Archive of SID

Original article

Evaluation of antimicrobial substance produced by a bacterium isolated from *Parmacella iberica*

Gholamhossein Ebrahimipour, PhD[1]
Azam Moradi, MSc[1]
Morteza Mehrdad, PhD[2]
Abdorazagh Marzban, MSc[1]
Howry Alaee, MSc[1]

[1]Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran
[2]Department of Environmental and Resources Economics, Faculty of Environmental, Tehran, Iran

Address for correspondence:
Azam Moradi, Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran
Tel: +9821 29902706
Mobil: +989153047633
Fax: +9821 22431664
Email: Azammoradi93@yahoo.com

How to cite this article:
Ebrahimipour G, Moradi A, Mehrdad M, Alaee H, Marzban A. Evaluation of antimicrobial substance produced by a bacterium isolated from *Parmacella iberica*. Jundishapur J Microbiol. 2011; 4(3): 131-140.

Received: August 2010
Accepted: December 2010

Abstract

Introduction and objective: Nowadays, widespread application of antibiotics results in resistant microorganisms all over the world. Thus screening researches for the products with antimicrobial activity have been lead to probe natural sources of antimicrobial agents to find new pharmaceutical products. Here we describe the antagonistic activity of a bacterium isolated from digestive system of Iran endemic slug, *Parmacella iberica,* its biochemical identification and phylogenetic relationships and extraction of antimicrobial substance.

Materials and methods: Samples from digestive system of slugs were immediately transferred to broth medium. Antimicrobial agent-producing bacteria were isolated using serial dilution method on nutrient agar. One of them was selected for more investigation. Disk diffusion test was used against some laboratory standard strains to screen the antimicrobial potential. Identification of bacteria was done with 16S ribosomal DNA amplification and standard biochemical tests.

Results: Phylogenetic analysis of 16S rDNA gene of this bacterium showed similarity to *Pseudomonas aeruginosa* confirmed by the result of biochemical tests. The results of the antibiogram assay implied that the antimicrobial substance is broad-spectrum. This substance is nonpolar since it is extracted by different solvents. The cell growth measurements revealed that this compound was produced at first logarithmic phase.

Conclusion: High antimicrobial effects of the bacterium are evident. Due to increscent need to new antibiotics, purification and identification of this antimicrobial compound seem to be necessary for introducing new medicinal source.

Significance and impact of the study: The isolation of bacterial strain producing antimicrobial agent with the broad spectrum antimicrobial properties is proposed.

Keywords: Slug; *Pseudomonas aeruginosa*; Antibiogram; Antimicrobial agent

www.SID.ir

Archive of SID

Introduction

Today, increase in the number of drug-resistant pathogens, particularly the acquired multi-drug resistant strains, cause serious public health problem throughout the world [1]. Therefore, the need for antimicrobial discovery and better treatments of these infections, particularly in hospitals where resistance is immediately life threatening, is growing more urgent [2].

The study of different environments throughout the world has yielded a lot of antimicrobial agents that are of great value for the treatment of many infectious diseases. Animals are considerable to these researches, since they defend themselves against pathogenic microorganisms via production and secretion of antimicrobial components [3,4]. On the other hand, their intestinal tract is continuously exposed to bacteria present in the external environment. Work on soil invertebrates has resulted in the characterization of gut microbial populations from many common species and has provided much information on host-microbe interactions [5].

The frequency of bacteria functional groups in normal microflora of the digestive systems of different aquatic animals is unlike. Intestinal bacterioflora of aquatic animals primarily integrates into the protective function of the organism and decompose natural and extraneous, useful and harmful substances as well as the synthesis of the missing ones. Molluscs adaptation to the medium, their ability to consume alimentary substances, successful competition and procreation in particular conditions are among the principal factors determining the formation of a microbial population [6].

In the case of gastropod molluscs, most of the studies of gut bacteria have focused on their general ecology and potential pathogenic nature [5,6], and their antimicrobial compound-producing bacteria are less studied. Antibacterial component produced by bacteria seem to play an important role in the bacterial antagonism in aquatic ecosystems [7] and might ensure the predominance of a given strain in a bacterial niche against other bacteria of the same species or against other species [8]. Selected slugs are endemic of Iran and their intestinal bacterioflora are less studied, therefore they are potentially good environment for new detection.

In this work we investigate the evaluation of antimicrobial agent produced by a bacterium isolated from the digestive system of Iran endemic slugs *Parmacella iberica*, its biochemical and phylogenetical identification and extraction of antimicrobial substance.

Materials and methods

Sampling, culture and bacterium isolation

According to standard procedures, slugs collected from agronomy fields of Babol, a city in north of Iran, and taken directly to the laboratory. The skin of slugs was washed with sterile distilled water several times, and then they were dissected using sterile dissected set. Sampling was done from digestive system, and then the sample was inoculated into sterile nutrient broth (Oxoid, England) and incubated in a shaker incubator (Labtech, South Korea) at 120 rpm and 35°C for 3-4 days.

All bacteria were isolated by serial dilution which is done as in the following method described [4,9,10]. Ten tubes containing 4.5ml of physiologic serum (0.8% NaCl) were prepared, 0.5ml of inoculum was transferred to tube one and the content was mixed, then tube two was inoculated with 0.5ml of tube one. Using this method, we prepared dilution series with 1:10 steps. Next, 100µl of each dilution was spread on nutrient agar plates (Merck, Germany) and then were incubated overnight at 35°C. Different types of

www.SID.ir

Archive of SID

obtained colonies were streaked out on fresh plates and transferred at least three times for purification. Bacterial isolate were compared by colony morphology and colour [9]. Finally twelve bacterial isolate were purified and inoculated to nutrient broth separately and put into a shaker incubator at 120rpm and 35°C.

The agar disk diffusion method was performed for the detection of antimicrobial agent-producing bacteria of which one was selected for more investigation. Growth was determined microscopically and by monitoring turbidity in this process. This bacterium was grown in synthetic medium containing the following ingredients per litter: NH_4CL, 1g; $MgSO_4.7H_2O$, 0.2g; $CaC1_2.2H_2O$, 0.01mg; K_2HPO_4, 0.05g; $FeSO_4.7H_2O$, 0.01g; sodium acetate, 10g; micro elements 1ml ($ZnCl_2$, 70mg; $MnCl_2.4H_2O$, 100mg, $CoCl_2.6H_2O$, 200 mg, $NiCl_2.6H_2O$, 100mg, $CuCl_2.2H_2O$, 20mg, $NaMoO_4.2H_2O$, 50mg, $Na_2SeO_3.5H_2O$, 26mg, $NaVO_3.H_2O$, 10mg, $Na_2WO_4.2H_2O$, 30mg; and sterile distilled water, 1000ml); and enough NaOH to neutralize [11].

Since many bacteria exhibit production of secondary metabolites mainly in the late exponential or in the stationary phase, we investigated whether this is also the case for the production of antimicrobial agent by our strain [9].

Microorganisms

To detect antimicrobial activity, agar diffusion tests with standard test organisms were performed using *Bacillus subtilis* ATCC 465, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 10031, *Enterococcus faecalis* ATCC 29737, *Pseudomonas aeruginosa* ATCC 85327, *Candida albicans* ATCC 10231 and *Aspergillus niger* (isolated in our lab) [12]. *B. subtilis* was used as indicator strain in subsequent experiments because the antimicrobial compound produced the maximum mean diameter of inhibition zone against this bacterium.

Identification of bacteria

Isolated bacterium was identified based on biochemical and morphological tests and molecular method.

Polymerase chain reaction

Genomic DNA was purified of overnight culture of the bacterium using high pure PCR Purification kit (Roch, Germany). The following thermal cycle profile was used for the PCR: 94°C for 5mins, 30 cycles of 94°C for 1min, annealing at 55°C for 40S and 72°C for 1min, and a final extension step of 72°C for 5mins [13-15].

Universal primers fD1: 5'-AGAGTTT-GATCCTGGCTCAG-3' and rD1: 5'-TAAGGAGGTGATCCAGCC-3' were used in this process [16]. The PCR product was separated on 1% agarose gel and recovered by high pure PCR purification kit (Roch, Germany). Nucleotide sequencing analysis was performed with dideoxy chain termination method (SEQLAB, Germany) [15,17]. Sequences were compared with similar sequences of reference organisms by BLAST algorithm (www.ncbi.nlm.nich.-gov/BLAST/) [9,15].

Biochemical tests

Table 1 depicts the list of biochemical tests that were used [1,18,19].

www.SID.ir

Archive of SID

Table 1: Results of biochemical and morphological tests of strain Q

Biochemical tests	Result strain Q	*P. aeruginosa*
Cell shape	Rod	Rod
Gram's reaction	-	-
Motility	+	+
Glucose Oxidation	+	+
Glucone Fermentation	+	+
Mannitol	+	+
Lactose	-	-
Sucrose	+	+
Maltose	+	+
Catalase	+	+
Oxidase	+	+ / -
Growth on MacConkey	+	+
Citrate	+	+
Sodium acetate	+	+
Urease	+	-
Nitrate	+	+
DNAse	-	-
Indole	+	-
TSI slant	Alk/Alk	Alk/Alk
Amylase	-	-
H_2S (TSI butt)	-	-
Manitol salt agar	-	-
Gelatin	+	+
Pigment	+	+
Growth at 42◦C	+	+
Growth at 4◦C	-	-
Methyl red	+	-
Voges-Proskauer	-	-

Antimicrobial activity assay

Strains tested for antibacterial activity were grown in synthetic medium with sodium acetate as carbon source and nutrient broth at 120rpm and 35°C until the stationary phase was reached after 68-70h [9]. Cell-free culture supernatant was obtained by centrifugation at 12,000rpm for 10mins at 4°C and pH-adjustment to seven with 1M HCl [20]. Then sterile antibiotic assay disks (Padtanteb, Iran) (6.4mm diameter) were soaked with culture supernatant of the strain and placed on agar plates previously inoculated with a swab submerged in the suspension of indicator strains which corresponded to a 0.5 McFarland turbidity standard solution [9,21].

The plates were incubated at 35°C in the dark overnight and checked afterwards for inhibition zones in mm. In order to check the antimicrobial activity of our strain, a portion of medium and culture supernatant of the standard strain of *P. aeruginosa* were tested as control [9]. All the tests were performed in Muller-Hinton agar (Merck, Germany) [22].

Effects of temperature, pH and organic solvents on the stability of antimicrobial activity

The antimicrobial activity was tested for sensitivity to several organic solvents, heat and pH. To determine the effect of temperature on antimicrobial compound activity, aliquots of culture supernatant were prepared in screw capped tubes, adjusted to pH 7 and placed at 4, 35, 50, 60, 70, 80 and 100°C for 2h in water bath (Memmert, Germany) [1,4,22]. The antimicrobial compound was also subjected to autoclaving temperature (121°C for 15mins) [23].

Aliquots of the supernatant were incubated at different pH values (pH 2-12) at 35°C for 120mins in screw capped tubes, then neutralized to pH 7.0 [4,24]. To assay the effect of organic solvents on antimicrobial activity, organic solvents were added to the supernatant and the samples were incubated at 35°C for 120mins. Before and after each treatment the samples were tested for antimicrobial activity against *B. subtilis* as indicator organism [4,25] and an untreated cell-free supernatant served as control [9].

www.SID.ir

Archive of SID

Polarity of the antimicrobial agent
To extract the antimicrobial agent, culture filtrate was mixed with 10% (v/v) of organic solvents such as chloroform, ethyl acetate, ether and n-hexan, vigorously shaken and the mixture was allowed to separate. The lower phase (organic phase) was separated from the exhausted supernatant (upper phase) and this was repeated 15 times. The solvent phase was concentrated under reduced pressure by a vacuum pump at 30°C (R-114, Buchi, Switzerland) until no solvent was left [26]. Then, two phases were tested for their antimicrobial activity by the agar disk diffusion method.

Results

Four of the twelve isolated bacteria produced antimicrobial agent. One of them (hereafter Q) was selected for more studying on the basis of maximum optical density in nutrient broth and the most average diameter of the zones of microbial inhibition in disk diffusion method against tested microorganisms. Morphological and physiological characteristics of the isolated bacterium were compared with data from Bergey's Manual of Systematic Bacteriology. Summarized results of biochemical and morphological tests are presented in Table 1. These characteristics suggest the genus *Pseudomonas* that was confirmed by phylogenetic analysis of the 16S rRNA gene.

The sequence of 16S rDNA was obtained after DNA extraction and PCR amplification result in 1500bp (Fig. 1). The sequence was reversed, aligned and compared to similar database sequences using the Bioedit software. BLAST analysis demonstrated a high level of similarity (98%) to the sequence of *P. aeruginosa* EF433547.1 and several other strains (all showing 98% similarity).

Inhibition zones of this bacterium in agar diffusion tests showed that the production of antimicrobial agent started during the early logarithmic phase of growth reaching maximum values at the late-logarithmic phase (Fig. 2). This bacterium in synthetic medium, an OD650 of 1.2 was obtained and the pH value was nearly constant (pH 8.0-9.0) during cultivation. Figure 3 demonstrates the relationship between cell density (OD 650nm) and inhibition zone (mm) depicting maximum antibacterial activity which was observed after 52h. The results of antimicrobial activity assay are shown in Table 2. Synthetic medium and nutrient broth didn't show any antibiotic properties whereas the standard strain of *P. aeruginosa* inhibited the growth of *B. subtilis*, *E. coli*, *C. albicans* and *A. niger.*

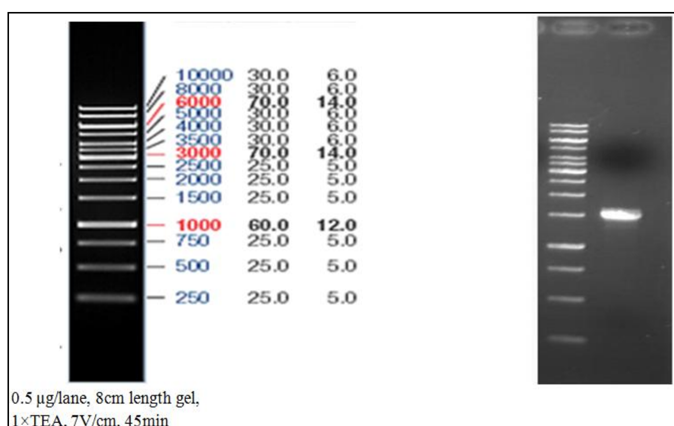

Fig. 1: Right: Electrophoresis marker; Left: Separation of PCR product of 16S rDNA on 1% agarose gel and the sharp band produced by the strain Q 16S rDNA on the right. 1kb marker used is shown on the left

www.SID.ir

Archive of SID

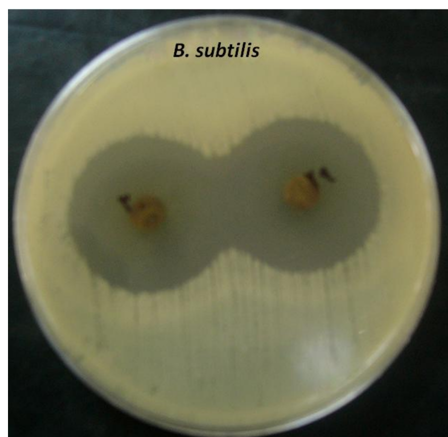

Fig. 2: Zones of inhibition produced by strain Q at 24h against the indicator microorganism, *B. subtilis*

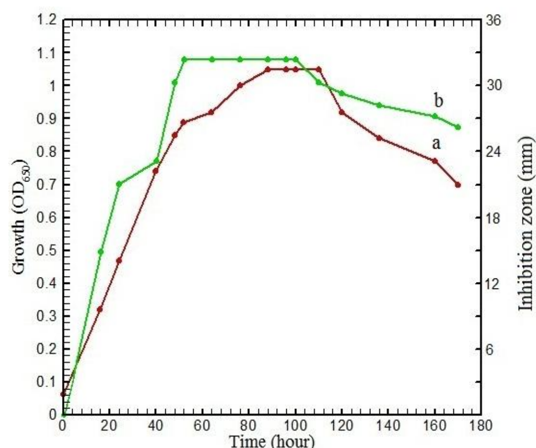

Fig. 3: Production of antimicrobial activity during growth of strain Q in synthetic medium at 35°C; turbidity (a) and antibacterial activity (b) were monitored.

Antimicrobial agent was stable at all six temperatures 4, 35, 50, 60, 70, 80°C and the antimicrobial activity decreased only when treated at 100°C and 121°C (Fig. 4). In addition, it was active at pH 4-9, preserving about 100% of its initial activity but the antimicrobial activity decreased at pH 2, 3, 10, 11 and 12 (Fig. 5). When treated with organic solvents, the antimicrobial activity was only sensitive to diethyl ether.

Table 2: Antimicrobial activity of strain Q against selected microorganisms

Standard strain	Zone of inhibition
P. aeruginosa	-
E. coli	+
S. aureus	++
B. subtilis	++
E. faecalis	++
C. albicans	++
A. niger	-
K. pneumoniae	+

(++) inhibition zone ≥ 25 mm, (+) inhibition zone ≤ 25 mm

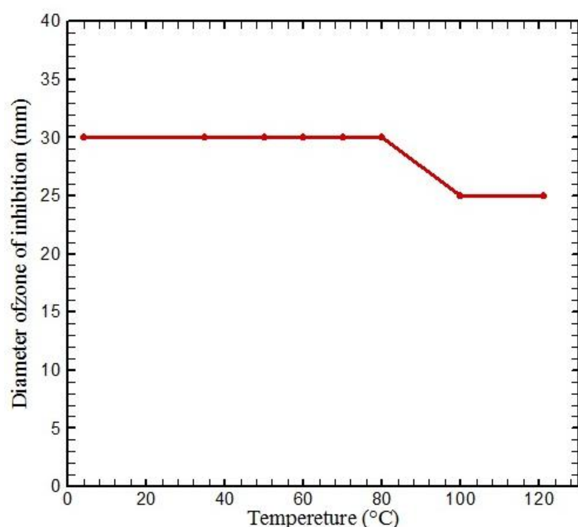

Fig. 4: Stability of antimicrobial activity at different temperatures

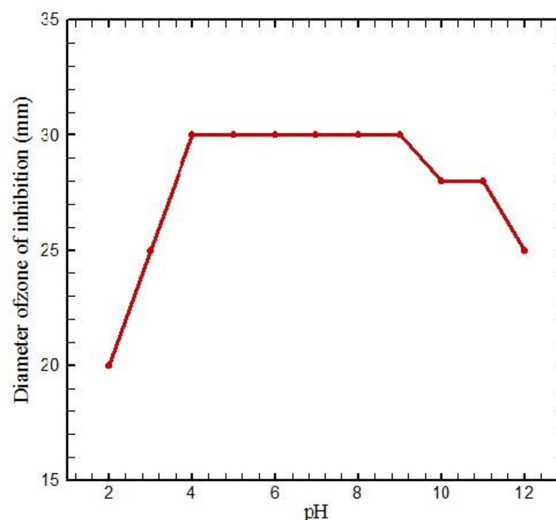

Fig. 5: Stability of antimicrobial activity at different pH

www.SID.ir

Archive of SID

The inhibition zone of solvents extract of bacterium against all the tested microorganisms showed in Table 3. The aqueous phase was not effective on indicator microorganism, however, the organic phase retained its antimicrobial activity and this shows that the antimicrobial compound is nonpolar.

Table 3: Antimicrobial spectrum of solvents extract of strain Q against all tested microorganisms

Solvent	Inhibition zone		
	Solvent	Solvent phase	Aquatic phase
Ethyl acetate	-	+	-
n-Hexan	-	+	-
Chloroform	-	+	-
Ether	-	+	-

Discussion

Throughout the last 50 years, antibiotics have been the main treatment modality used to fight bacterial infections. Prolonged and indiscriminate use of antibiotics to treat infections in both man and animals has resulted in the rapid emergence of antibiotic-resistant bacteria [27]. Therefore the search for bacterial strains producing antimicrobial agents has been intensified. However the discovery of new classes of antimicrobial agents with broad-spectrum antimicrobial properties has been slow [27,28].

In this work, the bacterium was isolated from digestive system of slug endemic in Iran, capable of producing a broad spectrum antimicrobial compound. So far, antibiotic producing bacterium from *P. iberia* has not been reported. Our strain was identified based on phenotypic and genotypic characteristics. Because of these characteristics, this species can be assigned to the species level as *P. aeruginosa*, which is a taxon with broad distribution on diverse environments [21].

Table 1 summarizes biochemical and morphological characteristics of the strain Q in comparison with *P. aeruginosa* in Bergey's Manual of Systematic Bacteriology. Antimicrobial agent shows an interesting spectrum of antimicrobial activity. Differences were observed in the susceptibility to the antimicrobial agent within different analysed strains. This may be due to the absence of receptors for the adsorption of the bacteriocin, or to some mechanism of bacterial resistance [29]. *P. aeruginosa* can produce a variety of inhibitory substances. Small antimicrobial substances such as pyocyanin and pyoverdin have been described for *Pseudomonas* spp. [30,31].

Pyoverdin has a good antifungal activity against the deleterious fungi and its maximum inhibition was observed against *A. niger* [32] whereas our antimicrobial agent is not able to inhibit *A. niger*. Pyocyanin has antifungal activity against *A. niger,* too [30,33]. Moreover, pyocyanin is a blue-green phenazine pigment [30] whereas our strain, when grown in a glucose-containing medium, produced antimicrobial substance but without any formation of green pigment. Also *P. aeruginosa* produces an antibiotic that its inhibitory activity has been observed against *E. coli*, *S. aureus* and *C. albicans* [34]. This substance, pyrrolnitrin, inhibits fungal pathogens among *A. niger* [33].

Pyocin AP41 was stable at 50°C for 30min, but it was rapidly inactivated above 70°C [35], while this reported agent is active at temperatures between 4-120°C. On the other hand, inhibitory activity of S-type pyocin is limited to *P. aeruginosa* strains and R-type pyocins are able to kill other gram negative bacteria, but our antimicrobial agent showed a broad inhibitory spectrum including several Gram-positive and Gram-negative except *P. aeruginosa* [36]. Activity spectrum of

www.SID.ir

Archive of SID

bacteriocin LlpA produced by *Pseudomonas* sp. strain BW11M1 is very different than our strain [37].

Moreover an antibiotic producer, *Pseudomonas* sp. of Persian Gulf was reported to show antibacterial activity against only Gram-positive bacteria, and not to the Gram-negative bacteria which were resistant to it [1]. In addition, the antimicrobial agent presents a broad antimicrobial spectrum and is more heat-stable than classical pyocins produced by *P. aeruginosa* [21]. The bacteriocin isolated from *P. syringae* was observed to be a pH and heat-sensitive compound with a narrow spectrum of activity when compared with the isolated bacterium [38].

The pH values of the culture supernatants of the strain Q indicate that the inhibitory effect was not due to the production of organic acids [21]. The stability of data and results of antimicrobial activity assay of this agent extracted by organic solvents, suggest that this substance may be a novel inhibitory agent and the nature of this substance merits future investigation. On the other hand, strain Q showed a broad inhibitory spectrum, including several pathogenic microorganisms such as *E. faecalis* and *S. aureus* that their resistance to drugs has been significantly identified [27]. The activity and stability of this antimicrobial agent in alkaline pH could be promising in potential clinical application due to mild alkalinity of human serum (pH 7.4).

The broad-spectrum inhibitory activity isolated strain may introduce an ecological benefit, due to its ability to inhibit some competing microorganisms. Yet, the role of antimicrobial substances produced by this bacterium is still under investigation. There are some intensive chemical and biochemical experiments in our lab to determine the exact chemical nature of this agent.

Conclusion

Our study showed that the isolated bacterium has excellent antimicrobial activity against the six tested microorganisms. High antimicrobial effects of the bacterium are evident, therefore investigation on antagonistic effect of this compound on other bacteria and purification and identification of agent are proposed in order to detect minimum inhibitory concentration and minimum bactericidal concentration of this pathogenic.

Acknowledgement

We thank Mojtaba Tushe for collecting samples, Soleiman Amiri for PCR and Mohammad Daraei for his guidance.

Conflict of interest statement: All authors declare that they have no conflict of interest.

Sources of funding: None

References

1) Darabpour E, Ardakani MR, Motamedi H, Ghezelbash G, Ronagh MT. Isolation of an antibiotic producer *Pseudomonas* sp. from the Persian Gulf. *Asian Pacific J Trop Med*. 2010; 3: 318-21.
2) Shlaes DM, Projan SJ, Edwards JE. Antibiotic discovery: state of the state. *ASM News*. 2004; 70: 275-81.
3) Amiche M, Seon AA, Wroblewski H, Nicolas P. Isolation of dermatoxin from frog skin, an antibacterial peptide encoded by a novel member of the dermaseptin genes family. *European J Biochem*. 2000; 267: 4583-92.
4) Abada EAE. Isolation and characterization of a antimicrobial compound from *Bacillus coagulans*. *Animal Cells and Systems*. 2008; 12: 41-6.
5) Walker AJ, Glen DM, Shewry PR. Bacteria associated with the digestive system of the slug *Deroceras reticulatum* are not required

www.SID.ir

Archive of SID

for protein digestion. *Soil Biol Biochem*. 1999; 31: 1387-94.

6) Šyvokien J, Mickėnienė L, Baršien J. Bacteria in the digestive system of molluscs from Lithuanian lakes. *Ekologija*. 2008; 54: 271-7.
7) Dopazo CP, Lemos ML, Lodeiros C, Bolinches J, Barja JL, Toranzo AE. Inhibitory activity of antibiotic-producing marine bacteria against fish pathogens. *J Appl Microbiol*. 1988; 56: 97-101.
8) Michel-Briand Y, Baysse C. The pyocins of *Pseudomonas aeruginosa*. *Biochimie*. 2002; 84: 499-510.
9) Brinkhoff T, Bach G, Heidorn T, Liang L, Schlingloff A, Simon M. Antibiotic production by a Roseobacter clade-affiliated species from the German Wadden Sea and its antagonistic effects on indigenous isolates. *Appl Environ Microbiol*. 2004; 70: 2560-5.
10) Betian HG, Linehan BA, Bryant MP, Holdeman LV. Isolation of a cellulolytic Bacteroides sp. from human feces. *Appl Environ Microbiol*. 1977; 33: 1009-10.
11) Schlegel HG. Allgemeine mikrobiologie. Auflage. Georg Thieme Verlag. 1992; 192.
12) NCCLS (National Committee for Clinical Laboratory Standards). Performance standards for antimicrobial susceptibility testing. 12th Informational Supplement 2002, M100-S12.
13) Doty SL, Oakley B, Xin G, *et al*. *Diazotrophic endophytes* of native black cottonwood and willow. *Symbiosis.* 2009; 47: 23-33.
14) Crost EH, Pujol A, Ladiré M, *et al*. Production of an antibacterial substance in the digestive tract involved in colonization-resistance against *Clostridium perfringens*. *Anaerobe*. 2010; 16: 597-603.
15) Brinkhoff T, Muyzer G. Increased species diversity and extended habitat range of sulfur-oxidizing *Thiomicrospira* spp. *Appl Environ Microbiol*. 1997; 63: 3789-96.
16) Batdorj B, Trinetta V, Dalgalarrondo M, *et al*. Isolation, taxonomic identification and hydrogen peroxide production by *Lactobacillus delbrueckii* subsp. lactis T31, isolated from Mongolian yoghurt: inhibitory activity on food-borne pathogens. *J Appl Microbiol*. 2007; 103: 584-93.
17) Charpentier S, Amiche M, Mester J, *et al*. Structure, synthesis, and molecular cloning of dermaseptins B, a family of skin peptide antibiotics. *J Biol Chem*. 1998; 273: 14690-7.
18) Mac Faddin JF. Biochemical tests for identification of medical bacteria. 2nd ed. Baltimore: Williams & Wilkins. 1980; 958.
19) Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's Manual of Systematic Bacteriology, Vol. 1, 2nd ed, Williams & Wilkins, USA, 1994; 559-65.
20) Basanta A, Sánchez J, Gómez-Sala B, Herranz C, Hernández PE, Cintas LM. Antimicrobial activity of *Enterococcus faecium* L50, a strain producing enterocins L50 (L50A and L50B), P and Q, against beer-spoilage lactic acid bacteria in broth, wort (hopped and unhopped), and alcoholic and non-alcoholic lager beers. *Int J Food Microbiol*. 2008; 125: 293-307.
21) Fontoura R, Spada JC, Silveira ST, Tsai SM, Brandelli A. Purification and characterization of an antimicrobial peptide produced by *Pseudomonas* sp. strain 4B. *World J Microbiol Biotechnol*. 2009; 25: 205-13.
22) Nowroozi J. Plasmid profile, antibiotic resistance, and phenotypic virulent strains of *S. flexneri*. *Iranian J Publ Health*. 2006; 35: 43-8.
23) Augustine SK, Bhavsar SP, Kapadnis BP. A non-polyene antifungal antibiotic from *Streptomyces albidoflavus* PU 23. *J Biosci.* 2005; 30: 201-11.
24) El-Adawy TA. Optimum production, stability, partial purification and inhibitory spectrum of antimicrobial compounds produced by *Pediococcus pentosaceus* DI. *Nahrung Food*. 2001; 45: 118-24.
25) Korenblum E, Der Weid I, Santos ALS, *et al*. Production of antimicrobial substances by *Bacillus subtilis* LFE-1, *B. firmus* H2O-1 and *B. licheniformis* T6-5 isolated from an oil reservoir in Brazil. *J Appl Microbiol*. 2005; 98: 667-75.
26) Shakeri J, Foster HA. Proteolytic activity and antibiotic production by *Trichoderma harzianum* in relation to pathogenicity to

www.SID.ir

Archive of SID

insects. *Enzyme Microb Technol.* 2007; 40: 961-8.
27) Filutowicz M, Burgess R, Gamelli RL, *et al*. Bacterial conjugation-based antimicrobial agents. *Plasmid*. 2008; 60: 38-44.
28) Yim G, Huimi Wang H, Davies J. The truth about antibiotics. *Int J Med Microbiol*. 2006; 296: 163-70.
29) Hubert L, Brevis P. Note: Purification and characterization of the bacteriocin PsVP-10 produced by *Pseudomonas* sp. *J Appl Microbiol*. 1998; 84: 910-3.
30) Baron SS, Rowe JJ. Antibiotic action of pyocyanin. *Antimicrob Agents Chemother*. 1981; 20: 814-20.
31) Visca P, Imperi F, Lamont IL. Pyoverdine siderophores: from biogenesis to biosignificance. *Trends Microbiol*. 2007; 15: 22-30.
32) Manwar AV, Khandelwal SR, Chaudhari BL, Meyer JM, Chincholkar SB. Siderophore production by a marine *Pseudomonas aeruginosa* and its antagonistic action against phytopathogenic fungi. *Appl Biochem Biotechnol*. 2004; 118: 243-51.
33) Kerr JR. Suppression of fungal growth exhibited by *Pseudomonas aeruginosa*. *J Clin Microbiol*. 1994; 32: 525-7.
34) Arima K, Imanaka H, Kousaka M, Fukuta A, Tamura G. Pyrrolnitrin, a new antibiotic substance, produced by *Pseudomonas*. *Agri Biol Chem*. 1964; 28: 575-6.
35) Sano Y, Kageyama M. Purification and properties of an S-type pyocin, pyocin AP41. *J Bacteriol*. 1981; 146: 733-9.
36) Sano Y, Matsui H, Kobayashi M, Kageyama M. Molecular structures and functions of pyocins S1 and S2 in *Pseudomonas aeruginosa*. *J Bacteriol*. 1993; 175: 2907-16.
37) Parret AHA, Temmerman K, De Mot R. Novel lectin-like bacteriocins of biocontrol strain *Pseudomonas fluorescens* Pf-5. *Appl Environ Microbiol*. 2005; 71: 5197-207.
38) Lavermicocca P, Lonigro SL, Evidente A, Andolfi A. Bacteriocin production by *Pseudomonas syringae* pv. ciccaronei NCPPB2355. Isolation and partial characterization of the antimicrobial compound. *J Appl Microbiol*. 1999; 86: 257-65.

www.SID.ir

Surf and download all data from SID.ir: www.SID.ir

Translate via STRS.ir: www.STRS.ir

Follow our scientific posts via our Blog: www.sid.ir/blog

Use our educational service (Courses, Workshops, Videos and etc.) via Workshop: www.sid.ir/workshop