

Identification, characterization enzymatic antagonist activity and antimicrobial resistance profile of bacteria isolated from Brazilian thermal aquifer

Identificação, caracterização enzimática, atividade antagonista e perfil de resistência a antimicrobianos de bactérias termotolerantes isoladas em aquífero termal Brasileiro

DOI:10.34117/bjdv7n1-559

Recebimento dos originais: 01/01/2021

Aceitação para publicação: 21/01/2021

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RESUMO

O objetivo deste trabalho foi isolar identificar, rastrear enzimas de interesse biotecnológico, traçar o perfil de resistência a antimicrobianos e verificar a atividade antagonista de microrganismos termotolerantes isolados em aquífero termal em Caldas Novas, Brasil. Para tal foram coletadas amostras de águas profundas em três poços artesianos, inoculadas em ágar nutriente e incubadas a 37°C durante 24 horas. Os isolados foram identificados ao nível de espécie por espectrometria de massa MALDI-TOF. As espécies identificadas foram submetidas ao *screening* enzimático para amilase, celulase, gelatinase, caseinase, lipase e pectinase conforme metodologias padrão. O perfil de susceptibilidade das bactérias aos antimicrobianos foi realizado por disco difusão e a atividade antagonista foi avaliada frente à *Escherichia coli* ATCC 8739 e *Staphylococcus aureus* ATCC 25923. Foram obtidas quatro isolados identificados como *Bacillus pumilus*, *Bacillus subtilis* e dois *Bacillus megaterium*. Os isolados produziram caseinase e gelatinase e foram sensíveis a todos os antimicrobianos testados. *Bacillus pumilus*, *Bacillus subtilis* e *B. megaterium* 3207 exibiram atividade antagonista frente ao *S. aureus*. A atividade antagonista frente à cepa de *S. aureus* aqui encontrada abre uma perspectiva para futuros estudos de identificação de metabólitos secundários com potencial atividade antimicrobiana

Palavras-chave: Antagonismo, Bioprospecção, Enzimas, Hot Springs, MALDI-TOF.

ABSTRACT

The aim of this study was to isolate, identify, search for enzymes of biotechnological interest, trace the resistance profile to antimicrobials and verify the antagonistic activity of thermotolerant microorganisms isolated in thermal aquifer in Caldas Novas, Brazil. For

this purpose, deep water samples were collected from three artesian wells, inoculated on nutrient agar and incubated at 37°C for 24 hours. The isolates were identified at species level by MALDI-TOF mass spectrometry. The identified species were *screened* for amylase, cellulase, gelatinase, caseinase, lipase, and pectinase enzymes according to standard methodologies. The profile of susceptibility bacteria to antimicrobials was performed by disc diffusion and the antagonist activity was evaluated against *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 25923 bacteria. The obtained four isolates were identified as *Bacillus pumilus*, *Bacillus subtilis* and two as *Bacillus megaterium*. All isolates produced caseinase and gelatinase and they were sensitive to all antimicrobials tested. *Bacillus pumilus*, *Bacillus subtilis* and *B. megaterium*-3207 exhibited antagonistic activity against *S. aureus*. The antagonist activity against the *S. aureus* strain opens a perspective for future studies to identify secondary metabolites with potential antimicrobial activity.

Keywords: Antagonism, Bioprospecting, Enzymes, Hot Springs, MALDI-TOF.

1 INTRODUCTION

The city of Caldas Novas located in the state of Goiás, Midwest of Brazil, holds the largest thermal underground water complex on the planet not associated with volcanism or another magmatism-related phenomenon. The hydrothermalism of this aquifer is exclusively by geothermal gradient and it has low mineralization due to the rocky composition of the groups Araxá and Paranoá that form these aquifer system (Andrade and Almeida, 2012; Campos and Cunha, 2015; Lunardi and Bonotto, 2018).

Natural hot springs represent unique environments, providing support for thermophilic microorganisms, which can be used as sources of new genes, molecules and/or enzymes to industrial interest (Saxena *et al.*, 2016). These sources occur in many regions of the world, with unique geophysical and biological characteristics, providing a diversity of microorganisms among sources from different locations (Peng *et al.*, 2017; Sahay *et al.*, 2017; Prieto-Barajas *et al.*, 2018). Thermophilic microorganisms have *attracted attention as sources of thermostable enzymes, such as amylases, cellulases, chitinases, pectinases, xylanases, proteases, lipases and DNA polymerases, which have* characteristics appropriate to the extreme processes used in industry (Mohammad *et al.*, 2017). The global demand for enzymes is growing rapidly and different thermo-active enzymes produced by microorganisms find its applications in various industries such as food, detergent, pharmaceutical and cosmetic, biofuels, leather processing, pulp and paper, textile industry and for studies in biology (Torimiro and Okonji, 2013; Gopinath *et al.*, 2017; Oumer and Abate, 2017; Sahay *et al.*, 2017; Yadav *et al.*, 2018). In addition to enzymes, a variety of other compounds with

pharmacological properties such as antibiotics, antifungals and anti-inflammatories have been identified in bacteria living in complex ecological communities, such as those found in terrestrial and aquatic environments (Jiménez-Delgado *et al.*, 2018). Physical-chemical parameters can influence microbiota in environments and prokaryotic communities of thermal springs are well adapted to high temperatures and chemical stress. Studies have been conducted investigating microorganisms living in extreme environments, including hot springs (Sahay *et al.*, 2017; Prieto-Barajas *et al.*, 2018).

The order *Bacillales* includes thermophilic members of several genera as *Geobacillus*, *Bacillus*, *Aeribacillus*, *Paenibacillus*, *Brevibacillus* and *Anoxybacillus* being more common in thermal sources (Aanniz *et al.*, 2015; Thebti *et al.*, 2016; Sahay *et al.*, 2017; Prieto-Barajas *et al.*, 2018; Yadav *et al.*, 2018). Members of the genus *Bacillus* are described to produce and secrete soluble antimicrobial molecules, which are used as biocontrol agents as well as in the prevention and control of infections (Mohammadou *et al.*, 2014; Kaki *et al.*, 2017).

The worldwide use of antibiotics contributes to the development of resistant strains of various pathogens, a global public healthcare concern (Zhao *et al.*, 2017; Banin *et al.*, 2017; Gómez-Ríos and Ramirez-Malule, 2019). Antimicrobial resistance is a multi-factorial process, usually through the acquisition of determinants of resistance already existing in the bacterial gene pool, through mobile genetic elements or DNA molecules, driven by the misuse of antibiotics in humans and animals (Banin *et al.*, 2017; Partridge *et al.*, 2018; Gómez-Ríos and Ramirez-Malule, 2019). It affects millions of people worldwide, implying additional costs in the order of trillion of dollars, and there is a consistent demand for more products with a single activity, not only as antimicrobials, but also as agents in the treatment of others health conditions (Mahajan and Balachandran, 2017).

Among the interactions that occur in biotic systems, antagonism is based on the release of toxic compounds, such as antibiotics and bacteriocins, in correspondence with interference competition, in which one species inhibits the development of the other, for greater access to food resources. These compounds have high potential in biotechnology, human or veterinary medicine (Moëne-Loccoz *et al.*, 2015).

The objective of this work was to isolate, identify, search for enzymes of biotechnological interest, trace the resistance profile to antimicrobials and verify the antagonist activity of microorganisms isolated in thermal aquifer in the city of Caldas

Novas, Goiás, Brazil.

2 MATERIALS AND METHODS

2.1 STUDY SITES AND SAMPLE COLLECTION

Deep water samples were collected from three thermal artesian wells located in the municipality of Caldas Novas- GO. The geographic coordinates of the collection points are indicated in table 1. The rationale of the choice of the artesian thermal wells was based on the record history of those with the highest temperatures according to reports of the Association of Thermal Water Miners of Goiás (AMAT). During sampling, the temperature and pH of the water collected directly from the artesian well runoff system were recorded. Five samples from each well were collected and transferred to sterile amber flasks and later submitted to laboratory analysis.

2.2 ISOLATION OF BACTERIA

Bacteria were isolated by adding 40 mL of each water sample in 50 mL of nutrient broth and incubated at 37°C for 48 hours. Then, a serial dilution was performed, followed by plating in nutrient agar and incubation at 37°C during 48 hours. Colonies were counted, selected according to the morphological characterization of the colonies by observation of color, appearance and presence of brightness or opacity and individual colonies were inoculated in 5 mL of nutrient broth and incubated at 37°C for 48 hours. The bacterial cells were centrifuged at 10,000 rpm for 2 minutes and the sediment was resuspended in nutrient broth plus 20% glycerol and stored at -80°C.

2.3 MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATIONS

The biochemical tests were performed according to MacFaddin (2003) and included: catalase, oxidase, mannitol, lecithinase, DNase, indole, tryptophan, sucrose, H₂S, glucose, urea, lysine, gas production from glucose and motility. The morphology of the isolates was investigated by optical microscopy. The colonies were stained Gram according to standard methodology and analyzed under Axio Lab optical microscope A1 Zeiss at magnification of 1000X and photographed in an AxioCamICc 5 digital ocular electronic camera. The optimal temperature was determined by incubation of bacteria in nutrient agar medium at temperatures range from 35 to 60°C and the pH was determinate, growing it in pH from 5.0 to 8.0 at a fixed temperature of 42°C. The

bacterial growth rate was determined by measuring the colony diameter with an analytical pachymeter.

2.4 BACTERIAL IDENTIFICATION BY MALDI-TOF

The proteomics identification by MALDI-TOF (*Matrix Assisted Laser Desorption Ionization - Time of Flight*) Mass Spectrometry was conducted by the AQUACEN laboratory of the Veterinary School of the Federal University of Minas Gerais (UFMG), using Bruker Daltonics' Microflex™ MALDI-TOF MS device following the protocol established by Assis et al (2017).

2.5 SCREENING OF ENZYMES-PRODUCING BY BACTERIAL ISOLATES

All isolates were investigated for the production capacity of commercial enzymes as amylase, pectinase, caseinase, lipase, cellulase and gelatinase. Appropriated culture media containing the specific substrate for each enzyme production was used as described below and the cultures were incubated at 42°C for 24-48 hours.

2.6 AMYLASE

Amylase activity was screened in plates containing starch agar (10 g tryptone, 10 g starch, 5 g meat extract, 15 g Agar-Agar, and 1000 mL distilled water, pH 7.4 ± 0.2). The plates were incubated for 24 hours at 42°C, refrigerated at 8°C for 24 hours and then submitted to the verification of the substrate degradation halo by staining with 2% iodine/potassium iodide solution for 15 minutes (Xavier *et al.*, 2017). A clear halo formation around the colony indicates amylase production.

2.7 CELLULASE

The screening of cellulolytic activity was done according to the method described by Bischoff et al. (2006), with modifications. The bacteria were inoculated in M9 medium supplemented with 0.5% w/v carboxymethyl cellulose (CMC) as the only carbon source and incubated at 42 °C for 24 hours. The enzymatic activity was verified by halo formation after staining with 1% (w/v) Congo-red solution for 15 minutes, followed by discoloration with 1 mol/L NaCl and treatment with 1 mol/L HCl. A clear halo formation around the colony indicates cellulase production.

2.8 GELATINASE

The screening of the gelatin-hydrolysis capacity was verified on nutrient agar containing 12 g/L of gelatin and incubation at 42°C for 24 hours. The revelation was performed by adding 10 mL of saturated Sulphate Magnesium solution into the plate and incubated for 10 minutes. After removal of solution excess, the formation of translucent halo around the colony was observed and halo detection indicates gelatinase production (McDade and Weaver, 1959).

2.9 LIPASE

The screening of lipolytic activity was performed by incubating the colonies in medium containing 10 g casein peptone, 5 g NaCl, 0.09 g anhydrous CaCl₂, 15 g Agar-Agar, and 1000 mL distilled water, pH 6.0 ± 0.1 and addition of 0.001 g/L Tween 80 after sterilization and cooling to 60°C. The plates were incubated at 42°C for 24 hours. Lipolytic activity was verified by the formation of calcium crystal halo around the colony (Sierra, 1957).

2.10 PECTINASE

Screening of the pectin hydrolysis capacity was verified on nutrient agar supplemented with 1% citric pectin and incubation for 24 hours at 42°C. The revelation was carried out by adding 5 mL of 1 mol/L anhydrous CaCl₂ solution and incubated for five minutes and appearance of translucent halos around the colony was observed, which indicates pectinase production (Minotto *et al.*, 2014).

2.11 CASEINASE

The casein degradation capacity was verified according to Minotto *et al.* (2014), using nutrient agar medium containing 25 g/L skimmed milk powder, 15 g/L Agar-agar, and 1% bromophenol blue. After incubation of the plates at 42°C for 24 hours, the formation of translucent halo around the colony was checked, which indicates casein degradation.

2.12 ANTIMICROBIAL SENSITIVITY PROFILE

Sensitivity profile to the various classes of antimicrobials was determined by the diffusion disc method, according to the guidelines from Clinical and Laboratory Standards Institute (CLSI, 2018), using the following antimicrobials: 10 µg Amikacin

(AMI), 30 µg Vancomycin (VAN), 10 µg Meropenem (MER), 1 µg Oxacillin (OXA), 10 µg Imipenem (IMP), 30 µg Cefoxitin (CFO), and 10 µg Sulbactam + 10 µg Ampicillin (SBA). The diameters of the inhibition halo were measured using a pachymeter and interpreted according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CASM/EUCAST, 2019). The isolate was considered sensitive if any zone of inhibition was observed around the antibiotic disc according to the methodology employed by Ismail *et al.*, (2016).

2.13 MICROBIAL ANTAGONISM TEST

The evaluation of the antagonistic effect was performed using the cross-test method described by Mohammadou *et al.* (2014), with modifications. Each individual isolate was inoculated in a single groove on a nutrient agar plate using a sterile 10 µL disposable strap. After incubation at 42°C for 24 hours, the bacteria were inactivated with chloroform for 10 minutes. A revealing bacterial suspension (*Escherichia coli* ATCC 8739 or *Staphylococcus aureus* ATCC 25923) at one in the MacFarland scale was then inoculated perpendicular to the inactivated isolate and incubated at 37°C for 24 hours. The antagonistic activity was verified by the formation of inhibition growth zone.

3 RESULTS AND DISCUSSION

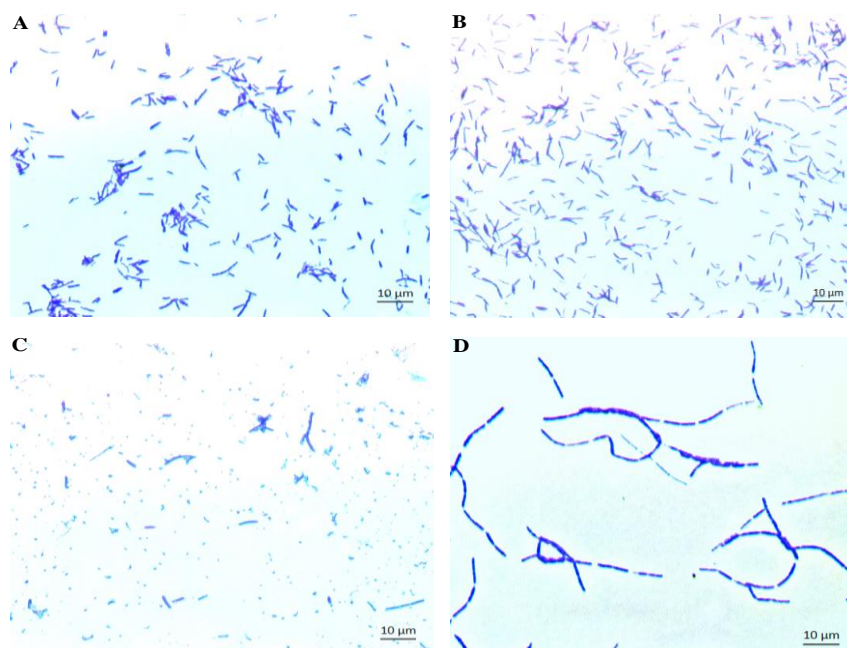
The geographical localizations of the artesian wells as well as data on depth, altitude, latitude, longitude, temperature and pH of the water at the time of sampling are shown in Table 1. The water temperature in the artesian wells selected for this study varied from 41 to 54°C with pH values from 6.0 to 7.0 (Table 1). The groundwater in the Caldas Novas region has low mineralization, temperatures in the range of 40 to 54°C, and depth more than 600 meters (Campos and Cunha, 2015; Lunardi and Bonotto, 2018). In these conditions, biodiversity can be reduced, since the organisms that inhabit this niche need to deal with high temperatures and low availability of nutritional compounds, due to the depth of the groundwater. However, some bacteria develop strategies to survive in these conditions (Mohammad *et al.*, 2017). From our knowledge so far is the first study with characterization of isolated microorganisms from thermal groundwater with exclusively geothermal heating in the region of Caldas Novas, Brazil.

Table 1: Geographical coordinates of collection sites and characteristics of the sampled groundwater. Sampling date: 2017.10.11

Artesian well	Depth (m)	Altitude (m)	Latitude	Longitude	Temperature (°C)	pH
Well 1	633	545	17°44'15,05"S	48°37'32,3"W	54	6,0
Well 2	679	676	17°44'19,6"S	48°37'28,9"W	41	7,0
Well 3	685	675	17°44'31,8"S	48°38'01,8"W	53	6,0

In this work, it was isolated 15 colonies from three sampled artesian wells, being 0 (none), 4 and 11 colonies from artesian well number 01, 02 and 03, respectively. Eight of those colonies could be recovered from -80°C, subsequently cultivated and submitted to the MALDI-TOF analysis at species level identification. Among these, four colonies presented reliable identification results at species level by MALDI TOF mass spectrometry, assigned as *Bacillus pumilus*, *Bacillus subtilis*, and 2 strains of *Bacillus megaterium* coded as *B. megaterium*-3207 and *B. megaterium*-3505 (Table 2). The isolates of *B. megaterium* differed in terms of indole production, urea degradation and optimal temperature and pH growth (Table 2). The result of morphological and tinctorial analysis revealed that the 4 identified isolates were Gram-positive bacteria (Table 2) in the form of rods (Figure 1).

Figure 1: Microscopic analysis of the selected four bacterial isolated. The bacteria were stained with Gram staining under the trinocular Microscope Axio Lab. A1, Carl Zeiss in the magnitude of 100X and captured in a digital electronic camera AxioCamICs 5 with FireWire Card 5 MP. A: *Bacillus pumilus*; B: *Bacillus megaterium*-3207; C: *Bacillus subtilis*; D: *Bacillus megaterium* – 3505.



The morphological and microscopic characteristics (Figure 1) of all isolates were similar to the characteristics of the genus *Bacillus*, confirmed by proteomic identification (Table 2).

The optimal pH growth was 8.0 for *B. megaterium*-3207 and *B. pumilus* after ten hours of incubation at 42°C, differently for *B. subtilis* and *B. megaterium*-3505 that present optimal growth at pH 7.0 (Table 2). The optimal temperature growth was 50°C for *B. subtilis*, *B. pumilus* and *B. megaterium*-3207 and 45°C for *B. megaterium*-3505 after 10 hours of incubation. The temperature of 42°C allowed good growth during 24 hours, being chosen to conduct the cited experiments. Although, the *B. megaterium* came from the same source, the two isolates grow differently and presented different patterns in the analyses performed (Table 2). These findings are in accordance to the literature on the absence of a monotonic relationship in an extreme temperature environment (Kumar *et al.*, 2014; Sharp *et al.* 2014).

These findings are consistent with studies conducted in several hot springs and suggest that the presence of *Bacillus* in the sampled sites may be due to the ability of the genus to withstand adverse environmental conditions (Connor *et al.*, 2010; Mohammad *et al.*, 2017; Sahay *et al.*, 2017; Yadav *et al.*, 2018). Furthermore, the physical and chemical characteristics of the aquifer, the possibilities of water replenishment and sampling site seem to determine the microbial community present (Chiriac *et al.*, 2017; Chiriac *et al.*, 2018).

In this work the result of enzymatic *screening* revealed that the isolates were able to produce the proteolytic enzymes caseinase and gelatinase, however, none of the isolates produced amylase, lipase, pectinase or cellulase (Table 2). The formation of halo around the colony observed indicates the ability of the microorganism to produce these proteases and to degrade the substrate present in the culture medium. The intensity difference observed among the halos in this study may be due to the amount of extracellular enzyme, also reported by Luz *et al.*, 2016. There is a close relationship between the niche occupied by a microorganism and the intra and extracellular enzymes produced by it, indicating the development of genetic and physiological mechanisms to use the available organic matter (Berrada *et al.*, 2012). The aquifer in the Caldas Novas-GO region has low mineralization and its depth also contributes to the scarcity of nutrients, which may explain the absence in the enzymes production against carbohydrates in the isolated strains, due to its adaptability to the environment. However, microbial proteases are among the most important hydrolytic enzymes with

great potential for use in various biotechnological processes, as food processing, pharmaceutical products and several other industries (Uddin *et al.*, 2017). Caseinase is used in the food industry as a coagulant in cheese manufacturing and in the reduction of milk allergens, as well as in the energy drinks, dietary foods and health care industry (Dalmaso *et al.*, 2015; Bhaturiwala *et al.*, 2017). Another important protease, gelatinase has played an important role in the food industry, as a meat tenderizer, in the beverage industry, cosmetics and in the development of drugs due to its ability to degrade connective tissue linked to tumor metastasis (Dalmaso *et al.*, 2015; Mohammad *et al.*, 2017).

Table 2: Phenotypic characteristics, proteomic identification, biochemical characterization and potential enzyme production of bacteria isolated from thermal groundwater collected from artesian wells in the city of Caldas Novas-GO, Brazil.

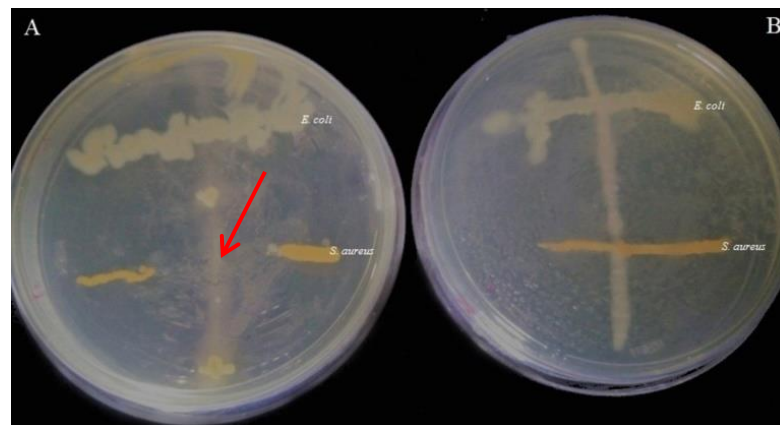
Characteristics assessed	Isolated 1	Isolated 2	Isolated 3	Isolated 4
Place of isolation	Well 2	Well 3	Well 3	Well 3
Morphological characteristics of the colonies	Opaque beige	Radiated beige	Dry beige	Shiny beige
Gram Staining Analysis	Gram -	Gram +	Gram	Gram +
Species identification (MALDI-TOF)	<i>B. pumili</i>	<i>B. megateri</i>	<i>B. subtili</i>	<i>B. megateri</i>
Catalase	+	+	-	+
Oxidase	-	-	-	-
Coagulase	+	-	-	-
Indole	-	-	-	+
Tryptophan	+	+	-	+
Sucrose	+	+	+	+
H ₂ S	-	-	-	-
Urea	+	-	+	+
Lysine	+	-	+	-
Motility	-	-	-	-
Gas Production	+	+	+	+
Amylase	-	-	-	-
Caseinase	+	+	+	+
Lipase	-	-	-	-
Cellulase	-	-	-	-
Gelatinase	+	+	+	+

Pectinase	-	-	-	-
Growth at 37°C	+	+	+	+
Growth at 50°C	+	+	+	+
Optimum temperature growth	50°C	50°C	50°C	45°C
Optimal pH growth	8.0	8.0	7.0	7.0

In the diffusion disc antimicrobial susceptibility test, the isolates were sensitive to all antibiotics tested (Table 3), and the results corroborates that microorganisms in natural environments are presumably sensitive to antibiotics (Grenni *et al.*, 2018) and a study performed by Selim *et al.* (2014) in which microorganisms isolated from water and hot spring soils were sensitive to all antibiotics tested. According to Jardine *et al.* (2019), hot springs are the only truly pure environmental niches, due to a constant flow in one direction, preventing water stagnation. Therefore, resistance to antibiotics in these sites should not increase as a result of human or animal action, but only due to natural evolution.

Regarding the antagonist activity, isolates *B. pumilus*, *B. subtilis* and *B. megaterium*-3207 were able to inhibit the growth of the standard strain of *S. aureus* (Figure 2), but none of isolated microorganism inhibited the growth of *E. coli* (Table 3).

Figure 2: Antagonistic activity of *Bacillus pumilus* (A) and *Bacillus megaterium*-3505 (B) isolates against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 8739. Growth inhibition in the crossbreeding region *Bacillus pumilus* in front of *Staphylococcus aureus* ATCC 25923 is indicated by the red arrow.



This suggests that these bacilli have inhibitory mechanisms capable of controlling Gram-positive pathogens by producing secondary metabolites that act as powerful antimicrobials. Members of the genus *Bacillus* are known to produce antimicrobial metabolites as antifungals and antibacterial (Bodhankar *et al.*, 2017; Pandya *et al.*, 2017; Boottanun *et al.*, 2017). These findings corroborate the results from Pednekar *et al.* (2011), where the authors concluded that most antimicrobials in bacilli, paenibacillus and streptomycetes are anti-Gram-positive bacteria. Also, Genomic analysis performed by Nascimento *et al.* (2020) revealed the presence of several genes producing antimicrobial compounds in the *B. megaterium* STB1 strain, some homologous to those produced by *B. subtilis* related to antagonist activity and production of antifungal and antibacterial compounds.

Table 3: Antibiotic susceptibility profile and microbial antagonism of bacteria isolated from thermal waters collected from artesian wells in the city of Caldas Novas-GO, against standard strains of *Escherichia coli* and *Staphylococcus aureus*.

Isolate	ANTIMICROBIALS						ANTAGONISM		
	VAN	IMP	CFO	SBA	OXA	MER	AMI	<i>S. aureus</i>	<i>E. coli</i>
<i>B. pumilus</i>	S	S	S	S	S	S	S	+	-
<i>B. megaterium</i> -3207	S	S	S	S	S	S	S	+	-
<i>B. subtilis</i>	S	S	S	S	S	S	S	+	-
<i>B. megaterium</i> -3505	S	S	S	S	S	S	S	-	-

S: sensitive; VAN: Vancomycin; IMP: Imipenem; CFO: Cefoxitin; SBA: Sulbactam + Ampicillin; OXA: Oxacillin; MER: Meropenem; AMI: Amikacin.

Thermal sources, especially groundwater, are still little explored in their microbial potential and may be a source of new bioactive compounds of pharmaceutical importance (Shanker *et al.*, 2014; Alrumman *et al.*, 2018).

4 CONCLUSION

From our knowledge, this is the first bioprospection of bacteria and characterization of enzymes of biotechnological interest performed in deep thermal water in one of the largest thermal aquifers in the world, located in South America. The results showed a bacterial microbiota similar to bacterial genera identified in other hydrothermal environments previously described in the literature. Enzymatic screening indicated candidate microorganisms for the production of two enzymes of biotechnological interest. Furthermore, the antagonistic activity against the *S. aureus* strain by isolated strains opens a perspective for future studies to identify secondary

metabolites with potential antimicrobial activity.

CONFLICT OF INTEREST

There are no conflicts of interest to declare

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