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ISOLATION AND CHARACTERISATION OF A NEW ALKALI-HALOTOLERANT *BACILLUS AQUIMARIS* STRAIN LGMT10, PRODUCING EXTRACELLULAR HYDROLASES, FROM THE EFFLUENTS OF A THERMAL POWER PLANT, IN ALGERIA

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ABSTRACT

Modern biotechnology takes into account cost, performance, and respect for the environment to set up an industrial process. In this sense, the study's goal is to highlight the presence of indigenous microbial microflora in the Terga thermal power plant effluents, which are capable of secreting extracellular hydrolases. Four different extracellular hydrolases classes, which are most of the time used in bioindustry, namely protease, amylase, lipase, and cellulase, were investigated in agar plate assay, to select microorganisms with an interesting enzymatic potential adapted to this type of environment. Consequently, the results have shown that among twelve isolated bacterial strains, three strains were screened based on their enzymatic potential, and were later identified by *16S* rRNA gene sequencing, i.e. showing that the strains belong to the genus *Pseudomonas aeruginosa*, and *Bacillus wiedmannii* with a similarity percentage of 99.33% and 100%, respectively, with their corresponding type of strains. Among them, the strain LGMT10 that belongs to the *Bacillus* genus, and is closely related to *Bacillus aquimaris*, showed a *16S* rRNA sequence similarity with the type of strain of 99.23%. This strain presents adequate characteristics to resist the harsh conditions of pH and NaCl. It could grow against wide ranges of NaCl concentrations between 0-12% (w/v), pH 5.5-12, and was able to produce extracellular hydrolases (i.e., protease, amylase, and cellulase) against pH ranges of 6.8-12 and NaCl concentrations between 0-12% (w/v) at 30 °C. This strain's intriguing enzymatic potential, as well as its pH and salinity tolerance, make it a promising candidate for various biotechnological applications in detergent, leather, textile, and food processing industries.

Keywords: amylase; cellulase; protease; lipase; alkali-halotolerant; *Bacillus aquimaris*

INTRODUCTION

The search for new eco-friendly means potentially involved in various industrial processes has directed the scientific community to microbial enzymes **(Ardakani *et al*., 2012)**. In recent years, research dealing with enzymes of extremophilic microorganisms has found great interest **(Shukla, 2019)**. However, many microbes such as bacteria, actinomycetes, fungi, and yeast extracellularly or intracellularly produce a group of versatile and attractive enzymes with a wide variety of structures and commercial applications. Many microbial enzymes, such as amylases, proteases, pectinases, lipases, xylanases, cellulases, and laccases are extracellularly produced **(Fiedurek and Gromada, 2000; Venkateshwaran *et al*., 1999)**.

The microbial enzymes have gained recognition globally for their widespread uses in various industrial sectors, e.g., food, agriculture, chemicals, medicine, and energy. Enzyme mediated processes are rapidly gaining interest because of reduced processing time, intake of low energy input, cost-effective, non-toxic and eco-friendly characteristics **(Li *et al*., 2012; Choi *et al*., 2015)**. In addition, the microbial enzymes have been given more attention due to their active and stable nature compared to enzymes extracted from plants and animals **(Anbu *et al*., 2013; Gopinath *et al*., 2013)**. Most microorganisms are unable to grow and produce enzymes under harsh environments that cause toxicity. However, some microorganisms have undergone various adaptations enabling them to grow and produce enzymes under harsh conditions **(Sardessai and Bhosle, 2004; Anbu, 2016)**. Recently, several studies have been initiated to isolate new bacterial and fungal strains from harsh environments such as extreme pH, temperature, salinity, heavy metal, and organic solvent, in order to the produce different enzymes having the properties to yield higher **(Anbu, 2016; Gopinath *et al*., 2005)**.

Halotolerant bacteria form a versatile group adapted to life at the lower range of salinities, with the possibility of rapid adjustment to changes in the external salt concentration **(Litchfield, 2002)**. This property of halotolerant bacteria makes them better candidates for bio-prospecting than their halophilic counterparts. Enzymes of the halotolerant bacteria find a very interesting field of application. However, halotolerant proteases are hydrolytic enzymes, which are mostly used in industries. For example, detergent industries add halotolerant proteases to their laundry detergent formulations in order to hydrolase proteinaceous stains **(Anwar and Saleemuddin, 1998)**. The tannery industry uses halotolerant proteases to assist in de-hairing of animal hides **(Abd Samad *et al*., 2017)**. Moreover, the identification of novel bacterial cellulases remains a currently explored route to the development of modern sustainable bio-industries for biofuel generation **(Novy *et al*., 2015)**. Thermo-alkali-stable cellulases isolated from extremophilic *Bacillus* strains have shown their potential within conditions that are appropriate for bioconversion processes **(Souii *et al*., 2020)**.

Marine microorganisms have been attracting more attention as a resource for new enzymes. The complexity of the marine environment involving high salinity, high pressure, low temperature, and special lighting conditions may contribute to the significant differences between the enzymes from marine microorganisms and homologous enzymes from terrestrial microorganisms **(Zhang and Kim, 2010)**. Besides that, new approaches such as metagenomics need to be performed to identify new groups of bacteria that remain unexplored in the seas and oceans **(Sharma *et al*., 2019)**.

In this context, the current research highlighted the promising potential of a newly isolated marine source bacterium; *Bacillus aquimaris* strain LGMT10, isolated from the effluents of the Terga thermal power plant, in western Algeria, after *16S* rDNA sequencing. This bacterial strain can produce three extracellular enzymes (i.e., amylase, protease, and cellulase). It is a preliminary screening study that uses a qualitative method of producing extracellular hydrolases by indigenous microorganisms isolated from a new underutilized site in Algeria.

MATERIALS AND METHODS

Sampling

The effluents of a well-known thermal power plant near the sea, in the Terga region of Ain Temouchent (GPS Coordinates: 35°27'43.2"N 1°13'40.5"W), were the subject of this study. Seawater is the main source for the operation of the thermal power plant, which consequently generates effluents. The samples were collected at 60 m before the discharge area into the sea (Figure 1), in sterile flasks, and then transferred directly to the laboratory and placed in a cold room (4 °C) for further analysis. The pH of the effluents at the time of collection was recorded to be 7.5-7.8, the temperature 25 °C, and the concentration of rejected chlorine 0.25 ppm.

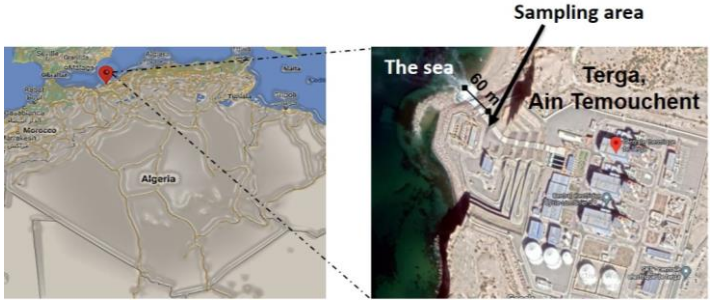

Figure 1 Sampling area of the Terga thermal power plant

Enrichment and isolation of microorganisms

One millilitre of the sample was transferred aseptically to 9 ml of nutrient broth (NB). After incubation at 30 °C for 1-2 days, decimal dilutions series were performed according to the method described by **Nandhini and Josephine (2013)**. For isolation, volumes of the bacterial cultures were diluted with a 0.85% (w/v) sodium chloride pre-sterilized. Decimal dilutions of 10^{-1} to 10^{-7} were made and 1 ml of the dilutions (10^{-5}, 10^{-6}, and 10^{-7}) was plated onto NB agar plates containing a nutrient agar composed of: Peptone (10g), yeast extract (5g), NaCl (5g), Agar (15g), and distilled water (1litre). The dishes were incubated at 30 °C under aerobic conditions for 2 days. After incubation, colonies of different morphologies were isolated and purified. The pure isolates were stored at (-20 °C) on a NB medium supplemented with glycerol 20% (v/v) for further studies.

Study of enzymatic activities of isolates

This paper is interested in the study of four hydrolase classes that have a wide range of biotechnology applications:

Protease activity

In order to select the proteolytic microorganisms, a milk agar medium composed of: Yeast extract (3g), agar (15g), and distilled water (1 litre), was used. This mixture was adjusted to pH 7.8 and autoclaved at 121 °C for 15 min. Then, 100 ml of skimmed milk (manufactured by Soummam, Algeria) was added sterilely after cooling the mixture. Microorganisms that hydrolyze milk casein show lightening halos around colonies **(Ardakani *et al*., 2012)**.

Amylase activity

The amylolytic activity of pure isolates was demonstrated in a starch-based agar medium composed of: peptone (10g), NaCl (5g), yeast extract (5g), starch (1%, w/v), agar (15g), and distilled water (1 litre). pH 7.8. Microorganisms, which hydrolyze the starch, show clear halos around the colonies after the addition of a Lugol solution for 15 min followed by two rinses with distilled water **(Ardakani *et al*., 2012)**.

Lipase activity

This activity was performed in tween 80 agar medium composed of (g/l): peptone (10g), NaCl (5g), $CaCl_2$ (0.1g), agar (15g), tween 80 (1%, v/v), distilled water (1 litre). pH 7.8. After incubation for 3-4 days, microorganisms that hydrolyze tween 80 show opaque halos around the colonies **(Hasan *et al*., 2009)**.

Cellulase activity

This medium was composed of two media as described by **Koraichi *et al*. (2015)**. A minimum medium M9 and a carboxymethylcellulose (CMC) medium. The M9 medium consists of Na_2HPO_4 (6g), KH_2PO_4 (3g), NH_4Cl (1g), NaCl (0.5g), and distilled water (1 litre). The medium was autoclaved for 15 min at 121 °C. Then, 1 ml of a 0.1 molar solution of $CaCl_2$ and 1 ml of a 1 molar solution of $MgSO_4$ are added. The CMC medium was composed of CMC (10g), yeast extract (5 g), glycerol (50%, v/v) (2 ml), agar (20 g) and the M9 buffer (quantity per litre). This final medium was adjusted to pH 7.8, autoclaved for 15 min at 121 °C, and then poured into Petri dishes. After incubation for 2 days at 30 °C, the cellulase activity was demonstrated by adding a solution of lugol for 15 min followed by three rinses with a molar solution of NaCl. Microorganisms that have cellulase activity show yellowish rings around the colonies.

Characterization of bacteria

The isolated strains were identified based on phenotypic and biochemical characteristics such as sugars fermentation (i.e., glucose, lactose, saccharose, and mannitol), citrate utilization test, mobility test and others, using the Bergey's Manual of Determinative Bacteriology as a guide, and on molecular characteristics by the sequencing of *16S* rRNA gene after extraction and Polymerase Chain Reaction (PCR) amplification, using the boiling method. This later consisted of bringing a pure colony to the boil for 10 min, and centrifuged at 15,000 rpm for 5 min at 4 °C **(Dutka-Malen *et al*., 1995)**. The amplification of the *16S* rRNA gene was carried out in a TC3000 Thermocycler using the following PCR program: Predenaturation 95 °C for 15 min, denaturation 94 °C for 1 min, hybridization 60 °C for 1 min, and elongation 72 °C for 1.5 min, folowwed by final elongation 72 °C for 10 min. The reaction mixture was composed of 2.5 µl of the buffer solution, 2 µl DNTP, 0.5 µl universal forward primer (27F: 5'-AGAGTTTGATCCTGGCTCAG-3'), 0.5 µl universal reverse primer (1492R: 5'-TACGGGTACCTTGTTACGACTT-3') **(Sato *et al*., 2003)**, 0.25 µl Taq polymerase, 5 µl of the bacterial cells, and 14.25 µl of distilled water. The PCR products were visualized after migration in an electrophoresis gel composed of 1.2 g of agarose per 100 ml of Tris-Borate-EDTA (TBE) buffer containing ethidium bromide. After the sequencing, according to the Sanger method, the nucleotide sequences of *16S* rRNA gene were aligned with other sequences via BLAST (Basic Local Alignment Search Tool) using NCBI (National Center for Biotechnology Information) database. The construction of the phylogenetic tree was performed using MEGA 7: Molecular Evolutionary Genetics Analysis **(Kumar *et al*., 2016)**. The *16S* rDNA nucleotide sequences of strains LGMT10, LGMT12, and LGMT8 have been deposited in GenBank/ENA/EMBL databases under the accession numbers : **MT337422**, **MT337423**, and **MT344187**, respectively.

Growth curves

Two growth curves of bacterial culture of the strain LGMT10 were plotted as a function of time at different pH (pH 5.5, 6.5, 8, 10, and 12) and concentrations of NaCl (0, 4, 8, 12, and 16 %, w/v) to study respectively the alkali tolerance and halotolerance in NB medium composed of (g/l): casein peptone (10g), yeast extract (5g), NaCl (at the studied concentrations) and distilled water (1 litre). The bacterial cultures were taken aseptically every 12 h and the optical density (OD) was measured at a wavelength of 600 nm (**Shivanand and Jayaraman, 2009)**. Results are expressed as the mean of two replicates tests ± standard deviation. Principal component analysis (PCA) was used to highlight the strain LGMT10's optimal pH and NaCl growth parameters.

Effect of pH and concentration of NaCl on the enzymatic activities

In order to investigate the influence of pH and NaCl concentrations on the production of extracellular enzymes (protease, amylase, and cellulase) by the strain LGMT10, different enzymatic assays were carried out in agar plate assay, with different pH (pH 6.8, 8.5, 10, and 12), and different concentrations of NaCl (0, 4, 8, and 12%, w/v) at a constant temperature of 30 °C. After incubation for 48 h, the secretion of enzymes was manifested by the formation of halos around the colonies. Another experiment on enzymatic activities was conducted by combining the optimal pH and NaCl growth parameters.

Statistical analyses

Principal component analysis (PCA) was used to compare the effects of different NaCl concentrations and pH ranges for the LGMT10 strain growth, using the XLSTAT® software (trial version). The LGMT10 strain's growth at different NaCl concentrations and pH ranges was performed at two replicates tests. Means and standard deviations were calculated using GraphPad Prism 9 (Trial version).

RESULTS

Isolation, screening, and characterization of bacteria

The isolation of microorganisms from the effluents, after enrichment in NB medium, showed a bacterial diversity with interesting enzymatic potential as shown in Tab 1. Among twelve bacterial isolates, three isolates designated LGMT10, LGMT12, and LGMT8 were screened based on their interesting enzymatic potential (Tab 1). The strain LGMT10 was selected for its enzymatic potential amylase, cellulase, and protease, by showing broad halos around the

colonies (Figure 2). It is short rod, Gram-positive, catalase-positive, oxidase-negative, which forms pale orange-yellow colonies on the surface of a nutrient agar medium after 24 h of incubation at 30 °C (Figure 3). It grows against wide ranges of NaCl concentrations between 0-12% (w/v) and pH 5.5-12 at 30 °C. Based on phenotypic and biochemical analyzes, and according to Bergey's Manual of Determinative Bacteriology; the strain was tentatively classified as *Bacillus* sp. (Tab 2). Phylogenetic analysis based on *16S* rRNA gene sequencing showed 99.23% homology with the type of strain *Bacillus aquimaris strain* DSM 16205^T (GenBank Accesion no.: AF483625) (Figure 4). For the strains LGMT12 and LGMT8, the percentage of similarity of the *16S* rRNA gene with their corresponding type of strains is 99.93% and 100%, respectively, for *Pseudomonas aeruginosa* strain DSM 50071^T (GenBank Accesion no.: HE978271) and *Bacillus wiedmannii* strain DSM 102050^T (GenBank Accesion no.: KU198626).) (Figure 4).

Table 1 Some phenotypic and enzymatic characteristics of isolates

Bacterial code	Microscopic observation	Gram's staining	Catalase test	Protease activity	Amylase activity	Lipase activity	Cellulase activity
LGMT1	Cocci	-	-	-	-	-	+
LGMT2	Rods	+	+	-	+	-	+
LGMT3	Rods	-	+	++	-	+++	+
LGMT4	Cocci	-	+	-	+	++	-
LGMT5	Cocci	-	+	-	-	+	+
LGMT6	Cocci	-	+	++	+	-	-
LGMT7	Cocci	-	-	++	+	-	-
LGMT8	**Rods**	**+**	**+**	**+++**	**-**	**++**	**-**
LGMT9	Rods	-	+	++	+	+	+
LGMT10	**Rods**	**+**	**+**	**+++**	**++**	**-**	**++**
LGMT11	Rods	-	+	++	+	+	-
LGMT12	**Rods**	-	+	++	-	++	+

Legend: (-) – no halos, (++) – medium diameter halos, (+++) – large diameter halos

Table 2 Biochemical characteristics of the strain LGMT10

Characteristics	Results
Gram's staining	+
Endospore staining	Central spores
NaCl growth range (%, w/v)	0-12
pH growth range	5.5-12
Sugars fermentation :	
1.Glucose	-
2.Lactose	+
3.Saccharose	+
4.Manitol	+
Citrate utilization test	-
Mobility	+
Catalase test	+
Oxidase test	-
Urease test	-
Indole production	-
Hydrolysis of tween 80	-
Hydrolysis of olive oil	-
Hydrolysis of skimmed milk	+
Hydrolysis of starch	+
Hydrolysis of cellulose	+

Legend: (+) – positive result, (-) – Negative result

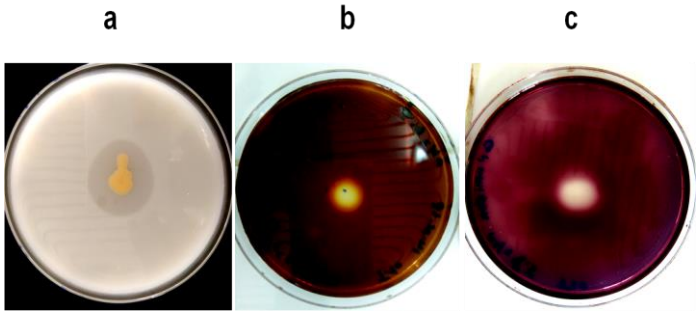

Figure 2 Enzymatic activities of the strain LGMT10 after 2 days of incubation at 30 °C, **(a):** Protease activity indicates hydrolysis of casein, **(b):** Cellulase activity indicates hydrolysis of cellulose**, (c):** Amylase activity indicates hydrolysis of starch

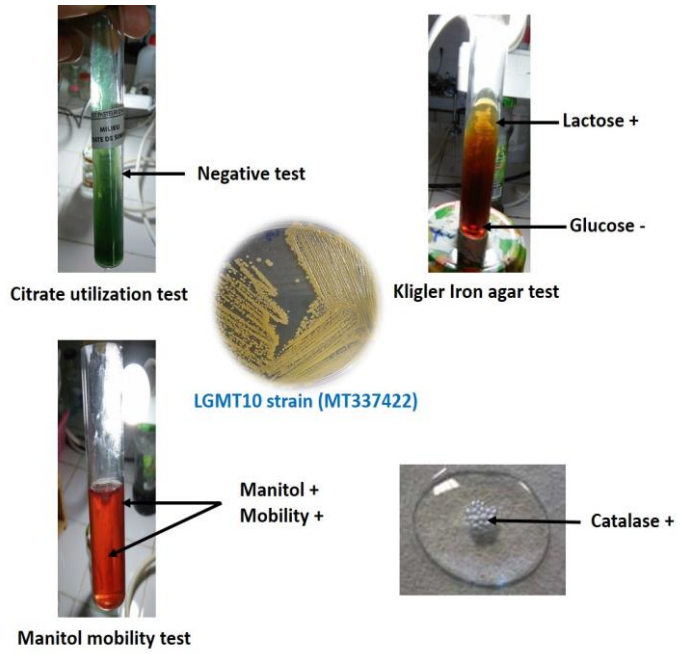

Figure 3 Some Phenotypic and biochemical characteristics of the strain LGMT10

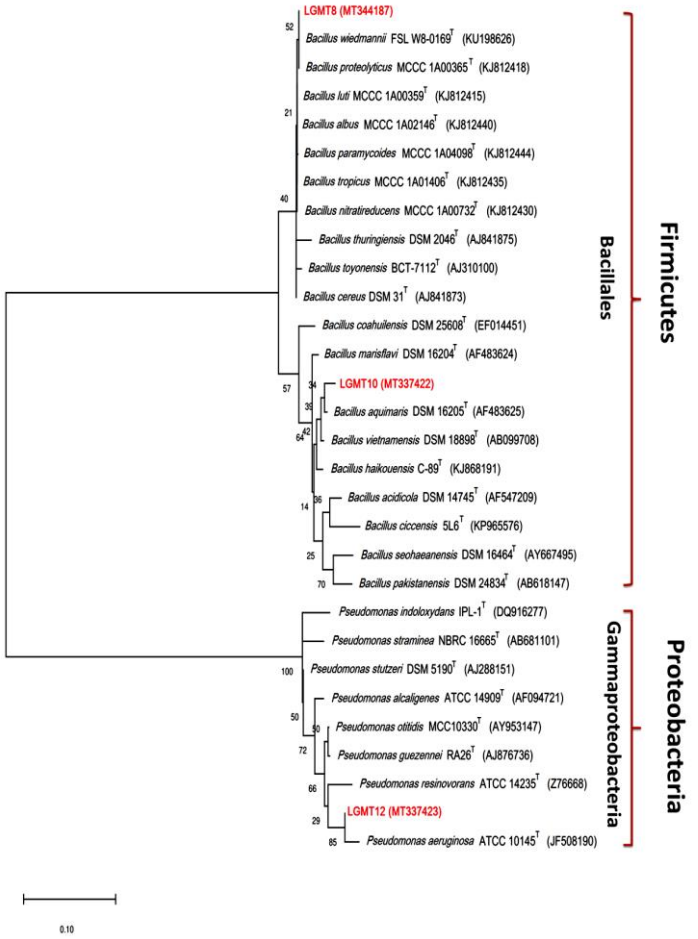

Figure 4 Phylogenetic tree by Maximum Likelihood method based on *16S* rRNA sequences of the strains LGMT8, LGMT12, LGMT10 and related species of BLASTn database. The trees were generated with 1000 repetitions and the percentages (%) at the node represent the probability values of the robustness of the similarity. Bar = 0.1 nucleotide substitution per site

Growth curve

Monitoring the growth of the strain LGMT10 over time has shown its capacity to grow on different concentrations of NaCl (0-12%, w/v), with optimal growth observed in NB medium at a concentration of 4% (w/v) NaCl, i.e., where the best rate growth was noted. This allowed the strain to be classified as moderately halophilic bacterium, indicating that it originated from the sea. (Shivanand and Jayaraman 2009) (Figure 5a). On the other hand, monitoring the growth against various pH ranges has shown that the LGMT10 strain can resist a pH range of 5.5-12, with an optimum growth pH observed at pH 8, i.e., where the best growth rate was recorded. As a result, the strain could be classified as an alkali-tolerant bacterium (Figure 5b). The effect of NaCl and pH on the LGMT10 strain's growth was demonstrated using principal component analysis. Accordingly, the best conditions for bacterial growth have been identified (i.e., 4% NaCl and pH 8) (Figure 6)

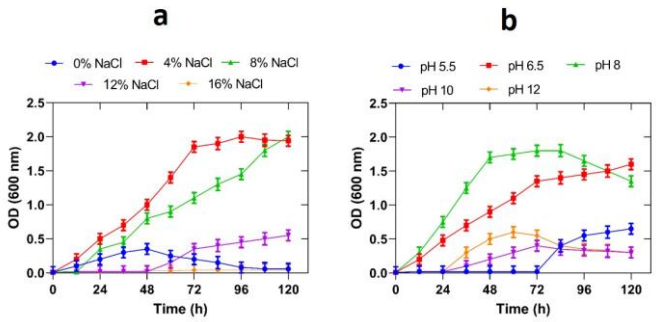

Figure 5 Growth monitoring (OD 600 nm) each 12 h at 0% NaCl (●), 4% NaCl (■), 8% NaCl (▲), 12% NaCl (▼), 16% NaCl (♦) (a); and in the pH 5.5 (●), pH 6.5 (■), pH 8 (▲), pH 10 (▼), pH 12 (♦) (b), of the strain LGMT10 on NB medium at 30 °C

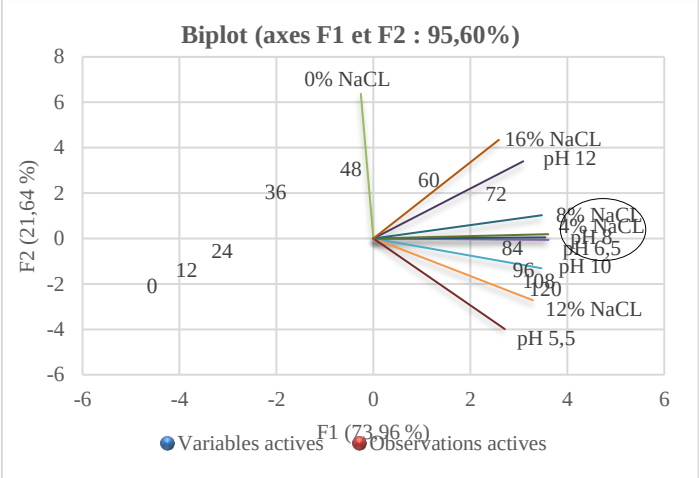

Figure 6: Principal component analysis of the turbidimetry measurements recorded for the LGMT10 strain at different salt concentrations and pH ranges, after 10 time of incubation. Projection of the variables on the factorial plane. The variables are the different salt concentrations (0% to 12%) and pH ranges (pH 5.5 to pH 12). Projection of samples corresponding to the 10 times of incubation

Effect of pH and NaCl concentration on the enzymatic activities

The production of the enzymes amylase, protease and cellulase was highlighted by the formation of halos around the colonies against wide ranges of pH 6.8-12 (with the exception of the amylase activity against pH 8.5-12), and NaCl concentrations between 0-12% (w/v) *(with the exception of the cellulase activity* against 4-12% (w/v) of NaCl) at 30 °C for 2-3 days of incubation (Tab 3).

Table 3 Effect of pH and NaCl concentration on the enzymatic activities of the strain LGMT10

Protease activity					Amylase activity					Cellulase activity				
pH 6.8	pH 8.5	pH 10	pH 12	pH 8.5 and NaCl 4%	pH 6.8	pH 8.5	pH 10	pH 12	pH 8.5 and NaCl 4%	pH 6.8	pH 8.5	pH 10	pH 12	pH 8.5 and NaCl 4%
++	++	++	+		-	++	++	+		++	++	++	+	
NaCl 0%	NaCl 4%	NaCl 8%	NaCl 12%		NaCl 0%	NaCl 4%	NaCl 8%	NaCl 12%		NaCl 0%	NaCl 4%	NaCl 8%	NaCl 12%	
+	++	+	+	+++	+	++	+	+	+++	-	++	+	+	+++

Legend : (-) – no halos, (+) – small diameter halos, (++) – medium diameter halos, (+++) – large diameter halos

DISCUSSION

In developing countries, microbial enzyme research is still in its infancy. However, the exploitation of certain discharge sites for the isolation of indigenous microorganisms should be carried out to better assess their microbial diversity and decipher their capacity for producing various enzymes classes. In this context, the present study highlighted the isolation and characterization of three bacteria designated LGMT10, LGMT12, and LGMT8 belonging respectively to the genus *Bacillus aquimaris*, *Pseudomonas aeruginosa*, and *Bacillus* sp. isolated from the effluents of the thermal power plant of Terga, Algeria. Since the first discovery of the novel strain *Bacillus aquimaris sp. nov*. by **Yoon *et al*. (2003)**, there are few reports regarding its enzymatic potential. However, the present study

highlighted the promising potential of new alkali-halotolerant *Bacillus aquimaris* strain LGMT10 producing three extracellular hydrolases (i.e., protease, amylase, and cellulase) able to resist different ranges of pH and NaCl concentrations. In the same context, the majority of studies in the literature have shown the halotolerant nature of *Bacillus aquimaris* and its ability to grow in NaCl concentration between 0-12% (w/v) (**Shivanand and Jayaraman, 2009)** in agreement with this study. Furthermore, strain LGMT10 has shown excellent properties to resist pH and NaCl for growth and production of the three studied enzymes compared to its counterparts among *Bacillus* spp and other bacterial genera.

Several bacterial genera that are capable of producing a variety of extracellular enzymes appear to be overly well described in the literature. However, a variety of bacteria has been reported for cellulase production. **Trivedi *et al*. (2011)** described the production of an alkalihalotolerant cellulase by *Bacillus flexus* strain NT. These authors showed that the enzyme, which has a molecular mass of 97 kDa, was stable in the pH range of 9-12 and at a range of NaCl concentration up to 15% (w/v). On the other hand, bacteria such as *Klebsiella* sp. produce cellulase active at 10 °C and pH 4.5 **(Bhat *et al*., 2013)**. Cellulase produced by *Marinobacter* sp. strain MSI032 was alkalotolerant, active at pH 9 **(Shanmughapriya *et al*., 2010)**. In contrast to alkaline cellulases, only a few salt-tolerant or halophilic cellulases have been reported **(Hirasawa *et al*., 2006; Voget *et al*., 2006)**. **Johnson *et al*. (1986)** have described a cellulase from halophilic actinomycetes, *Actinopolyspora halophila*, exhibiting optimal 15% (w/v) cellulase activity of NaCl. In addition, **Simankova *et al*. (1993)** characterized anaerobic eubacteria, *Halocella cellulolytica*, capable of producing cellulase enzyme at 20% (w/v) NaCl.

There are numerous available studies in the literature on alkaline protease production by *Bacillus* species. However, *Bacillus aquimaris* strain VITP4 has been reported to be active in the pH range of 7-10, with an optimum at pH 8 (**Shivanand and Jayaraman 2009, 2011)**. Currently, there are at least 29 *Bacillus* species and 17 fungal producers that have been reported to produce alkaline proteases **(Veloorvalappil *et al*., 2013)**. All the studies published on halotolerant microorganisms have shown that the enzymes from microorganisms which can grow over a concentration range of NaCl between 0-15% (w/v) are of great interest for their industrial use, because of their inherent ability to be active and stable both in the presence as well as in the absence of salt **(Chittoor *et al*., 2016)**. Moreover, some extracellular halophile proteases have maximum activity at near-neutral pH that has been reported **(Vidyasagar *et al*., 2006; Norberg and Hofsten, 1969)**. The *Bacillus aquimaris* strain VITP4 described by **Shivanand and Jayaraman (2011)** retained significant activity up to a concentration of 2M NaCl, in agreement with this study, although it exhibited the highest activity in the absence of NaCl. Even in the presence of 4 M NaCl, the protease retained about 40% of its activity, indicating the halotolerant behavior of the enzyme. It has been reported that the addition of NaCl up to a concentration of 5% (w/v) had no effect on the proteolytic function of *Bacillus cereus*. However, enzymatic activity decreased progressively upon further salt addition, and a 60% reduction in activity was reported when the NaCl concentration was increased to 10% **(Joshi *et al*., 2007)**. There are reports of salt-tolerant protease produced by mesophilic or thermophilic *Bacillus* spp **(Joo and Chang, 2005; Bhushan *et al*., 1999).**

In addition, the study of **Anupama and Jayaraman (2011)** on the production of extracellular amylase by the halotolerant strain *Bacillus aquimaris strain* VITP4 from the saltern of Kumta coast showed optimal activity at a pH range of 7.5 - 9.5 at 40 °C. Likewise, the partially purified a-amylase of *Bacillus aquimaris strain* MKSC 6.2 displayed optimum activity at pH 6.5 and 50 °C **(Puspasari *et al*., 2011).** It has been reported that amylases produced by certain halophilic microorganisms have optimal activity at high salinities and could, therefore, be used in many severe industrial processes where the concentrated saline solutions used would otherwise inhibit many enzymatic conversions **(Amoozegar *et al*., 2003; Prakash *et al*., 2009)**. It has also been reported that most of the halobacterial enzymes are considerably thermotolerant and remain stable at room temperature for long periods of time **(Mohapatra *et al*., 1998)**. The halophilic amylases have been characterized from halophilic bacteria such as *Chromohalobacter* sp. **(Prakash *et al*., 2009)**, *Halobacillus* sp. **(Amoozegar *et al*., 2003)**, *Haloarcula hispanica* **(Hutcheon *et al*., 2005)**, *Halomonas meridiana* **(Coronado *et al*., 2000)**, and *Bacillus dipsosauri* **(Deutch, 2002)**. Based on the literature, the main strains that are described in the production of extracellular enzymes among *Bacillus* spp are presented in Tab 4.

Table 4 Comparative study among some *Bacillus* strains on the production of extracellular hydrolases

Bacterial strains	Origin	Producing enzymes	Production proprieties	Study
Bacillus aquimaris strain LGMT10	Thermal power plant effluents, Algeria	Protease Amylase Cellulase	pH 6.8-12; NaCl: 0-12% pH 8.5-12; NaCl: 0-12% pH 6.8-12; NaCl: 4-12%	Our study
Bacillus aquimaris strain VITP4	Kumta coast	Protease Amylase	pH 7-10; NaCl: 0-2 M pH 7.5 - 9.5	(Shivanand and Jayaraman, 2009, 2011) (Shivanand and Jayaraman, 2011)
Bacillus subtilis (ATCC 6633)	nd	Amylase	pH 6-11	(Maity *et al*., 2015)
Bacillus cereus RSA1	Soil samples	protease	pH 5-10	(Sharma *et al*., 2020)
Bacillus licheniformis HI-08	The gut of building infesting termite *Heterotermes indicola*	Cellulase	pH 3-10	(Afzal *et al*., 2019)
Bacillus flexus NT	Green seaweed *Ulva lactuca*	Cellulase	pH 8-12; NaCl : 0-21%	(Trivedi *et al*., 2011)
Bacillus subtilis B22	Homemade kimchi	Protease	pH 7-10	(Elumalai *et al*., 2020)

Legend: nd – not determined

CONCLUSION

The microbial diversity of the Terga thermal power plant's effluents was investigated in this study, which revealed an intriguing enzymatic potential as well as the identification of a novel alkali-halotolerant bacterium, *Bacillus aquimaris* strain LGMT10 capable to produce three extracellular hydrolases (i.e., amylase, cellulase, and protease) in the pH range of 6.8-12, and at NaCl concentrations between 0-12% (w/v) at 30 °C. These findings bode well for better understanding the capabilities of novel microorganisms from this new site, in Algeria. In addition, the measurement of the enzymatic activities of purified enzymes and their biochemical characterization will be performed using more accurate techniques, such as sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and zymograhy, in order to provide the data necessary for their catalyzing capacities, for a possible affiliation of each enzyme towards the corresponding industry.

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