

Evaluation of the production potential of quorum quenching enzymes of extremophile bacteria from Antarctica

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Abstract— *Cell-cell communication mediated by the production of N-acylhomoserine lactone is one of the causes of the production of virulence factors in pathogens and biofouling. Although quorum-quenching is a useful method to disrupt quorum sensing signals, different QQ enzymes are required to block bacterial communication in specific environments. The aim of this study was to determine the QQ enzyme production potential of psychrophilic bacteria isolated from Antarctic marine sediments. This study was divided into three phases: (i) semi-qualitative evaluation of lipolytic activity, (ii) evaluation of resistance to β -lactam antibiotics, and (iii) evaluation of β -lactamase activity. In this study, the bacterial strains evaluated presented lipolytic activity and resistance to β -lactam antibiotics (ampicillin and penicillin). In addition, the strains UTB 167 and UTB 170, related to the genus *Psychrobacter*, presented β -lactamase activity (1.7 - 3.9 Umol/L), which indicates that their potential to produce QQ enzymes related to the metallo- β -lactamases superfamily.*

Keywords— *β -lactamases, N-acyl homoserine lactone (AHL) extremophiles, quorum-quenching, quorum-sensing.*

I. INTRODUCTION

Quorum sensing (QS) is a cell-to-cell communication system that allows bacteria to recognize cell density through the accumulation of signaling molecules (autoinducers) secreted by members of a community. When cell density is high, the accumulation of the signal in the extracellular medium allows the activation of a cellular response [1-2]. This system regulates various functions that require the concerted action of numerous cells such as bioluminescence, production of secondary metabolites, sporulation, biofilm formation, antibiotic resistance, and production of multiple virulence factors [3]. QS signal molecules are chemically diverse and characterized by having a low molecular weight [4].

One of the most common autoinducers by Gram-negative bacteria is acyl-homoserine lactone (AHL). AHL has a hydrophilic region consisting of a homoserine lactone ring and an amide group, and the hydrophobic region has a hydrocarbon chain that varies in length and level of oxygenation with a 3-oxo group depending on the microorganism [5].

One of the most common QS-mediated bacterial processes is the formation of biofilms. Biofilms are a microbial ecosystem, made up of one or several species that grow attached to a surface. Biofilms significantly reduce the sensitivity of bacteria to antibacterial agents and radiation [6]. Therefore, some infections are caused by pathogens that form biofilms, causing a negative impact on human health, agriculture, and food safety [4]. Different studies have shown that the blockade of QS signaling is considered an effective means to prevent the formation of biofilms of most pathogens, thus increasing the sensitivity of pathogens to antibacterial agents and improving the bactericidal effect of antibiotics [7].

One strategy for disrupting the QS system of pathogenic bacteria is to interfere with their signaling (quorum quenching - QQ) by inhibiting the biosynthesis of signal molecules, blocking the signal receptor, or enzyme-catalyzed degradation of signal molecules [8]. This strategy is less likely to select for resistance since it does not act directly on the growth of the pathogen [9]. The QQ enzymes act extracellularly to degrade AHL by degradation or cleavage of the homoserine lactone ring. The families of enzymes involved in this process include lactonase, decarboxylase, esterase, lactamase, or acylase [8] and [10].

The use of QQ enzymes has certain advantages including substrate affinity, catalytic efficiency, and stability. Due to the multiple applications of QQ enzymes, it is necessary to search for new QQ enzymes with high specificity for AHL molecules and variable stability under various conditions [11]. Therefore,

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the aim of this study was to assess the QQ enzyme production potential of Antarctic extremophile bacteria.

II. MATERIAL AND METHODS

A. Strains

The bacterial strains evaluated in this study were isolated from Antarctic marine sediments in previous studies [12] (Table 1). The strains were grown in LB broth and incubated at 10°C for eight days.

TABLE I
CHARACTERIZATION OF EVALUATED STRAINS

Sampling site	Code	Taxonomic affiliation	Colony description	Microscopic description	Gram stain
Base Artigas	UTB 170	<i>Psychrobacter cryohalolentis</i>	White, convex, entire and circular colony. 2mm	Cocci arranged in chain	Gram(-)
	UTB 171	<i>Psychrobacter glaciei</i>	White, convex, entire and circular colony. 5 mm	Rods short, thick, and are arranged in pairs	Gram(-)
Isla Decepción	UTB 167	<i>Psychrobacter glaciei</i>	White, convex, entire and circular colony. 2 mm	Rods short, thick, and are arranged in pairs	Gram(-)
Isla Herradura	UTB 173	<i>Psychrobacter glaciei</i>	White, convex, entire and circular colony. 2 mm	Rods short, thick, and are arranged in pairs	Gram(-)
	UTB 174	<i>Psychrobacter glaciei</i>	White, convex, entire and circular colony. 2 mm	Rods short, thick, and are arranged in pairs	Gram(-)
Península Byers	UTB 168	<i>Exiguobacterium</i> sp.	Yellow, convex, entire and circular colony. 1 mm	Long thick rods	Gram(+)
Punta Elefante	UTB 169	<i>Oceanisphaera marina</i>	White, convex, entire and circular colony. 3 mm	Rods short, thick, and are arranged in pairs	Gram(-)
Punta Elefante	UTB 172	<i>Oceanisphaera marina</i>	White, umbellate, entire and circular colony. 3 mm	Rods short, thick, and are arranged in pairs	Gram(-)

B. Semiquantitative lipolytic activity evaluation

To determine the lipolytic enzymatic activity, an MBS medium supplemented with Tween 80 or olive oil was used. The medium contained (g/L): Tween 80 or olive oil, 1; yeast extract, 1; and agar, 15. To evaluate the lipolytic enzymatic activity, the formation of a halo around the sensidisc indicated that the evaluated enzymatic activity is positive [13].

C. Evaluation of resistance to β -lactam antibiotics

The β -lactam antibiotics evaluated were penicillin (700 UI) and ampicillin (70 μ g/mL). For the antibiogram, previously inoculated LB agar was used, and filter paper discs (diameter 0.5 cm) impregnated with 20 μ L of each antibiotic evaluated were placed on the medium. The inoculated Petri dishes were incubated at 10 °C for 8 days [14]. All assays were performed in triplicate and sterile water was used as negative control.

Resistance to antibiotics was evidenced by presenting bacterial growth around the filter paper, and in case of susceptibility, inhibition halos were observed around each filter. The size of this inhibition zone reflected the sensitivity of the bacteria to the antibiotic evaluated, that is, the smaller the halo, the greater the resistance of the bacteria to β -lactam antibiotics [14].

D. β -lactamase activity evaluation

The beta-lactamase activity was evaluated in the intracellular fraction. To obtain the intracellular extract, the strains were cultivated in 20 mL of LB broth supplemented with each β -lactam antibiotic at a concentration of 70 μ g/mL (ampicillin) and 700 IU (penicillin) and were cultivated at 10°C for 8 days. Subsequently, the cultures were centrifuged at 8,000 rpm for 8 minutes at 4°C. The obtained pellet was washed three times with 50mM phosphate buffer (pH 7.0) and centrifuged at 8,000 rpm for 8 minutes at 4°C. Then, 20 μ L of 50mM phosphate buffer (pH 7.0) was added and it was sonicated for 1 minute at an amplitude of 40% during cycles of 8 seconds of sonication and 10 seconds of rest [15]. Subsequently, the samples were centrifuged at 9,000 rpm for 3 minutes, and the supernatant was recovered.

For the evaluation of β -lactamase activity, 103 μ L of 50 mM nitrocefin, 298 μ L of phosphate buffer (50mM-pH 7.0) and 100 μ L of the enzyme extract were used. The samples were incubated at 20 °C for 15 to 30 minutes. Finally, a spectrophotometric reading was performed at 480 nm. A β -lactamase unit was defined as the amount of enzyme necessary to hydrolyze 1 μ mol of substrate in each time under the evaluation conditions [16]. To calculate the concentration of enzymatic activity, the standard standard curve reported by [16].

III. RESULTS

A. Lipolytic activity

In this study it was determined that most of the evaluated strains presented lipolytic activity on the evaluated substrates (Table 2) (Fig. 1). The UTB 172 strain only presented lipolytic activity in the presence of the Tween 80.

TABLE II
LIPOLYTIC ACTIVITY OF THE EVALUATED STRAINS

Strain	Taxonomic affiliation	Hydrolysed substrates	
		T80	AO
UTB 167	<i>Psychrobacter glaciei</i>	+	+
UTB 168	<i>Exiguobacterium sp.</i>	+	+
UTB 169	<i>Oceanisphaera marina</i>	+	+
UTB 170	<i>Psychrobacter cryohalolentis</i>	+	+
UTB 171	<i>Psychrobacter glaciei</i>	+	+
UTB 172	<i>Oceanisphaera marina</i>	+	-
UTB 173	<i>Psychrobacter glaciei</i>	+	+
UTB 174	<i>Psychrobacter glaciei</i>	+	+

T80: Tween 80. AO: olive oil

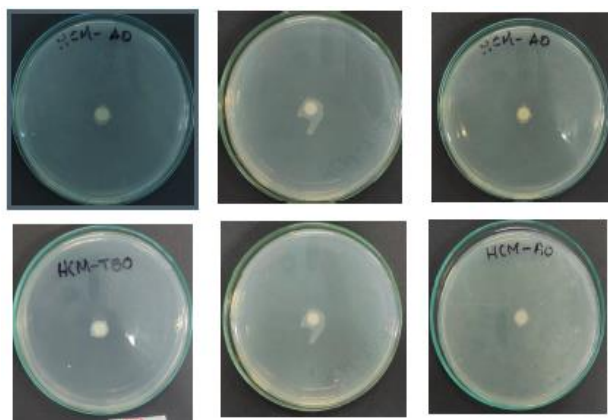


Fig 1: Semiquantitative lipolytic activity of the evaluated strains

B. Resistance to β -lactam antibiotics

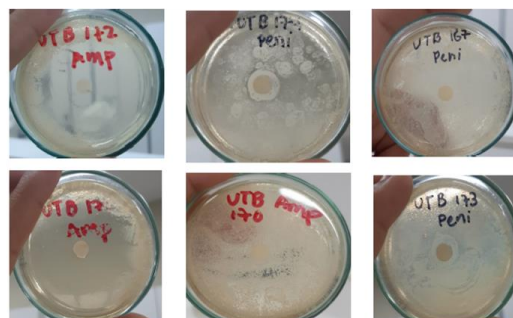
The results obtained allowed us to determine that strains UTB 167, UTB 170, UTB 171, and UTB 174 presented resistance to ampicillin and penicillin, which are related to the genus *Psychrobacter*. On the other hand, strains UTB 168 (*Exiguobacterium sp.*) and UTB 173 (*Psychrobacter glaciei*) were only resistant to penicillin, while strain UTB 172 (*Psychrobacter glaciei*) presented resistance only to

ampicillin. The results of resistance to β -lactam antibiotics are presented in Table 3 and Fig. 2.

TABLE III
RESISTANCE TO BETA-LACTAM ANTIBIOTICS OF THE EVALUATED STRAINS

Strain	Taxonomic affiliation	Ampicillin	Penicillin
UTB 167	<i>Psychrobacter glaciei</i>	+	+
UTB 168	<i>Exiguobacterium sp.</i>	4,2	+
UTB 169	<i>Oceanisphaera marina</i>	3	+
UTB 170	<i>Psychrobacter cryohalolentis</i>	+	+
UTB 171	<i>Psychrobacter glaciei</i>	+	+
UTB 172	<i>Oceanisphaera marina</i>	+	3,7
UTB 173	<i>Psychrobacter glaciei</i>	4,5	+
UTB 174	<i>Psychrobacter glaciei</i>	+	+

a



b

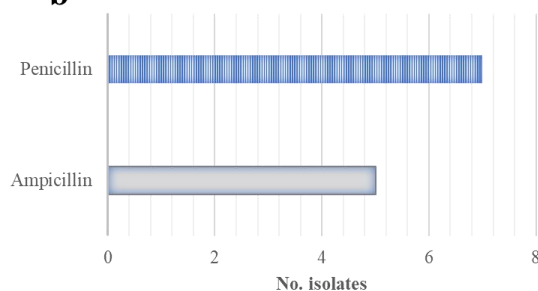


Fig 2: Evaluation of resistance to β -lactam antibiotics. a. Photographs inhibition halos. b. graph of the number of resistant strains detected in each antibiotic evaluated.

C. β -lactamase activity

To evaluate the beta-lactamase activity, the strains UTB 167 (*Psychrobacter glaciei*) and UTB 170 (*Psychrobacter cryohalolentis*) were selected since they presented lipolytic activity in the two evaluated substrates and multiresistance to β -lactam antibiotics. The UTB 170 strain presented a higher β -

lactamase activity in the presence of ampicillin (3,9 Umol/L), while in the UTB 167 strain a similar beta-lactamase activity was detected in the antibiotics evaluated (1,7 Umol/L). Fig. 3 presents the β -lactamase activity of the selected strains.

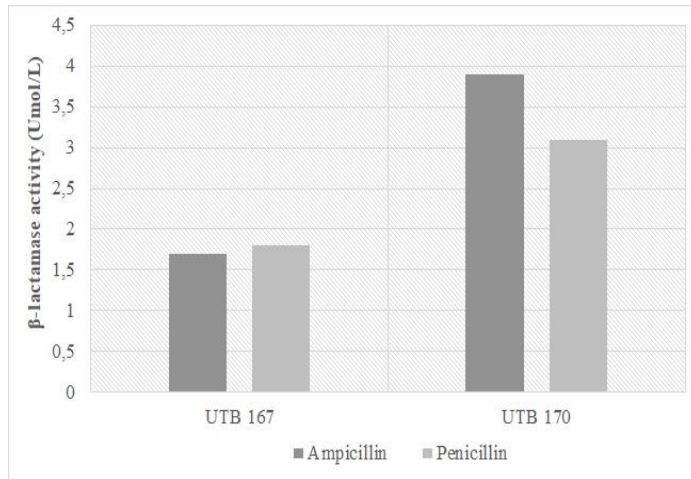


Fig 3: β -lactamase activity of the intracellular fraction of the strains UTB 167 and UTB 170

IV. DISCUSSION

QS is a communication system between bacteria that coordinates the expression of genes related to the secretion of pathogen virulence factors and the formation of biofilms in response to the density of the bacterial population [17]. The use of antibiotics has been the conventionally used strategy to inhibit the growth of pathogenic bacteria [18]. However, due to its excessive and inappropriate use, it has caused the proliferation, prevalence and persistence of antibiotic-resistant microorganisms and antibiotic resistance genes in the environment [19]. Therefore, strategies such as QQ can be an alternative for disease control, since its action is directed at stopping the virulence mechanisms of pathogens instead of inhibiting their growth, which reduces the emergence of resistance to antibiotics [20-21]. In recent years, interest in the discovery of QQ enzymes has increased due to their potential use in different biotechnological applications related to human health, aquaculture, agriculture, biofouling and biocorrosion [22]. However, this requires different QQ enzymes with variable substrate specificity, affinity, and stability under various conditions to eliminate communication mediated by specific AHL molecules in specific environments [23]. Therefore, the objective of this study was to identify the production potential of QQ enzymes of psychrophilic bacteria isolated from Antarctica, an environment little studied and with hostile conditions of temperature, radiation, and nutrient availability [24].

One of the most studied groups of QQ enzymes are the lactonases, which belong to the superfamily of metallo- β -

lactamases that possess the conserved HXHXDH motif required for AHL-degrading activity [21]. In this study we identified that the evaluated strains presented lipolytic activity. Previous studies have reported psychrophilic and psychrotolerant lipase-producing bacteria isolated mainly from the Antarctic and polar regions [25-27]. The lipases obtained from psychrophilic bacteria present high catalytic efficiency with low thermal stability with class C β -lactamase enzymes [28], therefore, the presence of lipolytic enzymes detected in the strains evaluated in this study indicates that they probably have the ability to produce β -lactamase enzymes. On the other hand, it has been reported that lipases obtained from psychrophilic bacteria have high catalytic efficiency with low thermal stability [29], parameters that are necessary for the application of QQ enzymes as anti-infective agents.

Resistance to β -lactam antibiotics is indirect evidence of β -lactamase production. Here, we identified that most of the strains evaluated present multiresistance to β -lactam antibiotics [30-31]. In the study carried out by [32] it was shown that *Psychrobacter* sp. is one of the genera associated with antibiotic resistance genes in sediments from Deception Island, Antarctica. In the case of genera *Oceanisphaera* and *Exiguobacterium*, they have not been previously reported as genera resistant to β -lactam antibiotics. The production of β -lactamase enzymes was detected in the strains UTB 167 and UTB 170, which are related to the genus *Psychrobacter*. These results coincide with previous studies where the ability of *Psychrobacter* spp. to produce β -lactamases [33-34]. On the other hand, [35] reported that the species *Psychrobacter* sp. TAE2020 exhibited high quorum quenching activity against a wide range of synthetic N-acylhomoserine lactone (AHL) at 4, 15, and 28 °C. Therefore, the results obtained in this study demonstrate the potential of psychrophilic bacteria from Antarctica to produce quenching enzymes.

V. CONCLUSIONS

The present study identified that the psychrophilic bacterial strains UTB 167 and UTB 170, related to the genus *Psychrobacter*, produce β -lactamases that could potentially be used as QQ enzymes. In addition, it was shown that polar ecosystems represent a reserve of biodiversity with the potential to produce QQ enzymes.

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