Bacteriocin production by *Staphylococcus epidermidis* the normal flora of outer ear: a potential probiotic against outer ear infections

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Several studies have reported that some infections are resulted from distortions of microflora, and hence, it was hypothesized that exploiting the defensive ability of normal flora may represent a promising approach to treat bacterial pathogens. This work seeks to bring attention to bacteriocin-producing bacteria that exist in the outer ear in order to exploit them to treat outer ear infections. Twelve bacterial isolates were collected from the outer ear of healthy individuals and subjected to a screening program in two steps: primary screening using agar plug diffusion assay to test the competitive activities against each other and secondary screening using well diffusion method. *Staphylococcus epidermidis* A3 showed an activity against different ear isolates with approximately 160 arbitrary unites (AU)/ml of bacteriocin activity. Tryptic soya broth was selected as the best medium to obtain maximum production of bacteriocin which showed a bactericidal action. In addition, 12 clinical isolates of common ear infections pathogens were used to test the activity of both *S. epidermidis* cells and its bacteriocin. Results revealed that bacteriocin was effective against 7 isolates, whereas *S. epidermidis* cells had an antagonism capability against 9 pathogens reflecting a competitive behavior. Moreover, interspecies interactions between *Staphylococcus epidermidis* A3 and clinical ear pathogens were investigated in liquid cultures which generally showed that bacteriocin production was increased in the presence of *Streptococcus pyogenes* and *Staphylococcus aureus*. The present study confirms previous findings and contributes an evidence on the possibility of using *S. epidermidis* and/or its bacteriocin to protect and against outer ear infections.

Keywords: Bacteriocin; normal flora; *Staphylococcus epidermidis*; otitis media.

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Introduction

Many microbes naturally exist on or inside the human body, which already serve as a particular microbial ecosystem occupied certain area from the skin to mucous membranes and the digestive tract. Due to the diversity of the surrounding environment, the microbial populations vary depending on their setting, and they usually struggle a wide range of bacteria and other microbes for colonizing a particular space in the human body [1]. The human ear can be considered as a unique environment with its own microbiome due to its distinct anatomy [2]. Naturally, the outer ear is protected by some physical elements such as skin, and chemical barriers mainly by earwax. These natural barriers prevent pathogens from entering inside the ear and causing an infection [3, 4]. Though, infections do occur in the ear as a result of several reasons mainly related to some environmental conditions. On the other hand, the nature and condition of earwax may play a role in creating the infection or developing bacterial growth. In this context, Brook [5] reported that the growth of *E. coli*, *S. epidermidis*, and *Corynebacterium* have significantly increased in the wet earwax. Moreover, it was found that the removal of

earwax may seriously raise the occurrence of ear infections because this compound naturally has antimicrobial properties [6].

In their populations, bacteria have to compete with each other to colonize a certain place and hence, they undergo a wide range of intra and inter-species interactions via utilizing some survival advantages that they may have. Production of bacteriocins is one of these survival advantages that used by bacteria to compete with other microorganisms [7]. Many Gram positive and negative bacteria produce a variety of bacteriocins that have a particular inhibiting role against other microbes. Since the outer ear is in contact with the atmosphere, it may involve a significant competitive microbial ecosystem. Therefore, it can be assumed that bacterial species isolated from such ecosystem produce inhibitory substances particularly bacteriocins.

One of the most significant discussions on the treatment of bacterial infections is that: is it possible to utilize the defensive bacteria which already exist on the human body as a probiotic to treat bacterial pathogens? It was widely reported that several kinds of bacterial normal flora produce antimicrobial compounds such as bacteriocin to kill other bacteria. In fact, several attempts have been made to use these bacteria instead of using their products to treat some infections such as eczema and atopic dermatitis [8], the oral cavity health and respiratory tract infections [9], vaginal candidiasis [10], peptic ulcer disease [11], and gastroenteritis therapeutics [12]. In this work, we tried to obtain an isolate of a normal bacterial flora from the outer ear, which has an ability to produce an effective bacteriocin against some bacterial pathogens that cause otitis media. In fact, this study set out with the aim of assessing the importance of bacteriocin-producing bacteria that exist in the outer ear and the possibility of exploiting them to treat or prevent outer ear infections.

Materials and Methods

Isolation of outer ear normal flora

Twenty-four (24) samples were collected from the outer ear of healthy persons using disposable sterile cotton swaps which then put in BHI broth and incubated overnight at 37°C. After incubation, serial dilutions to 10^{-6} from the culture were prepared and an aliquot of 100 µl from the last three dilutions were plated on BHI agar and mannitol salt agar, and incubated overnight at 37°C. Bacterial colonies with distinct morphology and color were picked and subsequently cultured on BHI agar plate.

Isolation of ear pathogenic bacteria

In this study, the spectrum activity of the selected bacterium isolates as a prospective probiotic and its bacteriocin was determined against 12 clinical bacterial isolates. These isolates were collected from patients suffering from ear infections admitting to Baghdad hospital in Iraq. The samples were taken by sterile swabs and put in sterile tubes containing BHI broth. All isolates obtained were then streaked on a suitable agar which then incubated at 37°C for 24h. All these isolates were identified in the hospital which were: *Streptococcus pyogenes* (C1), *Streptococcus sp* (C2), *Staphylococcus aureus* (C3), *Staphylococcus aureus* (C4), *Staphylococcus aureus* (C5), *Klebsiella pneumonia* (C6), *E. coli* (C7), *Proteus sp* (C8), *Pseudomonas aeruginosa* (C9), *Pseudomonas aeruginosa* (C10), *Pseudomonas aeruginosa* (C11), and *Pseudomonas aeruginosa* (C12).

Screening of isolates for bacteriocin production

The collected outer ear normal flora was subjected to a screening program in two steps: the primary screening using agar plug diffusion and the secondary screening using a well diffusion method. Briefly, plugs of about 0.6 cm in diameter were made with a sterile cork borer at progressive distance from the producer bacteria grown overnight on BHI agar. These plugs were then placed on Muller-Hinton agar plates streaked with another outer ear isolate as an indicator. The plates were then incubated overnight at 37°C and the diameters of inhibition zones were used as a measure of bacteriocin

production of the isolate [13]. Well diffusion method was used to determine the antimicrobial activity of bacteriocin in the secondary screening according to the method described by AL-Saeedi and Luti [13]. Bacteriocin activity was estimated using the critical dilution assay [14]. Briefly, a twofold dilution series of free cells culture supernatant of the selected isolate were prepared and bacteriocin activity was determined in each dilution against the indicator bacterium using agar well diffusion assay. The highest dilution generating an obvious inhibition zone reflected the total bacteriocin activity (arbitrary unites AU).

Preparation of bacterial inoculate and culture condition

Inoculum of the selected isolate of outer ear normal flora was prepared as follows: a few loopful of the bacterial growth from an overnight culture was inoculated in 50 ml of BHI broth and then incubated overnight at 37˚C. After the incubation, the number of cells in the inoculum was adjusted to be approximately 1×10^8 cells/ml. BHI medium was used for the cultivation of the out ear normal flora with an inoculation level of 2% (v/v). The flasks were then incubated at 37°C for 24h in an orbital shaker at 150 rpm. Thereafter, samples were taken for the determination of bacteriocin activity.

Mode of bacteriocin action

The studied bacteriocin was first precipitated from an overnight culture supernatant of *S. epidermidis* by saturation with different concentrations of ammonium sulfate (30, 40, 50, 60 ,70, and 80% w/v) under slow constant stirring at 4°C. The resulted precipitates were collected and dialyzed, and then, bacteriocin activity was estimated in order to determine the best saturation level to obtain the bacteriocin.

The mode of action of the bacteriocin produced in this work was studied. 0.5 ml of the extracted bacteriocin with a total activity of 640 AU/ml was added to 10 ml of an overnight culture of the indicator (*Staphylococcus aureus*) grown in tryptic soya broth contained 4.5×10^6 cells/ml. The control culture was prepared without adding bacteriocin. The viable cells (cfu/ml) were detected using the plate count method at zero time and after 10, 30, 60, and 120 min of incubation for both tested and control culture [14].

Antagonism simulation in liquid culture

Antagonism simulation between the selected isolate of out ear normal flora with ear pathogenic isolates was achieved in a mixed liquid culture. Briefly, culture of the out ear normal flora was prepared as described above. Then, a 1% inoculum level of live cells suspension from an overnight culture of each pathogenic bacterial isolate containing approximately1×10^6 cells/ml was added separately to the culture of ear normal flora at zero time. This level of inoculation was chosen arbitrarily as being half inoculation level of the selected ear normal flora (2%) to avoid any possible overcome. Samples were taken after the incubation for the determination of bacteriocin activity.

Results and discussion

In order to occupy a certain place, microflora shows an extensive interaction that confers them with some survival advantages over any neighboring microorganisms. Since the outer ear is in contact with the surrounding environment, it can be speculated that bacterial species exist in such part of the human body have a competitive behavior with an ability to produce some inhibitory compounds against each other. This work seeks to bring attention to the normal flora that naturally exists in the outer ear and can produce bacteriocins in order to utilize them to treat outer ear infections. For this purpose, 12 isolates were collected from the outer ear canal of healthy individuals which already identified based on morphological and biochemical tests.

Screening of isolates for bacteriocin production

Our strategy for screening isolates was based on testing the competitive activities against each other via using the agar plug diffusion method

Table 1. Primary screening of outer ear canal normal flora for bacteriocin production by agar plug diffusion method.

		Ear microflora as indicator											
		A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
Ear microflora as producer	A1	-	-	-	+	+	-	-	+	+	+	+	-
	A2	-	-	+	-	+	+	-	-	+	-	+	+
	A3	+	-	-	++	+	+++	-	+	++	-	-	+
	A4	+	+	-	-	-	-	-	+	+	-	-	+
	A5	-	-	-	-	-	-	-	-	-	-	-	-
	A6	-	-	-	-	-	-	-	-	-	-	-	-
	A7	+	-	-	+	+	-	-	-	-	-	+	+
	A8	+	-	+	++	-	-	-	-	-	-	-	+
	A9	+	+	-	-	+	-	-	-	-	-	+	+
	A10	-	-	-	-	-	-	-	-	-	-	-	-
	A11	+	-	-	-	-	+	++	-	-	-	-	+
	A12	-	-	-	-	-	-	-	-	-	-	-	-

+: ≥ 10 mm; ++: 10-20 mm; +++: < 20 mm.

Table 2. Secondary screening of outer ear canal normal flora for bacteriocin production by well diffusion method.

		Ear microflora as indicator (Bacteriocin activity AU/ml)											
		A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
Ear microflora as producer	A1	-	-	-	20	40	-	-	20	20	40	-	-
	A2	-	-	40	-	-	-	-	-	20	-	-	20
	A3	20	-	-	80	20	160	-	-	40	-	20	20
	A4	40	-	-	-	-	-	-	-	-	-	-	-
	A7	-	-	-	40	-	-	-	-	-	-	-	20
	A8	40	-	-	40	-	-	-	-	-	-	-	20
	A9	20	20	-	-	80	-	-	-	-	-	-	20
	A11	-	-	-	-	-	-	-	-	-	-	-	-

which usually used to exam the antagonism between different microorganisms. During their growth, microbial cells secrete antimicrobial molecules that diffuse in the agar medium from the plug which then detected by the appearance of an inhibition zone around the agar plug [15]. Results presented in table 1 shows that, out of 12 isolates, 8 had the ability to produce inhibitory compounds with different size of inhibition zones, and hence, were chosen for the second round of screening in liquid culture.

The secondary screening was performed by a well diffusion method to estimate the production of bacteriocin by isolates which showed an inhibitory effect in the agar plug diffusion method. As can be seen in the table 2, the bacteriocin produced by the isolate A3 was significantly active against 7 isolates with an arbitrary activity ranging from 20 to 160 AU/ml. Consequently, the isolate A3 was selected as a bacteriocin producing isolate in order to use it in this study. Moreover, the isolate A6 was selected to be used as an indicator to detect the bacteriocin activity. In addition, it can be noticed that isolates A11 and A7 that gave positive results in primary screening, showed no bacteriocin production in the well diffusion assay. This is of course, due to the difference between the two methods and the role that antagonism may play in the bacteriocin production which can be presented in the agar plug method. Next, the two

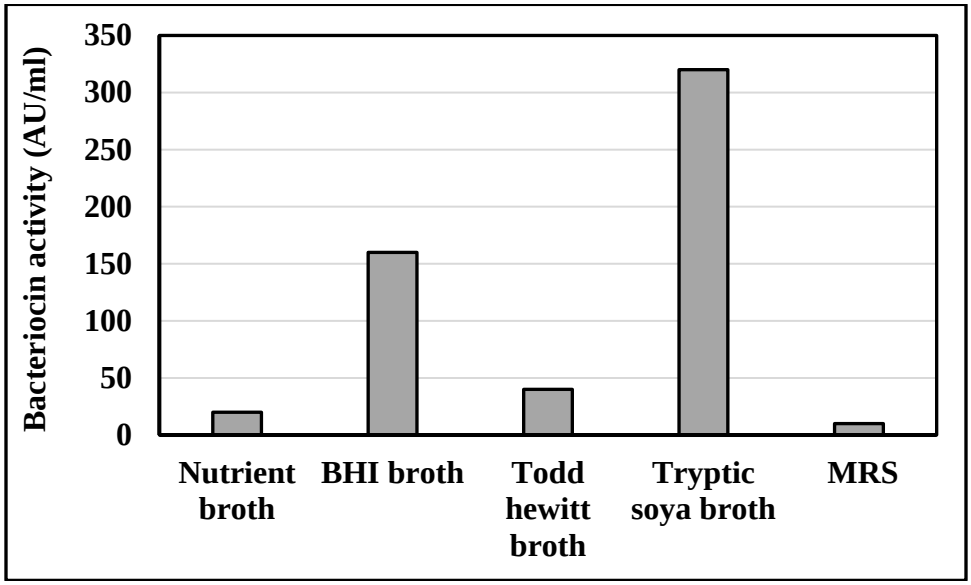

Figure 1. Production of bacteriocin by *S. epidermidis* (A3) in different media.

selected isolates were subjected to some biochemical tests as well as to the analytic profile index which revealed that the bacteriocin producing isolate A3 was *Staphylococcus epidermidis* and the isolate A6 was *Staphylococcus aureus* (data not shown).

S. epidermidis is naturally found as a part of the normal flora of human skin, Therefore, it is logical that this bacterium is existed in the auricle as a result of the similarity in the environmental conditions with the human skin. Dibb [16] mentioned that *S. epidermidis* was the commonest bacteria in 83% of individuals, whereas no growth was found in 20% of the persons sampled.

Based on the above results, *S. epidermidis* (A3) was chosen for the production of bacteriocin in liquid culture. For this purpose, different culture media were used (Nutrient broth, BHI, MRS, Todd Hewitt Broth, Modified Tryptic soy broth, and MRS) in order to choose the medium that supports the maximum production of bacteriocin. Lisboa *et al*. [17] hypothesized that biosynthesis of peptides from bacteria such as bacteriocins might be induced in a complex rich medium. From the figure 1, it can be seen that the production of bacteriocin was significantly enhanced in the tryptic soy broth with a maximum activity of 320 AU/ml. Todorov and Dicks also reported that tryptic soya broth gave the highest production of two bacteriocins ST461BZ and ST462BZ by *Lactobacillus rhamnosus* [18].

Mode of bacteriocin action

A considerable amount of literatures was published on the role of bacteriocin that might serve in the microbial populations. These studies suggest that bacteriocins may help as anti-competitors helping the invasion of microbes into an established population. However, bacteriocins may also play an important defensive role in terms of preventing the invasion of other microorganisms into an occupied niche or even limiting the development of neighboring bacteria. In both cases, the mechanism of bacteriocins mode of action is mainly based on creating desiccation of cell via increasing the membrane permeability of ions that consequently lead to the breakdown of proton-motive force causing cell death. In general, most bacteriocins show a bactericidal effect with or without cell-lysis [19]. However, in some cases this effect was found to be bacteriostatic. In this

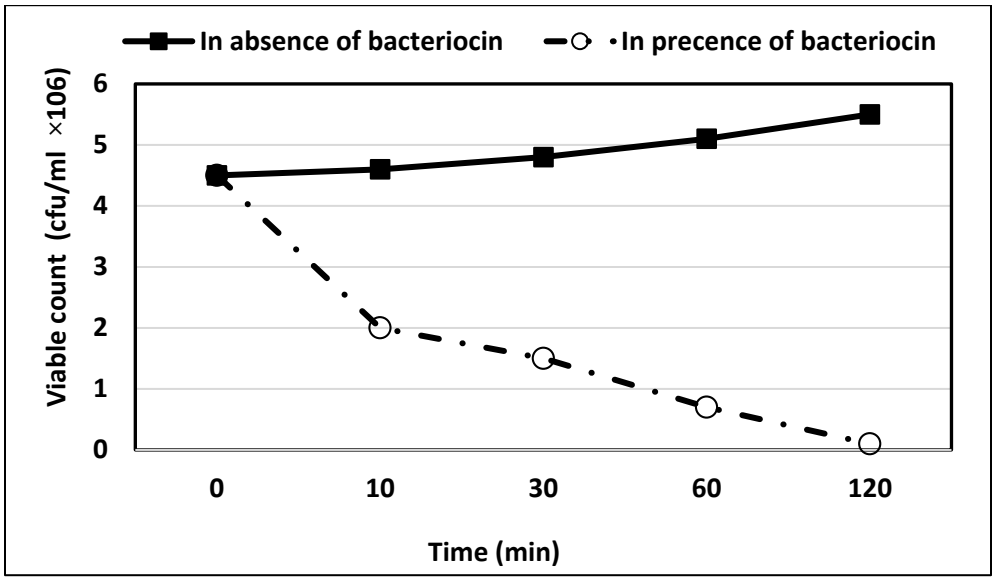

Figure 2. Mode of action of bacteriocin produced by *S. epidermidas* (A3) against *S. aureus* (A6).

work, the mode of action of bacteriocin produced by *S. epidermidis* (A3) was investigated using *S. aureus* (A6) as an indicator. The studied bacteriocin was first precipitated from the culture supernatant by saturation with different concentrations of ammonium sulfate. It was found that maximum bacteriocin precipitation was gained at an 80% saturation level, in which the bacteriocin activity was 640 AU/ml. The obtained bacteriocin was then used to investigate the mode of action. Based on results presented in figure 2, a rapid decrease was observed in the number of viable cells in the culture contained *S. aureus* (A6) with bacteriocin. The number of viable cells was decreased from 4.5×10^6 cells/ml at the beginning of the experiment to approximately zero within 2 hours. Whereas, in the control tube, the growth of *S. aureus* (A6) was almost followed its normal trend. These results confirmed the bactericidal effect of the bacteriocin produced by *S. epidermidas* (A3) rather than bacteriostatic. Basically, the bactericidal mode of action leads to kill the bacterial pathogens, and therefore, it is possible to eradicate the main population of undesirable microorganisms [20].

The antimicrobial activity of *S. epidermidas* and its bacteriocin against ear pathogens

Bacteria produce a variety of antimicrobial compounds which certainly improve their capability to compete against other microorganisms and inhibit their growth. Production of bacteriocin was usually used as an important criterion in the selection of suitable bacteria that can be utilized as a probiotic. So far, research has tended to focus on the role of bacteriocin-producing bacteria mainly in the digestive tract and their applications as probiotics. However, our knowledge and understanding are still very limited to explore a possible role that many bacteriocin-producing bacteria exist in the outer ear can play. In our study, the bacteriocin produced by *S. epidermidas* was tested against some clinical bacterial isolates collected from patients suffering from ear infections. In addition, the competitive behavior between *S. epidermidas* as a bacteriocin producer from the outer ear with ear pathogens was investigated using the agar plug diffusion method.

In this study, 12 clinical isolates of ear infections were used, which all are known as a common ear

Table 3. The antimicrobial activity of *S. epidermidas* (A3) (agar plug diffusion) and its bacteriocin (well diffusion method) against some otitis media pathogens.

Clinical isolate	Inhibition zone (mm)	
	Bacteriocin	Agar plug assay
Streptococcus pyogenes (C1)	+	+
Streptococcus sp (C2)	+	+
Staphylococcus aureus (C3)	+++	+++
Staphylococcus aureus (C4)	+	+
Staphylococcus aureus (C5)	+	+
Klebsiella pneumonia (C6)	-	+
E. coli (C7)	-	-
Proteus sp (C8)	-	-
Pseudomonas aeruginosa (C9)	-	+
Pseudmonas aeruginosa (C10)	+++	+++
Pseudomonas aeruginosa (C11)	+	++
Pseudomonas aeruginosa (C12)	-	-

+: ≥ 10 mm; ++: 10-20 mm; +++: < 20 mm.

pathogenic bacterium. Ngo *et al*. [21] reported that *Streptococcus pneumoniae* is the predominant bacterial pathogen for the majority reports from acute otitis media patients. In addition, recent evidence suggested that 30-50% middle ear bacterial cultures in patients with chronic middle ear effusion involved *Streptococci* [21]. However, the rates of bacterial pathogens were diverse in patients suffering from spontaneous perforation. The most frequent pathogens were *Streptococcus pyogenes*, *Haemophilus influenzae*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* [22].

Table 3 shows the results obtained from the two methods, well diffusion and agar plug. It can be seen that bacteriocin produced by *S. epidermidas* (A3) exhibited significant activity against 7 out of 12 isolates with different sizes of inhibition zones. However, results of the agar plug method confirmed that the *S. epidermidas* (A3) cells had an antagonism capability against 9 ear pathogenic isolates which certainly reflect a competitive behavior for this isolate. In addition, the results presented in table 3 revealed that bacteriocin produced by *S. epidermidas* (A3) was effective against 3 out of 4 isolates of *P. aeruginosa*. One of the most common outer ear infections is acute diffuse otitis externa, which is usually called swimmer's ear and the main bacterial causing agent is *P. aeruginosa*. This infection can be caused by swimming in contaminated water or even by bad ear cleaning [23]. Although *P. aeruginosa* may exist in the outer ear of healthy persons, it does not generally cause infection and hence is described as an opportunistic bacterium [24]. Though once these bacteria have the ability to get access to the outer ear, for example in case of earwax deficiency, it may be able to cause infection.

Antagonism simulation in liquid culture

In order to test the effect of interspecies interaction on bacteriocin production, mixed liquid cultures of *S. epidermidis* (A3) with living cells of the ear pathogenic bacteria were prepared. This investigation was performed against the same isolates that showed sensitivity against the bacteriocin produced by *S. epidermidis* (A3). The pathogenic isolates were added to *S. epidermidis* (A3) culture at an inoculum size of 1% (1×10^6 cells). In fact, the absent or the insufficient amount of bacteriocin production was observed in higher inoculum sizes (more than 2 %) of the added pathogens, and hence, it assumed that the pathogenic bacteria might overcome the growth of the *S. epidermidis* (A3) or even consumed some

Table 4. Bacteriocin production by *S. epidermidis* (A3) in co-culture with some ear bacterial pathogens.

Clinical isolate	Bacteriocin activity (AU/ml)
Control (pure culture of *S. epidermidis* (A3))	160
Streptococcus pyogenes (C1)	320
Streptococcus sp (C2)	160
Staphylococcus aureus (C3)	320
Staphylococcus aureus (C4)	40
Staphylococcus aureus (C5)	160
Pseudomonas aeruginosa (C9)	160
Pseudomonas aeruginosa (C10)	40
Pseudomonas aeruginosa (C11)	80

nutrients or precursors necessary in bacteriocin production. Table 4 shows the concentrations of bacteriocin in the presence of ear pathogens in the same culture compared with pure culture of *S. epidermidis* (A3) (control). Based on results, an increase in the production of bacteriocin than the control was observed in cultures supplemented with *Streptococcus pyogenes* (C1) and *Staphylococcus aureus* (C3). However, the co-cultivation of *S. epidermidas* with *Staphylococcus aureus* (C4) and *Pseudmonas aeruginosa* (C10) showed a significant decrease in the amount of bacteriocin produced (40 AU/ml). No changes were observed in cultures supplemented with *Streptococcus sp* (C2), *Staphylococcus aureus* (C5), and *Pseudomonas aeruginosa* (C9) as bacteriocin production was similar to control.

Interspecies interactions and the presence of competing microorganisms have been reported as a critical environmental factor that affects bacteriocin production in several researches. It was mentioned that *Lactobacillus sp*, for example, could confer resistance against infection with enteric pathogens [25]. Likewise, the production of Lactacin B by *L. acidophilus* N2 was enhanced in the presence of the inducer *L. delbrueckii* ATCC 4797 strain [26]. In addition, Divercin by *Corynebacterium* AS7 was enhanced in the presence of the inducer *C. piscicola* NCDO 2765 strain [27]. Moreover, Rojo-Bezares *et al*. found that bacteriocin production was detected in liquid media only when *L. plantarum* J23 was co-cultivated with some inducing bacteria [28]. Abd and Luti [29] reported an increase in the production of bacteriocin from *Bacillus subtilis* NK16 due to interaction with live cells of *S. aureus*, *Bacillus sp*, *E. coli*, *Saccharomyces cerevisiae*, and also with *Aspergillus niger*. In their work, they obtained an 8-fold increase of bacteriocin in the culture supplemented with *S. aureus*. However, far too little attention has been paid to examine this phenomenon in *S. epidermidis* or in general the normal flora of human skin.

Probiotic application of the natural microflora

A large and growing body of literature has investigated the relationship between the natural microflora and the human body in order to explore the beneficial aspects that may be gained. In fact, researchers started to appreciate the importance of this relation seeking to exploit it in order to solve some challenges, in particular, the resistance of antibiotics [1]. The rapid increase and spread of antibiotics resistant pathogens have motivated scientists to consider unusual strategies for combating infections. Nowadays, several antibiotics that describe as broad-spectrum antibiotics are widely used to treat infections. Certainly, using such antibiotics can provide a fast recovery, but on the other hand, it may destroy the natural microbial ecosystem of normal flora in the human body. Furthermore, broad-spectrum antibiotics may, in turn, be the reason for developing the resistance in both pathogens and natural microflora. One of the solutions suggested to solve this problem is

involved in developing a more rational antimicrobial agent with narrow spectrum activity. Bacteriocin is considered as a designer drug due to its specific narrow spectrum activity that targeting a specific microbial pathogen. In addition, because of the variety of bacteriocins production by microorganisms, it becomes possible to find bacteriocin that actively against a particular pathogen.

In the human body, the existence of bacteriocin-producing bacteria has been shown to reduce the occurrence of different pathogenic bacteria. Therefore, there has been an increasing interest in applying bacteriocin-producing normal flora to treat or reduce some pathogens. In a previous report, an isolate of *Streptococcus salivarius* K12, which produced bacteriocin Salivaricins A and B, was used as a dietary supplement and as a throat guard spray in order to keep a healthy throat via preventing or reducing throat infections [30]. In an interesting study, Park *et al*. [31] used *S. epidermidis* to prevent the colonization of Methicillin-resistant *S. aureus* in the nose. They performed their work by using mice via nasal inoculation with cells suspension of *S. epidermidis* suggesting that resident bacteria play an important role in reducing the growth of *S. aureus*. The findings of this study suggested that this logic could be extended to involve the treatment of infections of ear. *S. epidermidis* is naturally present in the outer ear, and according to results obtained in this study, it has the ability to interfere effectively with the activity of some common ear pathogens such as *Streptococcus pneumonia*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. In this way, *S. epidermidis* can be used to combat infections of outer ear by basically replacing the bacterial flora that may be found in the outer ear with such an active bacterium.

Conclusion

For controlling microbial pathogens, bacteriocin may provide a great chance to deal with the challenging problem of multi-drug resistant bacteria that obviously become a serious problem. Some of the normal flora exist in the outer ear can synthesize bacteriocins that can noticeably be occurred when tested against each other. In our study, *S. epidermidis* which is a common skin microflora showed an important inhibitory activity against different clinical ear pathogenic bacteria. Therefore, using *S. epidermidis* and/or its active bacteriocin as probiotics in ointment or emul gel, for example, may provide protection against outer ear infections. On the other hand, doctors have to be aware of beneficial properties of the microflora naturally found in the human body such as outer ear, and their treatment policies should concentrate on the control rather than the elimination of this bacterial microflora.

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