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Purification of the Antibiotic Gramsidin from the Marine Genus Bacillus

*Rami Basem Hammod**

High Institute of Marine Research, Latakia University, Syria

Abstract

The study included the determination of the antibiotic (Gramsidin) and its proportions in the organic extracts of marine species of the genus Bacillus, namely (*B. cereus, B. subtilis, B. circulans, B. polymyxa*), which were isolated from beach sediment samples from the Afamia region with geographical coordinates (35°76'19.1"N; 35°54'19.3"E). It is an open area affected by tourist activities, sewage canals, and ship waste. To obtain organic extracts of bacterial isolates, the optimal conditions for separating and purifying these antibiotics were determined at the lowest economic cost, given the importance of these antibiotics in the pharmaceutical industries and their effective impact on pathogenic microbes and cancer cells. The results showed that after separating the low-density liquid layer (the upper layer) from the high-density liquid layer (the lower layer) and measuring the absorbance using a spectrophotometer for each antibiotic, the optimal conditions for purifying and isolating antibiotics were at a centrifugation speed of 4000 rpm, a centrifugation time of (30) minutes, and a temperature of -5 °C.The results showed that the percentage of the antibiotic Gramsidin upon centrifugation at -5 °C for 30 minutes was 91.30% for the extract of B. subtilis, 97.91% for the extract of *B. polymyxa* and 88.23% for the extract of *B. circulans*.

Keywords: Bacillus; Antibiotic; Spectrophotometer

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Author : Dr. Rami Basem Hammod

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Correspondence:
Dr. Rami Basem Hammod, High Institute of Marine Research, Latakia University, Syria;
E-mail: ramihammod1133@gmail.com

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Introduction

Despite the great success achieved by the discovery of antibiotics and the development of the pharmaceutical industry in overcoming many diseases resulting from microbial infections, a number of diseases still await effective treatment. This is due, on the one hand, to the emergence of new diseases whose treatment still depends on the discovery and development of suitable new drugs, and on the other hand, to the development of bacterial isolates resistant to or with multiple resistance to traditional antibiotics. Hence, the need for continued research and development in this field is urgent [1]. The improper use of antibiotics leads to the development of resistance in some Gram-negative [2] and Gram-positive [3] pathogenic bacteria. This has prompted the World Health Organization in recent years to issue warnings about the dangers of antibiotic misuse to human health [4].

The emergence of antibiotic-resistant pathogens has posed a significant challenge to disease treatment, increasing treatment costs and rendering many common illnesses untreatable and potentially fatal [5]. The use of chemical drugs is almost always accompanied by side effects on human health when used to treat bacterial infections, and often leads to long-term secondary complications [6]. This has prompted researchers to conduct studies aimed at obtaining antimicrobial agents from natural sources as alternatives to commercial antibiotics, which would be more effective and less harmful to human health and the environment. This is especially true for natural products extracted from marine organisms such as seaweed [2].

Marine fungi [4], marine animals such as sponges [7], and marine microorganisms of vital importance to life [8] are all valuable resources. The most clinically effective antibiotics are those derived from natural marine products [2].

Given this, it is crucial to research and investigate marine bacteria, obtain their organic extracts, identify the active chemical compounds present in these extracts, and determine their biological efficacy against many diseases and pathogens that have recently eluded scientists and researchers in finding successful treatments. This is especially important after the depletion and testing of most available terrestrial resources, making the sea a potential new reservoir of untapped natural resources.

Characteristics of Polypeptide Antibiotics

One of the most important types of antibiotics produced by bacteria of the genus Bacillus belongs to the polypeptide class [9]. Gramsidin is a key polypeptide antibiotic produced by Bacillus species and is characterized by the following:

Gramsidin antibiotic

Chemical formula: $C_{99}H_{140}N_{20}O_{17}$. Molecular mass: 1882.3 g/mol. Chemical structure: A peptide composed of five amino acids of the L-type (valine, ornithine, leucine, proline, and alanine). Spectrum of action: Gramsidin is highly effective against both Gram-positive and Gram-negative bacteria.

Uses: It is used in surgery as a primary treatment for wounds and in the treatment of infected wounds, burns, and other abscesses. Mechanism of action: It binds to phospholipids in the bacterial cell membrane, leading to changes in cell membrane permeability and ultimately cell death [8].

Research significance and objectives

Given the importance of Bacillus extracts in the production of antibiotics and chemical compounds, and the limited number of studies on Bacillus species, this study aimed to investigate the chemical composition of an organic extract of Bacillus and to identify the biological significance and bioactivity of the chemical compounds isolated from the coastal marine sediments of the Apamea area in Latakia.

Research Materials and Methods

The study location

The study was conducted on marine sediments from the Latakia city beach, specifically from the Apamea area (35°76'19.1"N; 35°54'19.3"E). This area represents an open area affected by tourism activities and sewage drainage. Samples of the marine sediments were collected from the aforementioned location using sterile 500 ml glass containers and transported directly to the laboratories of the Higher Institute for Marine Research at Latakia University.

Laboratory study

Preparation of the organic extract of the bacterial isolates: Marine sediment samples were collected from the Afamia area using sterile 500 ml glass containers and transported directly to the laboratories of the Higher Institute for Marine Research at Latakia University for microbiological analysis. Marine isolates of various Bacillus species were obtained and genotyped: *B. cereus, B. subtilis, B. circulans*, and *B. polymyxa* [9].

The antibiotic gramsidin and its concentration in the methanolic extracts of four Bacillus species isolated from the Afamia area were determined. These extracts showed higher activity than dichloromethane extracts of species isolated from other locations. The optimal conditions for isolating and purifying this antibiotic at the lowest cost were also established [10].

Given the importance of this antibiotic in the pharmaceutical industry and its potent effect against pathogenic microbes and cancer cells, and because the manufacture of some antibiotics requires secondary compounds involving different chemical groups, obtaining these compounds from their natural sources is crucial [3]. The concentration of the antibiotics was studied using a spectrophotometer according to the method of Hammod *et al.,* [11], which relies on the following steps: Extracts of Bacillus species were centrifuged in an Eppendorf 5810r centrifuge (Germany) at 4000 rpm at -5°C for 10, 20, and 30 minutes, separating the lower, lower-density liquid layer from the upper, lower-density layer. To determine the absorption wavelength of each antibiotic, the spectrophotometer was zeroed using methanol (as a control). The appropriate wavelength was experimentally determined by scanning the wavelength range of 190-800 nm for the antibiotic Gramsidin [12].

To determine the optimal wavelength at which the antibiotic exhibits the highest absorbance, a spectrophotometer was used. A standard series of antibiotics at different concentrations (mg/ml) (0.01, 0.02, 0.05, 0.1, 1) was used to construct a standard curve and derive the equation for calculating antibiotic concentrations and their ratios based on the absorbance values of these different concentrations. The concentration of each antibiotic in the organic extracts was determined in each of the two layers formed: the low-density liquid layer and the high-density sublayer. The percentage of each antibiotic in each isolate (purification percentage) was calculated using the following formula: (Antibiotic concentration in the separated layer/Antibiotic concentration in the original sample) × 100

Results and Discussion

Isolation and concentration of the antibiotic gramsidin from Bacillus extracts:

The wavelength of the antibiotic Gramsidin was determined using a spectrophotometer, yielding a value of 430 nm. The concentration of the antibiotic in Bacillus extracts isolated from the Afamia region during the summer was then determined, as it exhibited higher inhibitory activity against pathogenic bacteria and a broad spectrum of biological activity against various pathogenic bacteria and bioactive compounds, as demonstrated in previous studies.

Discussion

Isolation and determination of the antibiotic gramsidin from extracts of the genus Bacillus:

The results showed that the total concentration of the antibiotic Gramsidin in the organic extract of each of the four species (*B. cereus, B. subtilis, B. circulans, and B. polymyxa*) belonging to the genus Bacillus was 2.1 mg/l for the B. subtilis extract, 4.7 mg/l for the *B. polymyxa* extract, 2.5 mg/l for the *B. circulans* extract, and 1.8 mg/l for the *B. cereus* extract (Table 1). However, when the liquid layer (low-density liquid - high-density liquid) of the methanolic extract was analyzed upon centrifugation at different temperatures (5, 0, and -5°C) during different centrifugation periods (10, 20, and 30 minutes), the results showed that:

Upon centrifugation at 0°C for 10 minutes, the low-density liquid layer showed the following Gramsidin concentrations in the extracts of Bacillus species: 0.4 mg/l for *B. subtilis*, 3.2 mg/l for *B. polymyxa*, 2.9 mg/l for *B. circulans*, and 0.09 mg/l for *B. cereus* (Table 1). In the high-density liquid layer, the Gramsidin concentration was 0.1 mg/l for *B. subtilis*, 2.6 mg/l for *B. polymyxa*, 2.2 mg/l for *B. circulans*, and 0.05 mg/l for *B. cereus* (Table 1). The results showed that the highest percentage of the antibiotic Gramsidin, at 55.31%, was recorded for the extract of *B. polymyxa* upon centrifugation at 0°C for 10 minutes, while the lowest percentage was recorded for the extract of *B. cereus* at 0.09% (Table 1).

Table 1: Concentrations of the antibiotic Gramsidin for each of the liquid layer of density (low-high) of extracts of the four species belonging to the genus Bacillus at different temperatures °C (5, 0, -5) and different centrifugation periods (10, 20, 30) minutes.

The Concentration of the antibiotic polymyxin Estimated in mg/l				
	B. cereus	***B. circulans***	***B. polymyxa***	***B. subtilis***
Total concentration of the antibody in the abstract	1.8	2.5	4.7	2.1
Centrifugation at 0°C for 10 minutes				
Low density liquid layer	0.09	2.9	3.2	0.4
High density liquid layer	0.05	2.2	2.6	0.1
Purification percentage	0.09%	50%	55.31%	38.09%
Sedimentation at temperature (-5)°C For a20 minute				
Low density liquid layer	0.06	2.5	2.7	0.2
High density liquid layer	0.1	2.8	3.1	0.5
Purification percentage	5.55%	63.63%	65.95%	23.80%
Sedimentation at temperature (-5)°C For a 30 minute				
Low density liquid layer	0.04	1.8	2	0.08
High density liquid layer	0.2	3.2	3.6	0.8
Purification percentage	11.11%	72.72%	76.59%	38.09%

However, upon centrifugation at 5°C for 10 minutes in the low-density liquid layer, the concentration of Gramsidin in the extracts of the Bacillus species was observed to be 0.2 mg/l for the extract of *B. subtilis*, 2.7 mg/l for the extract of *B. polymyxa*, 2.5 mg/l for the extract of *B. circulans*, and 0.06 mg/l for the extract of *B. cereus*. For the high-density liquid layer, the concentration of Gramsidin was observed to be 0.5 mg/l for *B. subtilis* extract, 3.1 mg/l for *B. polymyxa* extract, 2.8 mg/l for *B. circulans* extract, and 0.1 mg/l for *B. cereus* extract (Table 1).

The results showed that the percentage of the antibiotic Gramsidin upon centrifugation at 5°C for 10 minutes was 23.80% for *B. subtilis* extract, 65.95% for *B. polymyxa* extract, 63.63% for *B. circulans* extract, and 5.55% for *B. cereus* extract (Table 1). Upon centrifugation at -5°C for 10 minutes, the low-density liquid layer showed that the gramsidin concentration was 0.08 mg/l for *B. subtilis* extract, 2 mg/l for *B. polymyxa* extract, 1.8 mg/l for *B. circulans* extract, and 0.04 mg/l for *B. cereus* extract. For the high-density liquid layer, the results showed that the gramsidin concentration was 0.8 mg/l for *B. subtilis* extract, 3.6 mg/l for *B. polymyxa* extract, 3.2 mg/l for *B. circulans* extract, and 0.2 mg/l for *B. cereus* extract (Table 1). The results showed that the highest percentage of the antibiotic Gramsidin upon centrifugation at -5 °C for 10 minutes was recorded for the extract of *B. polymyxa* and reached 76.59%, followed by the percentage 72.72% for the extract of *B. circulans*, and 38.09% for the extract of *B. subtilis*. The lowest percentage of Gramsidin was 11.1% for the extract of *B. cereus* (Table 1). To determine the effect of centrifugation time on antibiotic concentration in organic extracts, centrifugation was performed for 20 minutes and 30 minutes at -5°C. The results were as follows: When centrifuged at -5°C for 20 minutes, the low-density liquid layer showed that the gramsidin concentration was 0.09 mg/l for *B. subtilis* extract, 2.2 mg/l for *B. polymyxa* extract, 1.3 mg/l for *B. circulans* extract, and 0.07 mg/l for *B. cereus* extract. As for the high-density liquid layer, the results showed that the concentration of the antibiotic Gramsidin was 1 mg/l for the extract of *B. subtilis*, 3.8 mg/l for the extract of *B. polymyxa*, 3.5 mg/l for the extract of *B. circulans* and 0.7 mg/l for the extract of *B. cereus* (Table 1).

Upon centrifugation at -5°C for 30 minutes, the gramsidin concentration in the low-density liquid layer was 0.5 mg/l for *B. subtilis* extract, 1.7 mg/l for *B. polymyxa* extract, 0.4 mg/l for *B. circulans* extract, and 0.4 mg/l for *B. cereus* extract. In the high-density liquid layer, the gramsidin concentration was 1.7 mg/l for *B. subtilis* extract, 4.3 mg/l for *B. polymyxa* extract, 4.1 mg/l for *B. circulans* extract, and 0.9 mg/l for *B. cereus* extract (Table 1). The results showed that the percentage of Gramsidin upon centrifugation at -5°C for 30 minutes was 80.95% for *B. subtilis* extract, 91.48% for *B. polymyxa* extract, and the highest percentage, 93.18%, for *B. circulans* extract. A percentage of 50% was recorded for *B. cereus* extract, while the percentage for *B. circulans* extract was 91.48% (Table 1).

The results, after separating the low-density liquid layer (upper layer) from the high-density liquid layer (lower layer) and measuring the absorbance using a spectrophotometer for each antibiotic, showed that the optimal conditions for purifying and isolating the antibiotics were at a centrifugation speed of 4000 rpm, a centrifugation time of (30) minutes, and a temperature of -5°C. The concentration of the antibiotic in each layer was determined, and we observed that the separation rate of some antibiotics exceeded 95%. The highest percentage for the antibiotic Gramsidin was recorded at 97.91% for the extract of *B. polymyxa*.

Conclusions and Recommendations

The most important polypeptide antibiotics and their proportions produced from Bacillus species were identified as Gramsidin. The highest concentration of Gramsidin was recorded in extracts of *B. polymyxa*. The optimal temperature for isolating antibiotics from methanolic extracts of bacterial isolates was -5°C and a centrifugation time of 30 minutes. This type of research is currently at the forefront of marine biotechnology research, aiming to leverage its applications in extracting therapeutically and pharmaceutically valuable substances from marine organisms. It also seeks natural sources for producing broad-spectrum, non-traditional, natural antibiotics against common human pathogenic bacteria, with significantly fewer side effects than conventional and synthetic antibiotics. Furthermore, it is essential to utilize marine Bacillus bacteria, which possess significant medical and pharmaceutical value, at a low cost.

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