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The Bioactivity of *bacillus sp*. Marine Bacteria Isolated From Marine Sediments of Lattakia City Towards Pathogenic Bacteria

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Abstract

The study included the selection of local marine bacteria isolates that were isolated from the sediments of the coastal waters of Lattakia, and their identity was determined using a number of agricultural, morphological, and biochemical tests as being species belonging to the genus Bacillus. The inhibitory activity of different concentrations of the organic extracts of these bacterial isolates against some pathogenic bacteria (Streptococcus pneumonia and Staphylococcus aureus) and Gram-positive bacteria (Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia) were studied using the gram-negative diffusion method. The inhibitory effect of this organic extract was then compared to that of some commercial antibiotics. The results showed that the biological activity of the organic extract of isolates of the genus Bacillus sp (B. subtilis, B. polymyxa, B. cereus, B. circulans) showed that the largest inhibition halo diameter of B. subtilis extract towards S. aureus was 26 mm, and it was the lowest halo diameter for bacteria. P. aeruginosa was 13mm. As for B. polymyxa extract, the highest inhibition halo diameter was 27 mm against S. pneumoniae and 25 mm against S. aureus, and the lowest diameter of inhibition was recorded as 13 mm against P. aeruginosa. In relation to the extracts of other species of the genus Bacillus sp (B. cereus, B. circulans), the inhibitory activity of their extracts was higher against Gram-positive bacteria than against Gram-negative bacteria. These bacterial extracts of Bacillus sp were more effective than various commercial antibiotics used.

Keywords*: Bacillus, Antibacterial activity, pathogenic bacteria.*

INTRODUCTION

H Oceans cover about 71% of the Earth's surface, and seas and oceans provide a tremendous diversity of microbial organisms [18]. The seas and oceans are an important resource of biodiversity that goes well beyond terrestrial environments. It possesses a unique and large group of diverse natural products produced by marine microorganisms [8], [9]. This marine biodiversity is reflected through the chemical diversity and efficacy of organic extracts against bacterial pathogens [7]. The rapid development of marine organisms chemistry over the past fifteen years has led to the discovery of a large number of chemical compounds, which have pharmacokinetic, medicinal, and toxicological properties towards previously unknown pathogenic human bacteria [16] ,[20]

Despite the great success achieved by the discovery of antibiotics and the development of the pharmaceutical industries in overcoming diseases resulting from microbial infections, many diseases are still waiting for effective treatment, and this is due to two main reasons; the first is the development of resistant or multi-resistant bacterial strains. The second is the emergence of new diseases whose treatment is still awaiting the discovery and development of suitable new drugs, hence the urgent need for continued research and development in this area [1], [7]

Therefore, it was necessary to study the effect of the organic extracts of the species of *Bacillus sp* bacteria isolated from marine sediments on some pathogenic bacteria and compare them with commercial antibiotics.

II. Aim and importance of research

Obtaining pure bacterial isolates of *Bacillus sp* from marine sediments.

The effect of organic extracts of *Bacillus sp* isolates on some pathogenic bacteria and their comparison with commercial antibiotics.

The importance of research stems from investigating new sources of microorganisms of marine origin, especially the species of the genus *Bacillus sp* that enjoy the production of chemical compounds that affect pathogenic bacteria. These new chemical compounds could be biologically effective and are the source for the development of many medicinal drugs, and thus are similar to effective antibiotics. It is important to investigate and obtain new natural materials from microorganisms, especially species of the genus *Bacillus sp* of marine origin.

III. Research materials and methods

Isolation and diagnosis of marine *Bacillus sp*:

Samples of marine water sediments were collected using sterile plastic bags from a depth of 10-20 cm from the beach of Lattakia city from Apamea region, as a site exposed to contamination with sewage water, during the sea launch in October of 2019. The sediment samples were transported directly to the laboratories of Tishreen University for laboratory analysis.

Taking 10 g from each sediment sample to obtain bacterial isolates from marine *Bacillus sp*, and adding to it 90 ml of sterile distilled water in a 250 ml glass beaker, mixed well, then heated the beaker in a water bath at a temperature of 80 ° C for half 1 hour to get rid of marine bacterial vegetative cells. Then 1 ml of the diluted samples was transferred to test tubes containing 9 ml of sterile nutrient broth. These tubes were then incubated at 30 ° C for 3 days. The tubes were examined by observing the formation of a thin white film close to the surface, which is a primary indicator of the growth of *Bacillus sp* [13], [21]. Then 0.1 ml of tubes that gave an indication of bacterial growth was taken and spread on the surface of a Petri dish containing nutrient agar medium, and the plates were incubated at a temperature of 30 ° C for 24 hours [6] The developing colonies were selected, purified, and identified by studying the shape, color and growth of the colonies on the medium, and by performing a number of biochemical tests and comparing the results with Berge's evidence [14]

Preparation of extracts of *Bacillus sp:*

The different isolated *Bacillus sp* colonies were transferred to a beaker containing 100 ml of peptone water medium and incubated at 30 ° C for 72 hours. The filtrate was separated from the sediment composed of bacterial cells after incubation using a centrifuge (5000 r / min). And for 10 minutes) according to Demain (1977). The top layer (the filtrate) was taken, which contains all the byproducts and antibiotics produced by the bacteria. It was stored at -20 ° C until its use, and its biological efficacy was tested against human pathogens [3], [11]

Pathogens used in this study:

The pathogenic bacterial isolates recorded in Table (1) were obtained from various disease samples from Tishreen University Hospital in Lattakia Governorate. Pathogenic bacteria isolates were diagnosed, and laboratory, microscopic and biochemical examinations were performed based on the scientific references used to diagnose the bacteria [4], [12], [23]. The initial diagnosis of pathogenic isolates was made by observing the colors, shape, nature, odor, and diameter of the colonies formed on the plate, and a set of biochemical tests were used according to the methods reported by Forbes and MacFaddin, based on Burji evidence [6] [13]. Also, bacteriostatic sentences based on biochemical tests were used to identify the germs (BioMérieux API Staph, API 20 Strep, API 20E System, France).

Table (1) Human pathogenic microbes and their sources

Source	Isolated pathogenic germs
Wound smear	***Staphylococcus aureus***
piss	***Streptococcus pneumonia***
piss	***Proteus vulgaris***
Wound smear	***Escherichia coli***
Cerebrospinal fluid	***Klebsiella pneumoniae***
Wound smear	***Pseudomonas aeruginosa***

Study of the inhibitory activity of extracts of *Bacillus sp*:

The sawing method was used in drilling (the cap assay method), according to Joreme and colleagues (2012). Liquid cultures of pathogenic bacteria were prepared in the middle of the nutritious broth and incubated at a temperature of 30 ° C for 24 hours, then l 100 were taken from these bacterial cultures and spread by sterile glass rod on the medium of Muller - Hinton agar (Merck Company, Germany), then the dishes were left to dry at a temperature Lab for 15 minutes. A sterile cork drill was used to make holes of mm6 in diameter in the medium distributed uniformly over the entire plate. Then I filled the holes with50 µl

of the organic extract of each of the different isolated *Bacillus sp* types (*B. subtilis* ،*B. polymyxa* ،*B. cereus* ،*B. circulans*). The plates were then incubated at a temperature of 30 ° C for 24 hours. The damping halos were then measured in millimeters around the holes to express the bioactivity of the organic extract [8][19]

Sensitization of pathogenic bacteria tested to antibiotics:

This was done using the Vatik device (company, state) in the National Hospital of Lattakia Governorate (a little explanation of the mechanism is preferred).

IV. Results and discussion

The results showed that the values of the biological activity of the organic extracts of *Bacillus sp* isolates against the pathogenic bacteria ranged between mm (7-28) and the lowest was against *P. Vulgaris*, and the highest was against Strep bacteria. Faecalis (Table 2). This inhibitory activity was higher against Gram-positive pathogens compared to Gram-negative bacteria for all extracts of *Bacillus sp* bacteria isolated (Table 2).

For the extract of *Bacillus subtilis* isolated from Apamea:

The results showed that the diameters of halos of growth inhibition of the pathogenic bacteria exposed to *Bacillus subtilis* extract ranged from 13-28 mm (Table 2 and Fig. 1). The largest observed halo diameter was 28 mm towards *Strep. Faecalis*, then to *Staphylococcus aureus*, was 26 mm, the diameter of inhibition against *E. coli* was 24 mm,

and 21 mm was against *K. pneumonia*, and 18 mm was against P. aeruginosa. As for P. vulgaris, it was 13 mm.

For *Bacillus polymyxa* extract isolated from Apamea:

The results are recorded in(Table 2 and Fig. 1). showed that the biological activity of this isolate extract against the pathogenic bacteria used ranged from 9-27 mm. The largest diameter of the inhibition halo was 27 mm towards Strep bacteria. Faecalis, then 25 mm toward *Staphylococcus aureus*. At the same time, the lowest biological activity was 9 mm against *P. aeruginosa*.

For *Bacillus cereus* isolated from Apamea:

1(Table 2 and Fig) show that the diameter of inhibition against *Staphylococcus aureus* was 23 mm and was 25 mm towards *Strep. Faecalis*, and that the diameter of the inhibition corona towards *E. coli* was 21mm, and that 20 mm was towards K. pneumonia, and that 10 mm was against *P. aeruginosa*. As for *P. vulgaris*, it was 12 mm.

For the extract of *Bacillus circulans* isolated from Apamea:

The results showed that the bioactivity of this organic extract ranged between 7-19 mm (Table 2 and Fig. 1). The diameter of the inhibition against *Staphylococcus aureus* was 16 mm and was 19 mm towards *Strep. faecalis*, and the diameter of the inhibition corona was 9 mm, 13 mm was towards *K. pneumonia*, and mm8 was against *P. aeruginosa*. As for *P. vulgaris*, it was 7 mm.

Table (2): The biological activity of the organic extracts of *Bacillus sp* isolates against the pathogenic bacteria was estimated by the diameter of the growth inhibition halos (mm).

P. vulgaris	***P. aeruginosa***	***K. pneumoniae***	***E. coli***	***S. faecalis***	***S. aureus***	
16	13	21	24	28	26	***B. subtilis***
10	9	18	22	27	25	***B. polymyxa***
12	10	20	21	25	23	***B. cereus***
7	8	13	9	19	16	***B. circulans***

The results showed that the inhibitory activity values of all organic extracts against Gram-positive pathogenic bacteria were higher than that of Gram-negative bacteria (Table 2).

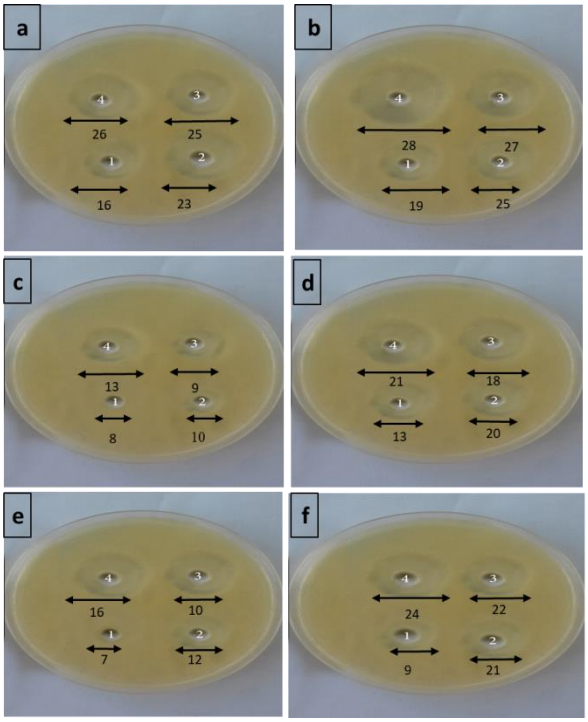

Figure (1): Effect of extracts of *Bacillus sp* species isolated from Apamea region (extract (1) *B. circulans*, (2) *B. cereus*, (3) *B. polymyxa*, (4) *B. subtilis*) on pathogenic bacteria ((a) S aureus, (b) *S. faecalis*, (c) *P. aeruginosa*, (d) *K. pneumoniae*, (e) *P. vulgaris*, (f) E. coli).

Results of pathogen sensitivity to antibiotics:

***Staphylococcus aureus*:**

Staphylococcus aureus showed sensitivity only to two antibiotics, Trimethoprime-Sulfamethoxazole and Vancomycin, and showed moderate sensitivity to Levofloxacin (Table 3).

***Streptococcus pneumoniae*:**

Streptococcus pneumoniae showed resistance to the following antibiotics Cloxacillin, Ciprofloxacin, Levofloxacin, and Amikacin and moderate sensitivity to Gentamycin (Table 3). This result is consistent with many studies that have shown resistance to beta-lactam antibiotics (penicillins and cephalosporins) of *staphylococci* and *streptococcus*, which are widely used in the treatment of various infections, in addition to their resistance to many other antibiotics [5] [2]

Common *Proteus vulgaris*:

Only the common Proteus showed moderate sensitivity to Ampicillin-Sulbactam (Table 3). Other studies have shown that most of the antibiotics commonly used to treat infections caused by *Proteus vulgaris* have become ineffective [15] [25]; note that all common *Proteus* strains are resistant to penicillin, ampicillin, and oxacillin. More than 80% of them were resistant to gentamicin and ciprofloxacin and 50% to amikacin, and this is due to the misuse of antibiotics or wrong prescriptions; and this pattern of resistance is associated with the occurrence of genetic mutations and the transmission of resistance genes between species through plasmids [22] [23]

***Escherichia coli*:**

Coli bacilli were only sensitive to Levofloxacin, Amikacin, and Ciprofloxacin (Table 3). The E. coli strains producing beta-lactamase in Dash *et al.* (2012), and Kaur and Anu (2016) showed resistance to gentamicin and amikacin, and showed moderate sensitivity to Ciprofloxacin. Coli bacilli in Cunha *et al.* (2016) also showed resistance to the antibiotic Trimethoprime-Sulfamethoxazole. These results give cause for concern as Ciprofloxacin is the most effective treatment for urinary infections .[21] [10]

***Pseudomonas aeruginosa*:**

The results showed that all strains of *Pseudomonas aeruginosa* were resistant to a large number of antibiotics, as in the study of Bernal-Rosas and colleagues (2015). It showed complete resistance to Cefazolin, Lincomycin, Cephalothin, Ampicillin, Clindamycin, and Chloramphenicol. It also showed moderate sensitivity to Sulfamethoxazole-Trimethoprim (Table 3).

The results of the study by Lin and colleagues (2012) and Rakesh et al. (2012) showed a low percentage of strains resistant to these two antibodies, while they showed moderate sensitivity to gentamicin, but they were sensitive to amikacin in the current study.

***Klebsiella pneumoniae*:**

Klebsiella pneumonia showed sensitivity to only two antibiotics, Levofloxacin and Amikacin (Table 3). This is in agreement with the results of a study by Cunha et al. (2016), which showed that most strains of Klebsiella are resistant to Ciprofloxacin and Trimethoprime-Sulfamethoxazol, and moderate sensitivity to Oxacillin. At the same time, it showed high sensitivity to the antibiotic Amikacin.

The fact that these bacteria possess resistant plasmids of penicillins and aminoglycosides and the occurrence of chromosomal mutations confer resistance to fluoroquinolones and narrow the field of choice of effective treatment [5], [14]

The proliferation of antibiotic-resistant bacteria is mainly attributed to their continuing effect on the same cellular sites (the fact that all the antibiotics used to affect the bacterial cell by one of the four known mechanisms: inhibition of cell-wall synthesis and metabolic processes, inhibition of bacterial protein synthesis, and finally, inhibition of synthesis of nucleic acids). This leads to mutations of bacterial genes [11], [26]. This is in agreement with recent data from the World Health Organization (2014) and the Centers for Disease Control (CDC).

Table (3). Allergy of pathogenic bacteria to a number of antibiotics

P. aeruginosa	*K. pneumoniae*	*E. coli*	*P. vulgaris*	*Strep.Faecalis*	*Staph.Aureus*	
0 R	11 I	0 R	7 R	19 S	0 R	AX
0 R	9 R	0 R	0 R	10 R	0 R	Cx
0 R	0 R	0 R	13 I	16 S	0 R	SAM
0 R	12 R	0 R	0 R	24 S	0 R	CE
0 R	9 R	0 R	0 R	22 S	12 R	CEC
10 R	0 R	9 R	0 R	27 S	0 R	CXM
12 R	0 R	0 R	9 R	18 S	0 R	CFM
10 R	11 R	0 R	10 R	22 S	9 R	CRO
23 S	8 R	18 I	12 R	12 R	11 R	CIP
19 S	18 S	24 S	10 R	10 R	15 I	LEV
18 S	18 S	17 S	10 R	14 R	9 R	AK
14 I	7 R	8 R	10 R	13 I	0 R	CN
9 R	0 R	10 R	0 R	26 S	17 S	VA
13 I	8 R	0 R	0 R	16 S	22 S	SXT

Conclusions and recommendations

From this study, we conclude that the extracts of species of the genus *Bacillus sp* (*B. subtilis*, *B. polymyxa*, *B. cereus*, *B. circulans*, *B. megaterium*) had higher inhibitory activity against Gram-positive bacteria than Gram-negative bacteria.

The extract of type *B. subtilis* showed higher bioactivity than the other organic extracts of the genus *Bacillus sp*.

Bacterial extracts of the genus *Bacillus sp* also showed higher bioactivity than various commercial antibiotics.

This result confirms the need to search and investigate natural sources of antibiotics that have an effective effect against pathogenic germs and have no harmful effects on human health.

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