources of novel biosurfactants. Given demonstrated biosurfactant production by ruminant isolates, further investigation into the composition of the biosurfactants and phylogenetic determination of BPB is suggested.

Acknowledgments

Funding for this work was provided by School of Veterinary Science of Shahrekord University as costs for DVM research project.

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