Diversity and enzymatic potential of thermophilic bacteria associated with terrestrial hot springs in Algeria

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Received: 25 May 2020 / Accepted: 3 September 2020 / Published online: 21 September 2020
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Abstract

This study aims to determine the diversity of culturable thermophilic bacteria isolated from eight terrestrial hot springs in Northeastern of Algeria using the conventional methods, SDS-PAGE fingerprinting of whole-cell proteins and 16S rRNA gene sequencing. In addition, their hydrolytic enzyme activities were also investigated. A total of 293 strains were isolated from the hot springs' water and sediment using different culture media. Overall, five distinct bacterial groups were characterized by whole-cell protein pattern analysis. Based on the 16S rRNA gene sequencing of 100 selected strains, the isolates were assigned to the following three major phyla: *Firmicutes* (93%), *Deinococcus-Thermus* (5%), and *Actinobacteria* (2%), which included 27 distinct species belonging to 12 different phylotypes, *Aeribacillus*, *Aneurinibacillus*, *Anoxybacillus*, *Bacillus*, *Brevibacillus*, *Geobacillus*, *Laceyella*, *Meiothermus*, *Saccharomonospora*, *Thermoactinomyces*, *Thermobifida*, and *Thermus*. The screening for nine extracellular enzymes showed that 65.87% of the isolates presented at least five types of enzyme activities, and 6.48% of strains combined all tested enzymes (amylase, cellulase, pectinase, esculinase, protease, gelatinase, lipase, lecithinase, and nuclease). It was found that *Bacillus*, *Anoxybacillus*, *Aeribacillus*, and *Aneurinibacillus* were the genera showing the highest activities. Likewise, the study showed an abundant and diverse thermophilic community with novel taxa presenting a promising source of thermozymes with important biotechnological applications. This study showed that a combined identification method using SDS-PAGE profiles of whole-cell proteins and subsequent 16S rRNA gene sequence analysis could successfully differentiate thermophilic bacteria from Algerian hot springs.

Keywords Thermal spring · Algeria · Thermophile · Culture-dependent method · SDS-PAGE fingerprinting · 16S rRNA gene · Biodiversity · Thermozyyme

Responsible Editor: Vania M.M. Melo

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s42770-020-00376-0>) contains supplementary material, which is available to authorized users.

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Introduction

Thermophiles are a group of heat-loving microbes thriving at high temperature. They are grouped according to their optimum growth temperature as moderate thermophiles (50–60 °C), extreme thermophiles (60–80 °C), and hyperthermophiles (> 80 °C) [1]. They are inhabitants of various environments, such as deep-sea hydrothermal vents, terrestrial hot springs, and other extreme sites, including volcanic region, tectonically active faults, and processing waste residues, like compost piles and deep organic landfills [2]. Thermophiles have attracted considerable attention because they present specific features with biotechnological and industrial interest, such as the production of different biomolecules (exopolysaccharide, antimicrobial, biosurfactant) [3–5] and thermostable enzymes (amylases, cellulases, chitinases, pectinases, xylanases, proteases, lipase, and DNA polymerases), for biotechnological applications in medical, industrial, and agriculture processes [1]. These organisms may also serve in an increasing number of potential bioremediation applications, mainly removing of heavy metals from waste [6], biodegradation of petroleum hydrocarbons, [7], and in renewable energy [8].

Geothermal springs are considered unique hot spots for large communities of thermophilic microorganisms, mainly belonging to Bacteria and Archaea domains [9]. Thermophile biodiversity can provide an overview of the potential, and such studies are required not only to understand the microbial community's organization and composition but also led to further discovery of novel taxa and to resolve the role of microorganisms in the community [10]. Microbial studies of hot springs have been extensively reported in many countries, particularly the Yellowstone National Park (USA) [11], New Zealand [12], India [13], Russia [14], Japan [15], China [16], Iceland [17], and Turkey [18].

Algeria is one of the world's richest countries in terms of geothermal sites, with over 240 hot springs across the country (temperature ranging between 22 and 98 °C) [19]. Moreover, it hosts Hammam Debagh, also named Hammam Challala that presents temperatures up to 98 °C, which offers the hottest terrestrial spring in the country [20] and the second in the world after those of Iceland [21]. Algerian hot springs formerly were only used as spas for balneology [22], regarding their therapeutic effects [23]. At these spots, researchers were mainly interested in geothermal potential, physicochemical composition, and bacteriological and fungal contamination investigations to ensure the safety of users [24–26]. Despite intensive studies on terrestrial thermal springs around the world, very little is known about the microbial biodiversity of hydrothermal springs in Algeria. Though recently some studies on thermophilic bacteria have emerged, they stay limited only to some hot springs and aspects [27, 28].

The identification of thermophilic species is usually performed by 16S rDNA sequence, DNA-DNA hybridization, PCR-RFLP profiles, and BOX-PCR [29–32]. Nevertheless, during isolation, multiple colonies are simultaneously detected and very often with very close morphologies within and between the strains, which makes the selection of different bacteria difficult; consequently, an efficient technique for species recognition is required before further identification by molecular approaches [2]. The analysis of SDS-PAGE whole-cell protein has demonstrated to be highly effective for the differentiation of thermophilic bacteria [33, 34]. Our study is a first attempt to classify and cluster thermophilic strains isolated from Algerian terrestrial hot spring using SDS-PAGE profiling.

Given these facts, the present study seeks to investigate the culturable thermophilic bacterial diversity using a culture-dependent approach based on SDS-PAGE whole-cell protein patterns and 16S rRNA gene sequencing from eight hot springs distributed over four cities in Northeastern of Algeria. The work was extended by investigating some industrially important hydrolytic enzymes (amylase, cellulase, pectinase, esculinase, protease, gelatinase, lipase, lecithinase, and DNase) from isolated thermophilic strains in order to evaluate their potential application in biotechnological processes.

Materials and methods

Site description

The sampling study was carried out in eight terrestrial geothermal springs distributed over four cities (Batna, Biskra, Guelma, and Khenchela) in Northeastern of Algeria. Among the hot springs studied, the following six sites have never been explored for microbial biodiversity: Belhachani Hot Spring (H24A), Guerfa Hot Spring (H24G), Saïda Hot Spring (H05S), Guerdjima Hot Spring (H05G), Sidi El Hadj Hot Spring (H07S), and El Knif Hot Spring (H40K). The hot springs included from moderate thermophilic to hyperthermophilic (40.6–96 °C) environments with a range of pH from 6.27 to 8.03 (Fig. 1).

Sample collection and isolation of thermophilic bacteria

In order to avoid possible bacterial contamination, water and sediment samples were collected aseptically in sterile flasks, at a depth of 30–50 cm from the surface. For water, physicochemical parameters as temperature and pH were recorded in situ by a portable multiparameter meter (Hanna HI 8314). The collected samples were immediately transported on ice to the laboratory for further analysis.

Fig. 1 Geographical location of the studied hot springs

Two techniques, liquid enrichment and dilution plating, were used to isolate the thermophilic bacteria on the following six different culture media: Thermus medium [35], Castenholz medium D, Thermus 162 medium [36], ATCC medium 697 [37], Nutrient medium (Liofilchem) [38], and Tryptic soy medium (BD, Difco) [39]. Solid media were obtained by adding 3% (w/v) of agar to liquid media. The plates were then incubated for 3 to 10 days at 55 °C in plastic bags. The plates were constantly observed for the appearance of bacterial colonies in the different employed media. Single colonies with distinct morphology were selected from each of the plates and purified by successive streaking on respective medium plates. Pure cultures were maintained at 4 °C as slant and in 20% glycerol stock at – 80 °C for further use.

Phenotypic features of isolates

All phenotypic tests were made in duplicate and repeated if inconsistent results were first observed. Growth tests, unless otherwise specified, were performed at 55 °C on Thermus media.

The colony morphology of the thermophilic isolates was checked by direct and stereomicroscopic observations of

single colonies. Gram staining was carried out by using the standard Gram's reaction [40] and was confirmed by a non-staining procedure using the KOH lysis test method [41]. Oxidase and catalase activities were performed as described by Maugeri et al. [42].

Temperature and pH range for growth were determined following incubation of the strains on Thermus agar at different temperatures (30–75 °C) and pH (5.0–9.0) with 5 °C and one unit intervals, respectively. Halotolerance was assayed by plating each culture onto Thermus medium supplemented with 0, 2, 2.5, 5, 7.5, 10, 15, and 20% (w/v) NaCl. Growth was determined by visual observation after three days of incubation [43].

Biochemical characteristics were screened by API 20E® strips system (bioMérieux, France) according to the manufacturer's instructions with some modifications. The number of bacteria was adjusted to one McFarland standard, and 200 µL of suspension was transferred into each well. Strips in incubation boxes were put into a plastic container filled into the bottom with sterilized water to minimize evaporation and then incubated at 55 °C. The API strips were observed at 4, 8, 16, and 24 h. At each observation point, water was replenished when necessary.

For further clarification of biochemical characters, a similarity dendrogram of API20E profiles was constructed using the MEGA6 software based on the UPGMA method and Jaccard coefficient.

SDS-PAGE of whole-cell proteins

Preparation of protein extracts

Thermophilic strains of 0.1 O.D. at 600 nm were incubated overnight at 55 °C in 50 mL of LB broth and centrifuged at 16,000 $\times g$ for 10 min at 4 °C. Each sample pellet was washed twice with distilled sterile water and suspended in 100 mM phosphate buffer (pH = 7.6). The suspensions were then sonicated with an ultrasonic homogenizer (LABSONIC M, Sartorius) at an output of 40% amplitude and pulse mode of 30 s with 10 s intermittence, for 5–10 min according to bacterial strains. Sample tubes were placed in an ice bath during sonication to prevent heat buildup. All lysates were clarified by centrifugation at 16,000 $\times g$ for 15 min at 4 °C. The supernatant obtained was stored at –20 °C [44]. Protein concentration was measured according to Bradford microplate protein quantification assay. The absorbance was measured at 595 nm with a microplate reader (SpectraMax® M2), and the protein concentration was determined from a standard curve using bovine serum albumin (Thermo-Fisher) as standard protein. Protein samples were then mixed with Laemmli sample buffer (Bio-Rad). For protein denaturation, samples were heated for 5 min at 95 °C and centrifuged at 12,000g for 5 min to prevent streaking during electrophoresis and kept on ice before loading into the wells.

SDS-PAGE electrophoresis

SDS-PAGE of whole-cell proteins was carried out as described by [45], on vertical slab gels (20 $\times$ 20 cm $\times$ 1 mm) in a Protean® II xi Cell gel electrophoresis apparatus (Bio-Rad Laboratories), using 12% (w/v) separating and 5% (w/v) stacking gels. A volume of a sample containing 40–50 ng of protein was layered on top of the gel. Electrophoresis was performed at a constant current of 120 V through the stacking gel and 200 V through the resolving gel for 4 h. In each gel, the broad range protein molecular mass marker (11 and 190 kDa, Prestained Protein Ladder, Biolabs) was used to determine the protein molecular mass. After electrophoresis, the gel was stained for 1 h with 200 mL of 0.1% (w/v) Coomassie Brilliant Blue R-250 (Sigma) with gentle continuous shaking and then destained twice with 100 mL of 10% (v/v) acetic acid and 50% (v/v) methanol aqueous solution first for 1 h followed by a second destained solution containing 5% (v/v) methanol, 7% (v/v) acetic acid, and 88% water overnight.

Computing numerical data

The destained gels were scanned and stored as JPEG files. The whole-cell protein patterns were compared with each other to group the thermophilic isolates. The densitometric analysis, normalization, and interpolation of the protein profiles, grouping of the bacterial strains by the *Pearson* product-moment correlation coefficient (*r*), and cluster analysis on the matrix of correlation values by the unweighted pair group method using arithmetic averages (UPGMA). Linkage was performed using the BioNumerics version 7.6.3 software package (Applied Maths NV).

Molecular identification by 16S rRNA gene sequencing and phylogenetic analysis

Genomic DNA extraction

Bacterial genomic DNA of promising isolates was extracted from 5 mL cultures grown overnight at 55 °C in *Thermus* broth and pelleted by centrifugation at 16,000 $\times g$ for 2 min using Wizard® Genomic DNA Purification Kit by Promega (USA). DNA was quantified using the Nanodrop 2000 spectrophotometer (Thermo Scientific), by measuring OD₂₆₀ and OD₂₈₀.

PCR amplification

The extracted DNA samples were used as a template for amplification of the 16S rRNA sequences gene using the universal primers UNI16S-L (5'-ATTCTAGAGTTTGATCATGGCTCA-3') and UNI16S-R (5'-ATGGTACCGTGTA CGGGCGGTGTGTA-3') [43]. PCR reactions were carried out in 50 μ L volume containing 10 μ L GoTaq Flexi Buffer (5 $\times$), 3 μ L MgCl₂ (25 mM), 1 μ L dNTPs (10 mM), 1 μ L reverse primer (10 mM), 1 μ L forward primer (10 mM), 0.25 μ L GoTaq G2 Flexi DNA polymerase (5 U/ μ L), and 1 μ L of the DNA template, and the volume was made up to 50 μ L with Milli-Q water. The PCR amplification conditions consisted of initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 2 min, and a final polymerization step of 72 °C for 5 min. PCR products were visualized in 1% (w/v) agarose gel electrophoresis at 120 V for 15 min.

Sequencing and phylogenetic analysis

The PCR products were cloned into the pGEM-T Easy vector system (Promega, USA), and then, the 16S rRNA gene sequence was determined with an Applied Biosystems model 373A DNA Sequencer, using the ABI PRISM® BigDye™ Terminator cycle-sequencing kit (Macrogen).

The sequences were compared with those in the databases of the National Center for Biotechnology Information (NCBI) website using the BLAST method and the EzTaxon server (<http://www.eztaxon.org/>) [46]. Phylogenetic analyses were carried out using MEGA software version 6 [47]. Phylogenetic trees were constructed using the neighbor-joining method [48]. Tree topologies were evaluated by bootstrap sampling expressed as a percentage of 1000 replicates. The nucleotide sequences determined in this study have been deposited in GenBank (NCBI database) under accession numbers from MN885696 to MN885795.

Extracellular hydrolytic enzyme screening

For preliminary qualitative screening of ten hydrolytic enzymes, the thermophilic isolates were grown on *Thermus* agar for 24–48 h. The relevant assays were performed on agar plates, including enzyme-specific substrates using drop plate technique after incubation in plastic bags at 55 °C for 1–5 days. All experiments were done in duplicate and conducted according to the standard protocols described subsequently.

Amylase activity

Amylase assay was determined on 1% (*w/v*) starch agar medium. The starch hydrolysis was detected by flooding plates with Lugol's iodine solution. The clear zone around the colony indicates a positive result [49].

Cellulase activity

For cellulase activity screening, 0.5% (*w/v*) carboxymethyl-cellulose (CMC)-based medium was used. The plates were flooded with Lugol's iodine solution. The clear halo around the colonies indicates the CMC hydrolysis [50].

Pectinase activity

The pectinolytic activity was detected on MP5 medium containing 0.5% pectin [51]. After incubation, the plates were flooded with iodine-potassium iodide solution (1.0 g iodine, 5.0 g potassium iodide, and 330 mL H₂O). Clear zones around the colonies showed pectinolytic activity [52].

Esculinase activity

Esculinase hydrolysis enzyme was screened according to [53] with some modifications using specific medium containing 10 g peptone, 1 g esculin/aesculin, 20 g ferric ammonium citrate, 30 g agar, and 1 L distilled H₂O. Visualizing a zone of black precipitate around the colonies indicates a positive result.

Protease activity

The proteolytic or caseinolytic activity was tested in 10% skim milk plate medium. Transparent clear zones around the growth were considered as an indication of casein activity degradation [54].

Gelatinase activity

Gelatin hydrolysis assay was conducted according to the method of Frazier [55] using 0.4% gelatin-based agar. After incubation, degradation of gelatin was seen as a clear zone around colonies, in the somewhat opaque agar. When the plate was flooded with a saturated aqueous solution of ammonium sulfate, a precipitate was formed that made the agar more opaque and enhanced the clear zones around the colonies [56].

Lipolytic and esterase activities

Lipolytic activity was detected according to the diffusion agar method on Tween-based medium containing 1% of tween (20 or 80), 10 g Difco Bacto-peptone, 5 g NaCl, 0.1 g CaCl₂·1H₂O, 30 g agar-agar, and 1 L distilled H₂O (pH 7.4). A well visible halo or turbid around the colonies indicated lipolysis [57].

Lecithinase activity

For determination of lecithinase enzyme, nutrient agar, including (5%, *v/v*) egg-yolk emulsion, was used. The formation of a white precipitate as opalescence around or beneath the colony revealed lecithinase formation [58].

Nuclease activity

DNase activity of the strains was revealed using the DNase test agar medium with methyl green. After incubation, DNase-producing bacteria exhibit a clear zone around growth against a green background [59].

Results

Isolation and distribution of thermophilic bacteria

A total of 293 thermophilic strains were isolated from the different hot springs. According to the distribution, 17.57% (52/293) of all the isolated strains were retrieved from Debagh Hot Spring (H24D), followed by Belhachani Hot Spring (H24B), Essalihine Hot Spring (H40S), Guerdjima Hot Spring (H05G), Sidi El Hadj Hot Spring (H07S), and Saïda Hot Spring (H05S) with 16.04, 14.68, 14.33, 12.29, and

11.95%, respectively. El Knif Hot Spring (H40K) and Guerfa Hot Spring (H05G) presented the lowest number of isolates, 6.83% (20/293) and 6.14% (18/293), respectively.

Isolation and purification procedures were carried out with both water and sediment samples. Sediment samples from all hot springs provided 51.32% (166/293) isolates, while water samples allowed the isolation of 48.68% (127/293) isolates. A total of six media were employed to retrieve the culturable bacteria. A total of 57.68% of total isolates have been cultured on specific media (Thermus medium, ATCC medium 697, Thermus 162 medium). In comparison, 42.32% were isolated from standard media distributed into 6.14% from a minimum medium (Castenholz medium D) and 38.18% from standard media (Nutrient agar and Tryptic soy medium).

Morphological and physiological characterizations of isolates

Morphologically, the isolates showed some variation in color; they were gray, black, creamy and creamy-gray, buff, white and off-white, yellow to bright yellow, pink, orange, and red (Fig. S1). The pigments produced by the isolated species were endocellular and nondiffusible. The colonies show a very wide range of colonial morphologies. Some isolates are circular or irregular in shape with a margin of entire to undulate or crenate uneven edges. They have wrinkled, rough, granular, crusty, or smooth textures, and some may appear finely wrinkled and adherent to the agar surface. They were either opaque or translucent. Another fast-growing mycelial colony was noted.

Based on Gram staining, 78% of the isolates were found mostly to be Gram-positive. A total of 182 isolates (62.12%) were catalase-positive, 71 isolates (24.23%) were oxidase-positive, while 16.72% (49 isolates) were oxidase- and catalase-positive.

All isolates screened for temperature tolerance were able to grow between 30 and 80 °C with variation among strains. It was noted that all isolates (293 isolates) grow at 55 °C within 24 h to 1 week. Of which, 40.20% were found to tolerate 65 °C, 10.87% could tolerate 75 °C, while only 5.11% of isolates could grow at 80 °C. Moreover, 52.82% of isolates could grow at 37 °C.

Salt was not required for growth since all the strains were able to grow in the unsupplemented medium. The isolates tolerated salt concentrations in medium supplemented with 1 to 15% NaCl (w/v). While none of the strains were able to grow in medium supplemented with 20% NaCl (w/v). The number of strains growing decreased at increasing salt concentration. In addition, the pH values tested ranged from 5.0 to 9.0. Almost all strains grew in pH ranging between 6.5 and 8.5.

Fig. 2 Dendrogram of similarity based on SDS-PAGE of whole-cell protein pattern analysis from thermophilic bacterial isolates. The right side of the figure presents the strain number and the clustering classification

SDS-PAGE analysis

The electrophoretic patterns of whole-cellular proteins from all isolates, resulting from SDS-PAGE, showed that five groups could be delineated at a similarity level of 45% (Fig. 2). The cluster I includes 42 strains grouped into the following four sub-clusters: (A) six strains, (B) 15 strains, (C) 12 strains, and (D) nine strains. The cluster II also was grouped into four sub-clusters assembling 171 strains distributed as 134 strains from sub-cluster (C), followed by 23 strains in sub-cluster (B), and both sub-clusters (A) and (D) including seven strains. Finally, clusters III, IV, and V include only one strain for each one belonging to three different hot springs, distributed as 05S37, 07S35, and 24A17, respectively. A total of 100 representative strains from different clusters were selected for further molecular identification by 16S rRNA gene sequencing.

Characterization by 16S rRNA gene sequencing

Of 293 isolates, 100 bacterial isolates were selected for identification by complete 16S rDNA gene sequencing (~1400 bp). The phylogenetic analysis revealed that the strains were affiliated to 12 different genera (*Bacillus*, *Anoxybacillus*, *Aeribacillus*, *Aneurinibacillus*, *Brevibacillus*, *Thermoactinomyces*, *Meiothermus*, *Geobacillus*, *Thermus*, *Laceyella*, *Thermobifida*, and *Saccharomonospora*), grouping 27 distinct species with 96.09 to 100% of sequence similarity to closely related species (Table 1; Figs. 3, 4, and 5). The strains retrieved from hot springs were distributed over three phyla, *Firmicutes* (93%), *Deinococcus-Thermus* (5%), and *Actinobacteria* (2%). Blast analysis of the 16S rRNA gene sequence indicates that seven strains grouped as *Bacillus*, *Brevibacillus*, *Geobacillus*, *Aneurinibacillus*, and *Thermoactinomyces* may probably constitute new taxa on these genera (Table 1). They present between 96.09 and 98.79% nucleotide sequence similarity with 16S rRNA gene sequences of typed strains.

Biochemical characterization

The API 20E profiles of the isolates demonstrated biochemical diversity (Fig. S2). All strains were negative for indole and H₂S production. As one can see, the utilization of carbohydrates varies; negative assimilation results were obtained for rhamnose and saccharose overall strains. The carbohydrate assimilation test was positive for 20% of strains on

Fig. 2 continued.

Fig. 2 continued.

Table 1 Thermophilic bacteria isolated from Algerian hot springs and their best match results with 16S rRNA gene sequences of typed strains

Site	Genus	Isolates	Related bacterial strains (accession number)	Similarity (%)
Guerdjima Hot Spring (H05G) N = 15	<i>Aeribacillus</i> (n = 4)	05G38	<i>A. composti</i> N.8 (LT594972)	99.77
		05G14	<i>A. pallidus</i> KCTC3564 (CP017703)	99.60
		05G30	<i>A. pallidus</i> KCTC3564 (CP017703)	99.82
		05G40	<i>A. pallidus</i> KCTC3564 (CP017703)	99.36
	<i>Aneurinibacillus</i> (n = 4)	05G4	<i>A. thermoaerophilus</i> DSM 10154 (X94196)	99.82
		05G7	<i>A. thermoaerophilus</i> DSM 10154 (X94196)	99.86
		05G8	<i>A. thermoaerophilus</i> DSM 10154 (X94196)	99.72
		05G9	<i>A. thermoaerophilus</i> DSM 10154 (X94196)	99.88
	<i>Anoxybacillus</i> (n = 3)	05G21	<i>A. gonensis</i> G2 (CP012152)	99.22
		05G27	<i>A. gonensis</i> G2 (CP012152)	99.81
		05G37	<i>A. gonensis</i> G2 (CP012152)	100.0
	<i>Bacillus</i> (n = 3)	05G33	<i>B. sonorensis</i> NBRC101234 (AYTN01000016)	99.78
		05G32	<i>B. licheniformis</i> ATCC14580 (AE017333)	99.09
		05G35	<i>B. licheniformis</i> ATCC14580 (AE017333)	99.71
	<i>Thermoactinomyces</i> (n = 1)	05G17	<i>T. vulgaris</i> KCTC9076 (AF138739)	99.60
Saïda Hot Spring (H05S) N = 13	<i>Aeribacillus</i> (n = 3)	05S4	<i>A. pallidus</i> KCTC3564 (CP017703)	99.71
		05S8	<i>A. pallidus</i> KCTC3564 (CP017703)	100.0
		05S20	<i>A. pallidus</i> KCTC3564 (CP017703)	99.30
	<i>Anoxybacillus</i> (n = 4)	05S29	<i>A. gonensis</i> G2 (CP012152)	99.65
		05S32	<i>A. kaynarcensis</i> D1021 (EU926955)	99.14
		05S15	<i>A. pushchinoensis</i> K1 (jgi.1042845)	99.53
		05S24	<i>A. salavatliensis</i> A343 (EU326496)	99.70
	<i>Bacillus</i> (n = 1)	05S9	<i>B. hisashii</i> N-11 (AB618491)	99.40
	<i>Brevibacillus</i> (n = 1)	05S37	<i>B. aydinogluensis</i> PDF25 (HQ419073)	99.79
	<i>Meiothermus</i> (n = 1)	05S28	<i>M. ruber</i> DSM1279 (CP001743)	100.0
	<i>Thermoactinomyces</i> (n = 3)	05S3	<i>T. vulgaris</i> KCTC9076 (AF138739)	99.40
		05S19	<i>T. vulgaris</i> KCTC9076 (AF138739)	99.62
		05S21	<i>T. vulgaris</i> KCTC9076 (AF138739)	98.13
Belhachani Hot Spring (H24A) N = 15	<i>Aeribacillus</i> (n = 3)	24A34	<i>A. composti</i> N.8 (LT594972)	99.93
		24A13	<i>A. pallidus</i> KCTC3564 (CP017703)	99.50
		24A52	<i>A. pallidus</i> KCTC3564 (CP017703)	99.08
	<i>Anoxybacillus</i> (n = 4)	24A10	<i>A. flavithermus</i> DSM2641 (CP020815)	99.04
		24A 3	<i>A. gonensis</i> G2 (CP012152)	99.63
		24A46	<i>A. gonensis</i> G2 (CP012152)	99.81
		24A12	<i>A. thermarum</i> AF/04 (AM402982)	99.28
	<i>Bacillus</i> (n = 5)	24A37	<i>B. haynesii</i> NRRLB-41327 (MRBL01000076)	99.78
		24A11	<i>B. kokesii</i> MO-04 (JX848633)	99.21
		24A 6	<i>B. licheniformis</i> ATCC14580 (AE017333)	99.80
		24A27	<i>B. licheniformis</i> ATCC14580 (AE017333)	99.47
		24A31	<i>B. licheniformis</i> ATCC14580 (AE017333)	99.61
	<i>Laceyella</i> (n = 1)	24A21	<i>L. sacchari</i> KCTC9790 (AF138737)	99.25
	<i>Saccharomonospora</i> (n = 1)	24A43	<i>S. viridis</i> DSM43017 (CP001683)	99.36
	<i>Thermoactinomyces</i> (n = 1)	24A22	<i>T. vulgaris</i> KCTC9076 (AF138739)	99.17
El Knif Hot Spring (H40K) N = 8	<i>Aneurinibacillus</i> (n = 4)	40K10	<i>A. thermoaerophilus</i> DSM 10154 (X94196)	99.88
		40K14	<i>A. thermoaerophilus</i> DSM 10154 (X94196)	99.65
		40K15	<i>A. thermoaerophilus</i> DSM 10154 (X94196)	99.88
		40K18	<i>A. thermoaerophilus</i> DSM 10154 (X94196)	99.25
	<i>Anoxybacillus</i> (n = 2)	40K 8	<i>A. gonensis</i> G2 (CP012152)	99.03
		40K12	<i>A. gonensis</i> G2 (CP012152)	99.31

Table 1 (continued)

Site	Genus	Isolates	Related bacterial strains (accession number)	Similarity (%)
Essalihine Hot Spring (H40S) N = 15	<i>Bacillus</i> (n = 1)	40K 1	<i>B. licheniformis</i> ATCC14580 (AE017333)	99.11
	<i>Brevibacillus</i> (n = 1)	40K19	<i>B. aydinogluensis</i> PDF25 (HQ419073)	99.28
	<i>Aeribacillus</i> (n = 4)	40S28	<i>A. composti</i> N.8 (LT594972)	99.85
		40S35	<i>A. composti</i> N.8 (LT594972)	99.85
		40S30	<i>A. pallidus</i> KCTC3564 (CP017703)	99.56
	<i>Aneurinibacillus</i> (n = 6)	40S42	<i>A. pallidus</i> KCTC3564 (CP017703)	99.24
		40S14	<i>A. thermoaerophilus</i> DSM 10154 (X94196)	98.35
		40S16	<i>A. thermoaerophilus</i> DSM 10154 (X94196)	99.15
		40S23	<i>A. thermoaerophilus</i> DSM 10154 (X94196)	99.69
		40S43	<i>A. thermoaerophilus</i> DSM 10154 (X94196)	99.41
		40S44	<i>A. thermoaerophilus</i> DSM 10154 (X94196)	99.87
		40S45	<i>A. thermoaerophilus</i> DSM 10154 (X94196)	99.88
	<i>Bacillus</i> (n = 2)	40S13	<i>B. haynesii</i> NRRLB-41327 (MRBL01000076)	99.78
		40S20	<i>B. licheniformis</i> ATCC14580 (AE017333)	99.61
Sidi El Hadj Hot Spring (H07S) N = 15	<i>Brevibacillus</i> (n = 3)	40S24	<i>B. aydinogluensis</i> PDF25 (HQ419073)	99.64
		40S25	<i>B. thermoruber</i> DSM 7064 (Z26921)	99.54
		40S27	<i>B. thermoruber</i> DSM 7064 (Z26921)	96.81
	<i>Anoxybacillus</i> (n = 2)	07S31	<i>A. geothermalis</i> GSsed3 (KJ722458)	99.23
		07S16	<i>A. gonensis</i> G2 (CP012152)	99.90
	<i>Bacillus</i> (n = 11)	07S24	<i>B. haynesii</i> NRRLB-41327 (MRBL01000076)	99.35
		07S26	<i>B. haynesii</i> NRRLB-41327 (MRBL01000076)	99.78
		07S5	<i>B. hisashii</i> N-11 (AB618491)	99.88
		07S12	<i>B. hisashii</i> N-11 (AB618491)	99.90
		07S14	<i>B. hisashii</i> N-11 (AB618491)	99.52
		07S21	<i>B. hisashii</i> N-11 (AB618491)	99.77
		07S32	<i>B. hisashii</i> N-11 (AB618491)	99.51
		07S20	<i>B. licheniformis</i> ATCC14580 (AE017333)	96.09
	<i>Brevibacillus</i> (n = 2)	07S27	<i>B. licheniformis</i> ATCC14580 (AE017333)	98.51
		07S13	<i>B. paralicheniformis</i> KJ-16 (KY694465)	99.73
		07S15	<i>B. paralicheniformis</i> KJ-16 (KY694465)	99.52
		07S11	<i>B. aydinogluensis</i> PDF25 (HQ419073)	99.77
		07S35	<i>B. aydinogluensis</i> PDF25 (HQ419073)	99.48
Guerfa Hot Spring (H24G) N = 5	<i>Bacillus</i> (n = 3)	24G13	<i>B. haynesii</i> NRRLB-41327 (MRBL01000076)	99.50
		24G 1	<i>B. hisashii</i> N-11 (AB618491)	99.40
		24G17	<i>B. sonorensis</i> NBRC101234 (AYTN01000016)	99.64
	<i>Geobacillus</i> (n = 2)	24G 6	<i>G. icigianus</i> G1w1 (KF631430)	98.02
		24G 5	<i>G. stearothermophilus</i> NBRC 12550 (AB271757)	98.79
Debagh Hot Spring (H24D) N = 14	<i>Aeribacillus</i> (n = 1)	24D35	<i>A. composti</i> N.8 (LT594972)	99.41
	<i>Aneurinibacillus</i> (n = 1)	24D 1	<i>A. thermoaerophilus</i> DSM 10154 (X94196)	99.22
	<i>Anoxybacillus</i> (n = 2)	24D20	<i>A. kaynarcensis</i> D1021 (EU926955)	99.41
		24D29	<i>A. kaynarcensis</i> D1021 (EU926955)	99.49
	<i>Bacillus</i> (n = 4)	24D46	<i>B. haynesii</i> NRRLB-41327 (MRBL01000076)	99.57
		24D45	<i>B. hisashii</i> N-11 (AB618491)	99.47
		24D 6	<i>B. kokesiiformis</i> MO-04 (JX848633)	99.41
		24D26	<i>B. thermocopriae</i> SgZ-7 (JX113681)	99.51
	<i>Brevibacillus</i> (n = 1)	24D 3	<i>B. aydinogluensis</i> PDF25 (HQ419073)	99.56
	<i>Meiothermus</i> (n = 2)	24D25	<i>M. ruber</i> DSM1279 (CP001743)	99.92
		24D28	<i>M. ruber</i> DSM1279 (CP001743)	98.98
	<i>Thermobifida</i> (n = 1)	24D39	<i>T. fusca</i> NBRC14071 (BCWB01000075)	99.73

Table 1 (continued)

Site	Genus	Isolates	Related bacterial strains (accession number)	Similarity (%)
	<i>Thermus</i> (n = 2)	24D9	<i>T. antranikianii</i> HN3-7 (Y18411)	99.78
		24D24	<i>T. antranikianii</i> HN3-7 (Y18411)	99.23

amygdaline and inositol, 18% for mannitol, and 15% for glucose, sorbitol, and arabinose, respectively. Only 8% assimilate melibiose. The number of positive tests displayed by the isolates ranged from one to ten tests. Around 32% of strains were positive only for one character.

Thermophilic bacterial diversity

Among the different sites, Debagh Hot Spring (H24D) and Belhachani Hot Spring (H24A) presented the highest species richness with its isolates being closely related to 11 genera. The second position of more diverse sites belonged to Saïda Hot Spring (H05S) with nine represented genera, followed by Guerdjima Hot Spring (H05G), Sidi El Hadj Hot Spring (H07S), and Essalihine Hot Spring (H40S) with seven genera for each site. Guerfa Hot Spring (H24G) and El Knif Hot Spring (H40K) resulted in five and four genera, respectively.

In the current study, the genus *Bacillus* (30%) was isolated from all sampled geothermal sites. A total of 7% of isolates represented by *Geobacillus*, *Laceyella*, *Saccharomonospora*, *Thermobifida*, and *Thermus* were uniquely distributed in the different hot springs. The other genera were distributed variably. *Aeribacillus*, *Aneurinibacillus*, *Anoxybacillus*, and *Brevibacillus* were isolated from five hot springs, while *Thermoactinomyces* and *Meiothermus* were cultured from three and two sites, respectively. At each sampling point of the four studied areas, we determined exclusive species (detected at a single sampling point) and common species (found in at least two sampling points). Venn diagram illustrates that Guelma's hot springs were more diverse than the other sites in terms of species exclusivity, hosting ten exclusive species. The analysis revealed that three species were shared among all locations, whereas one to four other species were classified as common species for the different combinations of regions (Fig. 6).

Hydrolytic enzyme analysis

Bacterial isolates collected from hot springs were screened for amylase, cellulase, pectinase, esculinase, protease, gelatinase, lipase, lecithinase, and nuclease activities (Fig. S3). The frequency occurrence of nine hydrolytic extracellular enzymes produced by thermophilic bacteria at hot springs is shown in Fig. 7. A total of 143 isolates (48.81%) showed amylase activity, 156 isolates (53.24%) produced proteases, 122 isolates

(41.64%) produced lecithinase, 197 isolates (67.24%) reduced CMC-cellulose, 174 isolates (59.39%) produced pectinase enzyme, 200 isolates (68.26%) produce nuclease, 143 isolates (48.81%) produced esculinase, 189 isolates (64.50%) produce lipase, and 173 isolates produced (59.04%) gelatinase.

Among the 293 thermophilic bacteria, 99.32% produced at least one extracellular hydrolytic enzyme. The combined hydrolytic activity was also detected in most strains. Indeed, 19 isolates (6.48%) combined all tested enzymes. Jointly, 18.43% (54 isolates), 65.87% (42 isolates), and 15.36% (45 isolates) produced, respectively, four, five, and six hydrolytic enzymes simultaneously. In addition, 65.87% of bacterial isolates showed at least five enzymatic activities.

Discussion

The present study searched for the diversity of thermophilic culturable bacteria in Algerian hot springs. Thermophilic bacteria were present in all analyzed samples, and the occurrence of isolates in different locations may be a result of the prevailing environmental conditions and the chemical properties of the water and sediment of the different hot springs, suggesting the environment selects those strains better thriving under those specific conditions, mainly temperature, pH, salinity, and mineral elements [60, 61]. Almost water and sediment allowed the isolation of a similar abundance of thermophilic bacteria. However, sediment samples presented higher diversity than water samples. Water samples showed more stable culturable communities, which were dominated by a low number of the most representative species. Microbial community composition and abundance of sediments are influenced by high nutrient content especially, organic matter, dissolved oxygen, nitrogen, and phosphorus levels, which are key factors causing differences in microbial community structure comparing to water [10, 62]. The number of thermophilic bacterial populations recovered was dependent on the media composition. Nutrient agar and Tryptic soy medium were employed for isolating a large proportion of the available diversity mainly the thermotolerant isolates; both media support the growth of bacteria from hot springs when incubated at high temperature [39]. Furthermore, *Thermus* medium, ATCC medium 697, and *Thermus* 162 medium were suitable for isolation of thermophilic bacteria, including novel species [63].

Fig. 3 Neighbor-joining phylogenetic tree showing the relationship based on 16S rRNA gene sequence comparison of (a) *Deinococcus-Thermus* and (b) *Actinobacteria*. Bootstrap values (expressed as

percentages of 1000 replications) greater than 50% are shown at nodes. The scale bar represents 2% estimated sequence divergence. The sequence of *Bacillus thermoamylovorans* was used as an outgroup

There were very significant differences among the 293 presumptive thermophilic isolates in terms of morphological, biochemical, and physiological properties. Isolates had characteristic phenotypic characters; they were commonly Gram-positive rod-shaped cells, and they were generally oxidase-

and catalase-positive. Similarly, Narayan et al. reported the dominance of Gram-positive thermophilic bacteria from Savusavu hot Spring in Fiji [64].

Temperature tolerance of the isolates allowed their classification as thermophilic bacteria [1]. It has shown that 100%

Fig. 4 Neighbor-joining phylogenetic tree showing the relationship between *Firmicutes* isolates (*Paenibacillaceae* and *Thermoactinomycetaceae*) and closely related species based on 16S rRNA gene sequence comparison. Bootstrap values expressed as percentages of 1000 replications; only bootstrap values > 50% are shown at nodes. The scale bar represents 2% estimated sequence divergence

of isolates grow variably at 55 °C. Although, around 50% of the bacteria can grow at 30 °C, but their optimal temperature was 55 °C, the same temperature of their isolation. A total of 128 isolates were moderately thermophilic, while 5.11% isolates were extremely or obligate thermophilic. Salt-tolerant range revealed that all the isolated strains were halotolerants [65]. The number of strains growing decreased at increasing salt. Almost all strains grew at near neutral to slightly alkaline pH ranging between 6.5 and 8.5. The physiological and morphological characteristics turned too difficult to differentiate isolates. This is well known and in accordance with previous microbiological descriptions [30].

The analysis of SDS-PAGE whole-cell protein patterns has proven to be highly reliable for comparing and clustering a large number of Gram-positive and negative mesophilic bacteria growing on identical conditions and belonging to different genera [66, 67]. However, there are very little researches about the application of this technique on thermophilic species. First, it was proved that thermophilic bacteria can be identified by their amino acid composition deduced from their complete genome sequences [33]. Later on, another research suggested that the whole protein profiles by SDS-PAGE of thermophilic bacteria isolated from deep-sea hydrothermal fields in the Pacific Ocean provide discrimination between species with high resolution [34]. Inan et al. [18] suggested the classification of strains belonging to the *Anoxybacillus* genus based on SDS profile. Both results showed great efficiency and rapidity in the differentiation of thermophilic species prior to further identification by genomics tools.

Based on SDS-PAGE fingerprinting of whole-cell protein results combined with physiological and biochemical data, of 293 isolates, 100 bacteria were selected for identification by complete 16S rDNA gene sequencing (~1400 bp). The *Bacillus* genus dominated the thermophilic isolates (30%) with high species diversity: *B. haynesii*, *B. hisashi*, *B. kokeshiiformis*, *B. licheniformis*, *B. paralicheniformis*, *B. sonorensis*, and *B. thermocopriae*. Other research found that *Bacillus* was the most dominant isolated genus [30, 38].

Anoxybacillus was ranked in second position (17%) with the following seven different species: *A. flavithermus*, *A. geothermalis*, *A. gonensis*, *A. kaynarcensis*, *A. pushchinoensis*, *A. salavatliensis*, and *A. thermarum*. The genera *Aeribacillus* and *Aneurinibacillus* presented 15% of the isolates. One genus with two species *A. composti* and *A. pallidus* and one species *A. thermoaerophilus* for the

second one. They were followed by *Brevibacillus* (8%), *Thermoactinomyces* (5%), and *Meiothermus* (3%) with two species *Brevibacillus aydinogluensis*, *Brevibacillus thermoruber*, and only one species *Thermoactinomyces vulgaris* and *Meiothermus ruber*, respectively. A low isolation rate of 2% characterized two genera *Geobacillus* represented by *Geobacillus icigianus* and *Geobacillus stearothermophilus*, with *Thermus* comprising one species *Thermus antranikianii*. Other species were also isolated, such as *Laceyella sacchari*, *Saccharomonospora viridis*, and *Thermobifida fusca*, representing 1% each of the total number of isolates. Yohandini et al. reported that the genera *Geobacillus*, *Anoxybacillus*, *Brevibacillus*, and *Bacillus* were the commonly found in thermal environments, within Tanjung Sakti Hot Spring, in South Sumatera, Indonesia, and these results are consistent with our findings [31]. Moreover, these results also indicate that Algerian hot springs located in the Eastern part of the country harbor a wide variety of thermophilic species, some already known or potentially novel waiting further identification. Interestingly, we notice the isolation and identification of strains belonging to six genera, *Aneurinibacillus*, *Laceyella*, *Meiothermus*, *Thermus*, *Thermobifida*, and *Saccharomonospora*, which have never been reported previously from any hot springs in Algeria [27, 28].

The members of *Firmicutes* dominated among the bacteria isolated from all sampled hot springs; the results provide evidence that *Bacillus* and related genera (*Aeribacillus*, *Anoxybacillus*, and *Geobacillus*) represent the family *Bacillaceae* with 64% of total isolates were ubiquitous. This is likely due to endospore-forming ability of these bacteria representing a successful survival strategy and dominance under high temperature conditions [68]. Similarly, many reports indicate the abundance of the phylum *Firmicutes* in extreme environments [9]. A total of 23% of *Firmicutes* were represented by *Paenibacillaceae* (*Aneurinibacillus* and *Brevibacillus*), following the finding by Kumar et al. [69]. Gram-negative bacteria were represented by the *Deinococcus-Thermus* phylum harboring *Meiothermus* and *Thermus*. Both genera have so far been isolated from terrestrial hot springs around the world [70, 71]. A reduced number of *Actinobacteria* was obtained. Two genera (*Thermobifida* and *Saccharomonospora*) were isolated from thermal spring sediments on the *Thermus* medium. Many researchers reported the actinobacterial diversity in hot springs by culture-independent methods. However, there are rare reports on culturable actinobacteria in geothermal fields [72].

Furthermore, six isolates exhibited < 98.51% nucleotide sequence similarity with reported type strains in the EzTaxon server. These values were lower than 98.65% 16S rRNA gene sequence similarity, threshold needed for differentiating two bacterial species, and to describe a new species without performing the DNA–DNA hybridization [73].

Fig. 5 Neighbor-joining phylogenetic tree showing the relationship between *Bacillaceae* isolates and closely related species based on 16S rRNA gene sequence comparison. Bootstrap values expressed as percentages of 1000 replications; only bootstrap values > 50% are shown at nodes. The scale bar represents 2% estimated sequence divergence

Among the potentially novel strains, 07S20 and 07S27 showed sequence similarity with the type strain *Bacillus licheniformis* of 96.09 and 98.51%, respectively. The strain 40S27 indicated the highest sequence similarity with *Brevibacillus thermoruber* (96.81%). The two strains 24G6 and 24G5 exhibited 98.02 and 98.79% similarity with the species *Geobacillus icigianus* and *Geobacillus stearothermophilus*, respectively, while the strain 40S14 showed the highest 16S rRNA gene sequence similarity with the type strain *Aneurinibacillus thermoaerophilus* (98.35%), and the strain 05S21 revealed 98.13% sequence similarity with the species *Thermoactinomyces vulgaris*. These results indicate that they probably represent new species belonging to four different hot springs among the eight prospected, explaining the richness in new taxa of Algerian thermal springs. Previous studies describe novel species isolated from different Algerian terrestrial geothermal sites. Mainly represented by *Pyrococcus* sp. HT3, *Caldicoprobacter algeriensis*, and *Thermoactinomyces khenchelensis*, respectively [74–76].

The traditional methods, including morphological, physiological, and biochemical characterization, used for a preliminary classification of the isolates are troublesome, uncertain, difficult for interpretation, and may involve substantial subjective judgment [77]. At the same time, SDS-PAGE results allow clustering the isolates enabling rapid and easy discrimination as the first step for mandatory molecular identification. The 16S rRNA gene sequencing provides an important contribution to identifying the strains successfully to the genus

and sometimes even to species level. Although 16S rRNA gene sequencing is highly accurate and useful regarding bacterial classification, it may be insufficient to distinguish between some closely related species and has low discriminatory power for some genera [78]. This is the case for species within some genera, like *Bacillus*, *Anoxibacillus*, *Aeribacillus*, and *Geobacillus* [79–81]. Thus, the identification of bacteria, and specifically thermophiles, could benefit from the addition of other fingerprinting methods for strain and species discrimination [10, 18–82].

The limits of traditional culture-based methods to describe the enormous diversity existing in the hidden microbial world have forced to currently shift towards culture-independent approaches (i.e., based on DNA sequencing) which complement physiological analyses to explore functional and taxonomical diversity in hot springs [83].

The constructed UPGMA dendrogram of the biochemical characteristics of the selected 100 bacterial strains isolated from eight hot springs showed three clusters (numbered from I to III). Overall, all the isolates displayed a minimum similarity coefficient of approximately 0.68 or displayed phenotype profiles that were approximately 68% similar. A group of 14 isolates belonging to the eight hot springs formed cluster I. Three isolates from the sub-cluster (IA) showed identical biochemical profiles (isolates 05S28, 24D28, and 24D25 identified by 16S rDNA sequencing as *Meiothermus ruber*), same (isolates 07S13 and 07S15 identified as *Bacillus paralicheniformis*) from sub-cluster (IB), and cluster II was a mixture of 10 isolates from six different sites, exhibiting similar profiles for (40S24, 24D3, 05S37, and 07S35 attributed to *Brevibacillus aydinogluensis*). Finally, cluster III grouped 76 isolates into two sub-clusters, IIIA harboring six isolates and IIIB gathering 70 isolates. They corresponded to *Anoxybacillus gonensis*, *Geobacillus icigianus*, *Aneurinibacillus thermoaerophilus*, and *Brevibacillus aydinogluensis*. The biochemical characterization has certain limitations in the classification of isolates, as it is hard to distinguish closely related bacteria. Molecular identification is more reliable and could assist in definitive identification of isolates as part of a polyphasic taxonomic approach [84].

Thermophilic bacteria are expected to be a potential source of interesting hydrolytic enzymes with exceptional condition [1]. The present study revealed that the main enzyme producers were related to *Firmicutes* phylum (Fig. 7). Furthermore, nuclease, cellulase, lipase, and pectinase activities ranked in the first place of the common enzymes. Maybe due to the fact that the hot spring biotopes present a significant pool for special organic molecules inducing the bacterial enzymatic production [65]. Almost all the selected strains showed at least one extracellular enzyme among the tested activities, and 65.87% of bacterial isolates showed at least five combined enzymatic activities. The dominant isolates belonged to *Bacillus* and *Bacillus*-like genera, namely

Fig. 6 Venn diagram distribution illustrates the number of exclusive and common thermophilic bacterial isolates among the sampled sites. Each ellipse represents a sampling point

Fig. 7 Distribution of extracellular hydrolytic enzymes among isolated thermophilic bacteria. *N*, the number of isolates presents activities for each enzyme

Anoxybacillus, *Aeribacillus*, and even *Aneurinibacillus* genus known for their significant production of enzymes [85, 86]. *Bacillus* sp. was the highest producer of all targeted enzymes ranging between 39.53 and 20.69%; indeed, *Bacillus* species are considered as workhorse industrial bacteria in applied and industrial microbiology due to the high rate of growth of these bacteria and their ability to secrete a large volume (20–25 g/L) of extracellular enzymes. Besides, most of these species are “generally regarded as safe” (GRAS) [87]. According to our findings, up to 28.81% of protease and 32.61% of amylase were produced by *Bacillus*. *Bacillus* proteases have several remarkable characteristics and make up approximately 60% of the total worldwide enzyme sales [88]. Different bacterial species can produce alpha-amylase, but for commercial applications; they mainly originated from the genus *Bacillus* [89]. Our results showed the highest rate of 30.43% for amylase activity produced by *Anoxybacillus* spp. (Fig. 7); from this genus, several new amylases with different properties were identified and characterized. Viksø-Nielsen et al. [90] reported a new α -amylase that hydrolyzes raw starch granules.

Ecological studies on Algerian hot springs led to the isolation and identification of a large number of thermophilic bacteria capable of producing a mixture of hydrolytic enzymes that may be valuable for biotechnological purposes [28]. Extremophile microorganisms can thrive under extreme conditions of high temperature, salinity, pH, and pressure.

Particular interest on industrial applications of extremophiles emerges during the past few decades, because they are able to produce novel biocatalysts functioning under extreme conditions, resulting in unique products and higher production efficiency [65]. Thermozyms offer unique characteristics, such as temperature, chemical, and pH stability, resulting to technological interest in diverse industrial applications, including food, pharmaceutical, chemical, paper, textile processing, bio-fuel production, pulp, and waste treatment [91]. Consequently, thermophilic bacteria are a good source of novel catalysts suggesting new application or improving the existing processes of industrial interest [92].

Conclusion

The present study aimed to analyze the culture-dependent diversity of thermophilic bacteria from terrestrial geothermal springs in Eastern-Algeria, using conventional taxonomic methods combined with molecular approaches. Interestingly, high taxonomic diversity was observed in Algerian hot springs, and several phylotypes, grouped as *Aneurinibacillus*, *Bacillus*, *Brevibacillus*, *Geobacillus*, and *Thermoactinomyces*, may constitute new taxa with novel hydrolytic potentials. Bacterial species diversity is a resource that can translate into novel biocatalysts. In fact, these

thermophilic isolates exhibit promising sources of hydrolytic extracellular enzymes serving various industrial, agricultural, and medicinal purposes, especially that present optimal activities at high temperatures.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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