



Isolation of Biosurfactant Producing Bacteria From Oily Skin Areas of Small Animals

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ABSTRACT

Background: Biosurfactants or microbial surfactants are surface-active biomolecules that are produced by a variety of microorganisms. They are a structurally diverse group of surface-active molecules and are highly sought after biomolecules for both present and future applications.

Objectives: The aim of the present study was to isolate and identify biosurfactant producing bacteria from the ear canal and inguinal areas (oily skin areas) of dogs and cats.

Materials and Methods: Eighty inguinal area and ear canal samples were collected from stray and owned dogs and cats (10 animals each, 20 samples) and screened for biosurfactant-producing bacteria using criteria such as hemolysis, oil spreading and E 24 emulsification index tests. The isolated strains were identified at genus level.

Results: 42 hemolytic bacterial strains (20 from dogs and 22 from cats) were isolated. The owned animal's samples had a higher population of positive strains than the stray ones. In total 11 isolates (26.2%) were positive for all examinations, out of these 9 (21.1%) isolates belonged to owned animals. 9 isolates (out of 11) (82%) were gram positive of which 4 (44.4%) were *Bacillus* spp. and 3 (27.2%) *Lactobacillus* spp.

Conclusions: The results showed that biosurfactant producing bacteria are distributed in the oily skin areas of both dogs and cats. Further investigation into the composition of the biosurfactants and phylogenetic determination of biosurfactant producing bacteria is suggested.

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► Implication for health policy/practice/research/medical education:

Oily skin areas of small animals may be a source of novel biosurfactant-producing bacteria.

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1. Background

Biosurfactants are unique amphipathic molecules with properties that have been explored for a variety of industrial and bioremediation applications (1). They are amphiphilic compounds produced on living surfaces, mostly on microbial cell surfaces, or excreted extra cel-

lularly 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 (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. Recently, several groups have presented intriguing data suggesting that biosurfactants are important for microbial growth and survival in the environment. For example, surfactin production is necessary for fruiting body formation by *Bacillus subtilis* (4).

Apart from their obvious role as agents that decrease

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surface and interfacial tension, thus promoting the formation and stabilization of emulsions, surfactants can have several other functions. They improve the consistency and texture of fat-based products (5). Several biosurfactants have shown antimicrobial action against bacteria, fungi, algae and viruses (6). There are many advantages of biosurfactants compared to their chemically synthesized counterparts. Research on this subject, will make them highly sought after biomolecules for present and future applications such 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, based on our knowledge there is no report on the screening and isolation of these microorganisms from animal skins.

2. Objectives

The aim of the present study was to investigate biosurfactant producing bacteria (BPB) habitats in ear canal and inguinal areas (IA) (oily skin areas) of dogs and cats.

3. Materials and Methods

3.1. Sample Collection

The study was carried out between September 2010 and April 2011 on 40, stray and owned dogs and cats. They were randomly selected from the stray dogs and cats in areas around Shahrekord University. Owned animals were referred to veterinary clinics in Esfahan for routine checking and vaccination. 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 rubbing on the inguinal areas. The surfaces were swiped thoroughly by rolling the wet swabs to attain maximum contact. The swabs were put

in separate sterile test tubes containing Stuart transport media (Quelab cat. QB-65-5015), labeled and kept in a cool box then transported to the veterinary microbiology laboratory at the veterinary college of Shahrekord University for further processing. For the bacteriological examination, the swabs were removed from the tubes and streaked over plates of blood agar-base (Scharlau 01-352, EU) supplemented with 7% sheep blood. The streaking was further spread with an inoculating loop to aid colony isolation. The plates were labelled and incubated aerobically at 37°C for 24-48 h (7). One colony was selected from those colonies that had similar morphologies and sub-cultured on blood agar plates for further analysis.

3.2. Screening Methods

The first screening test for the identification and isolation of BPB was a hemolysis test (8). In order to assay the hemolytic activity each strain was streaked onto a blood agar medium and incubated for 48 h at 37°C. The plates were visually inspected for zones of clearing around the colonies, indicative of biosurfactant production. For identification, Gram staining, catalase and oxidase tests were performed on the isolated haemolytic positive strains using a standard biochemical scheme according to Balows *et al* (9). Hemolytic isolates were inoculated into tubes containing Luria bertani broth (LB, Biomark-B699) media and incubated at 37°C for 72 h and shaken (~ 50 rpm). For each set of cultures one tube of sterile LB was considered as control. For the oil spreading technique (OS), 50 mL of distilled water was added to a large petri dish (25 cm diameter) followed by the addition of 20 µL of n-Decane (Merck, UN 2247) to the surface of the water. Ten microliters of LB culture cell-free broth (centrifuged at 10000 rpm for 10 min.) were then added to the surface of the oil (10). The diameter of the clear zone on the oil surface was measured. The diameters of tripli-

Table 1. Bacteria Isolated From Stray and Owned Dogs

Isolated Bacteria Ear/IA ^a	Ear			IA		
	E _{24h,%} ^a	E _{72h,%} ^a	O. S. SD (cm) ^a	E _{24h,%} ^a	E _{72h,%} ^a	O. S. SD (cm) ^a
Stray						
<i>Staphylococcus</i> spp. / <i>Bacillus</i> spp.	40	40	4.4 ± 0.5	52	52	6.3 ± 0.2
<i>Bacillus</i> spp. / <i>Bacillus</i> spp.	48	48	5.5 ± 0.2	40	40	4.3 ± 0.6
<i>Chromobacterium</i> spp. / <i>Bacillus</i> spp.	36	36	7.5 ± 0.3	52	52	5.4 ± 0.2
<i>Staphylococcus</i> spp. / <i>Staphylococcus</i> spp.	31	31	4.8 ± 0.3	48	48	2.7 ± 0.16
<i>Bacillus</i> spp.	36	36	5.6 ± 0.3	-	-	-
<i>Staphylococcus</i> spp.	40	40	3.3 ± 0.4	-	-	-
Control	50	50	4.45 ± 0.05	50	50	4.45 ± 0.05
Owned						
<i>Bacillus</i> spp. / <i>Staphylococcus</i> spp.	52	52	4.65 ± 0.07	54.5	63.6	5.65 ± 0.21
<i>Lactobacillus</i> spp. / <i>Bacillus</i> spp.	54.5	59	6.7 ± 0.28	45.5	47.2	6.1 ± 0.56
<i>Bacillus</i> spp. / <i>Lactobacillus</i> spp.	59	68.2	2.5 ± 0.28	59	59	5.5 ± 0.04
<i>Chromobacterium</i> spp. / <i>Bacillus</i> spp.	60	56	6.4 ± 0.14	36	44	7.0 ± 0.04
<i>Capnocytophaga</i> spp.	30.4	39.2	2.2 ± 0.14	-	-	-
<i>Staphylococcus</i> spp.	47.8	56.5	7.0 ± 0.28	-	-	-
Control	50	50	4.7 ± 0.14	50	50	4.7 ± 0.14

^a Abbreviation: IA, Inguinal area; O. S. SD - Oil spreading and the Standard Deviation; E, Emulsification index

cate samples from the same culture of each strain were determined.

The emulsifying capacity was evaluated by an emulsification index (E_{24}). The E_{24} of the culture samples was determined by adding 1.5 mL of kerosene and 1.5 mL of the cell-free broth to a test tube, spun at high speed for 2 min and allowed to stand for 24h and 72h. The E_{24} (and E_{72}) index is given as a percentage of the height of the emulsified layer divided by the total height of the liquid column (cm). The percentage of the emulsification index is calculated by using the following equation (11), E_{24} = height of emulsion formed x 100 / total height of solution. For each test strain, centrifuged samples of incubated tubes of sterile LB were used as a control.

4. Results

After culture and incubation of 80 samples (20 from each animal species, 10 ear canal and 10 IA) 42 hemolytic strains, 20 from dogs and 22 from cats were isolated. OS and bioemulsifying activities were measured for all isolates (Tables 1 and 2). In total 11 isolates (26.2%) were positive for all examinations, out of these 9 (21.1%) isolates belonged to owned animals. Nine isolates of the 11 (82%) were gram positives of which 4 (44.4%) were *Bacillus* spp. and 3 (27.2%) *Lactobacillus* spp. (Table 1 and Table 2)

5. Discussion

Hemolytic activity appears to be a good screening criterion in the search for BPB (8) 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 the ability to emulsify oils and dispersing or solubilization activity (12). Comparatively high numbers of surfactant-producing bacteria were isolated from the owned cats and dogs

so that five (22%) and four (20%) isolates respectively of these owned animals were positive for all examinations. In contrast, stray animals had lower surfactant producing bacteria. From 23 isolates of stray animals only 2 (from dogs) were positive for all examinations.

This distribution of BPB may be a response to the type of environmental contaminants present in the studied skin areas of the examined animals. The contaminants may have inhibitory effects on establishing BPB in the studied skin areas and stray animals might be more exposed to these contaminants. Adria et al. (13) showed that the distribution of biosurfactant-producing bacteria in soils was dependent on the soil conditions, with gram-positive biosurfactant-producing isolates tending to be from heavy metal-contaminated or uncontaminated soils and gram-negative isolates tending to be from hydrocarbon-contaminated or co-contaminated soils.

Further investigation is needed to determine whether this pattern holds for other species of owned and stray animals. However, some skin areas that were not included in this present study may contain even more surfactants produced by BPB when compared to the studied areas. We could not find reports regarding BPB isolation from animals; however our previous work indicates that BPB are also present on oily areas of ruminant's skin (unpublished observations). The presence of BPB has also been described in the guts of some marine invertebrates (14).

The relative domination of the biosurfactant producing *Bacillus* spp. and *Lactobacillus* spp. is represented in the isolated strains. This distribution may represent the ability of the microorganisms to survive in these skin areas. The biosurfactant activity in *Bacillus* spp. isolated from diesel oil has been documented by Singh and Lin (15), Tabatabaee et al. (12) also supports the biosurfactant activity of this bacteria. Lgia et al. (16) showed the biosurfactant activity of *Lactobacillus* spp. and that cheese

Table 2. Bacteria Isolated From Stray and Owned Cats

Isolated bacteria Ear/ IA ^a	Ear			IA		
	$E_{24h, \%}$ ^a	$E_{72h, \%}$ ^a	O. S. SD (cm) ^a	$E_{24h, \%}$ ^a	$E_{72h, \%}$ ^a	O. S. SD (cm) ^a
Stray						
<i>Bacillus</i> spp. / <i>Staphylococcus</i> spp.	40	40	7.55 ± 0.5	46	46	6.4 ± 0.1
<i>Staphylococcus</i> spp. <i>Bacillus</i> spp.	48	48	4 ± 0.7	52	52	4.6 ± 0.5
<i>Capnocytophaga</i> spp. / <i>Staphylococcus</i> spp.	36	36	5.35 ± 0.5	40	40	5.5 ± 0.2
<i>Chromobacterium</i> spp. / <i>Bacillus</i> spp.	31	31	7.25 ± 0.5	56	56	2.85 ± 0.16
<i>Bacillus</i> spp. / <i>Chromobacterium</i> spp.	40	40	5.4 ± 0.16	48	48	5.3 ± 0.1
<i>Lactobacillus</i> spp. / <i>Bacillus</i> spp.	40	40	4.3 ± 0.16	40	40	6.25 ± 0.2
- / <i>Staphylococcus</i> spp.	-	-	-	40	40	5.5 ± 0.16
Control	50	50	4.4 ± 0.1	50	50	4.4 ± 0.1
Owned						
<i>Staphylococcus</i> spp. / <i>Lactobacillus</i> spp.	44	44	5.05 ± 0.35	47	54.5	5.25 ± 0.07
<i>Bacillus</i> spp. / <i>Bacillus</i> spp.	44	45.5	3.2 ± 0.56	48	52	3.4 ± 0.28
<i>Bacillus</i> spp. / <i>Bacillus</i> spp.	40	38.5	5 ± 0.14	34.78	40	6.1 ± 0.028
<i>Capnocytophaga</i> spp. / <i>Bacillus</i> spp.	42.3	50	5.5 ± 0.28	50	43.3	6.2 ± 0.07
<i>Bacillus</i> spp. /	44	51.9	5.2 ± 0.14	-	-	-
Control	40	40	4.4 ± 0.1	40	40	4.4 ± 0.1

^a Abbreviation: IA, Inguinal area; O. S. SD - Oil spreading and the Standard Deviation; E, Emulsification index

they can be used as an alternative medium for the biosurfactant production by this bacteria. Biosurfactant production by many of the isolated strains suggests that the resident bacteria could be a source of surfactants in the studied areas. 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 removal process of the surface fat layer by solubilizing the hydrophobic fat layer or preventing the destructive function of skin lytic substances. It may also dissolve the organic matter on the skin surface secreted by the different body systems or it may have some role in the formation of the bacterial community on the skin surfaces. From a clinical perspective, at least one biosurfactant rhamnolipid produced by *Pseudomonas aeruginosa*, has a role in the pathogenesis of this opportunistic pathogen (17).

Biosurfactants are often superior to commercial surfactants at solubilizing different chemicals and are more easily biodegraded (6). Viewing biosurfactant producing bacteria in Tables 1 and 2, the genera isolated from the studied areas are well documented to be present in different oily environments such as potato process effluents, cassava flour waste water and oil reservoirs for *Bacillus spp.* (6, 12). The results of this present study showed that biosurfactant-producing bacteria are distributed in the oily skin areas of both dogs and cats. 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 the biosurfactant producing bacteria is suggested.

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