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Original Article

Characterization of endospore-forming bacteria producing extracellular enzymes isolated from the Djurdjura Mountains in Algeria

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ABSTRACT

Biodiversity in mountains in Algeria appears scanty and has not been thoroughly investigated. However, the mountain soil has been shown as an almost entire reserve of novel enzymes with interesting properties for industrial and environmental applications. In the present study, thirty bacterial strains were isolated from the Djurdjura Mountains in Kabylia (Algeria) and were studied for their ability to produce enzymes to be possibly used in biotechnological processes such as amylase, caseinase, and chitinase. The characterization of these isolates was carried out using morphological, physiological, and biochemical characteristics. All the data obtained with regards to the phenotypical properties of the isolates, confirmed that the strains belonged to the *Bacillus* group. In addition, the 16S rRNA gene of the two retained strains KA15 and LK-DZ15 was also amplified and sequenced. Phylogenetic tree was, afterwards, constructed. The nucleotide sequences and blast analyses confirmed that the KA15 and LK-DZ15 strains were closely related to those of the *Bacillus altitudinis* (accession n°.: **MK874318**) and *Paenibacillus timonensis* (accession n°.: **MK734103**) strains. The presence of amylases, proteases, and chitinases in KA15 and LK-DZ15 isolates are an indicator of their pivotal application in a variety of biotechnological processes.

1. Introduction

Among the most common microorganisms in the environment, the "*Bacillus* group", which is characterized by its diversity, the ability of its species to survive in unfavorable conditions and to produce large quantities of extracellular enzymes and biomolecules of industrial and medical interest [1]. The term "aerobic endospore-forming bacteria" is used to encompass species of the genus *Bacillus* and related genera. Since 1990, 14 genera have been proposed to accommodate species previously assigned to *Bacillus*. In addition, 37 other new genera containing species not previously affiliated with *Bacillus* have also been proposed. Initially the Gram-positive

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bacteria were all affiliated with the phylum *Firmicutes* as described by Gibbons & Murray [2].

The genus *Paenibacillus*, initially included in the genus *Bacillus*, is characterized as rod-shaped Gram-positive or Gram-variable endospore forming aerobic or facultatively anaerobic bacteria [3]. Members of this genus are enzyme producers such as protease, amylase, and chitinase [4, 5, 6]. Indeed, extraordinary characteristics of these enzymes make them a promising renewable tool for industrial biotechnological processes. The global market for industrial enzymes is expected to reach $7 billion by 2018 with a compound annual Growth rate (CAGR) of 8.2% from 2013 to 2018 [7]. In addition, the global specialty enzymes market is forecasted to reach about $4 billion by 2018. This market will likely continue to grow for the foreseeable future as a result of advancements in the biotechnology industry, the continued need for a cost-efficient manufacturing process, and calls for greener technologies [8].

In this context, the presented research aims to isolate endospore-forming bacteria from the Djurdjura Mountains in Algeria, to determine the capacities of hydrolytic enzymes, and to identify them by using morphological, physiological, and biochemical methods. Also, the most important strains were identified *via* molecular methods.

2. Materials and Methods

2.1. Sample collection

Samples were collected from the soil surface of Tamgut Aâlayen (Lalla Khedidja) (GPS coordinates: Latitude 36°27'0" N, Longitude 4°13'60" E) in the Djurdjura Mountains (Kabylia) (2,308 meters), Tizi-Ouzou, Algeria (Fig. 1.), using 1 L sterile thermal glass bottles. Samples were stored in the laboratory at room temperature. The sampling was carried out by Pr. Jaouadi B. (university of Sfax, Tunisia), Dr. Yahiaoui M. (university of M'Sila), and Dr. Bouacem K. (university of Tizi-Ouzou).

2.2. Isolation of microorganisms

Isolation and enrichment cultures were performed in initial medium containing (in g/L): glucose, 2; NH_4Cl, 1.5; K_2HPO_4, 1; KH_2PO_4, 1; NaCl, 10; KCl 0.1; $CaCl_2 \cdot 2H_2O$, 1; $MgCl_2 \cdot 6H_2O$, 0.25; yeast extract, 1; and Biotrypcase, 2 at pH 7. The enrichment cultures were sub-cultured several times under the same conditions. Submerged cultures were carried out in 1000 mL shake flasks with 100 mL of medium. The flasks were inoculated and incubated in an orbital shaker at 30°C and 180 rpm for 72 h. From each sample, 100 µL aliquot was plated by spreading on initial medium plates (five replicates) and incubated for 12, 24, 36, 48, 60, and 72 h at 30°C. In order to obtain pure cultures, different colonies were selected and restreaked several times. Pure cultures were stored in nutrient agar (NA) until used, and also in glycerol under freeze at -20°C. All selected colonies were tested for amylase, protease and chitinase activities.

2.3. Characterization of the isolates

2.3.1. Morphological and biochemical studies

The colony morphologies were determined using cultures grown aerobically on nutrient agar. Cell morphology and motility were examined microscopically in exponentially growing liquid cultures after 18-24h of incubation at 37°C. The isolates were identified by the use of conventional tests. These latter were; Gram reaction, catalase and oxidase production. Acids production from carbohydrates and hydrolyses of some polymers were determined using API Gallery 50 CHB (bioMérieux) as recommended by the manufacturer.

2.3.2. Physiological tests

The temperatures tested were 30, 40, 50, 60, 70, and 80°C. The pH growth range was examined between 4 and 12. Salinity tolerance was investigated for 1 to 7 (w/v) NaCl. All the physiological tests were determined in nutrient agar the only exception of the pH dependence of growth at pH 4 and the temperature growth at 80°C, which were performed in nutriment broth [9].

Fig. 1. Site of sampling isolation.

2.3.3. 16S rDNA sequence and phylogenetic analysis

2.3.3.1. DNA purification

The genomic DNA of KA15 and LK-DZ15 strains was purified by the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) and then used as a template for PCR.

2.3.3.2. Amplification via the polymerase chain reaction (PCR)

The 16S rRNA genes were amplified by PCR using forward 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse 1525R (5'-AAGGAGGTGATCCAAGCC-3') primers. Amplification conditions included an initial denaturation step 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 s; annealing at 60°C for 45 s; and an extension at 72°C for 60 s, with a final extension at 72°C for 10 min. The amplified ~1.5 kb PCR product was cloned in the pGEM-T Easy vector (Promega, Madison, WI, USA), leading to pLK-DZ15-16S and pKA15-16S plasmids [10, 11, 12]. The *E. coli* DH5α [F^- *supE44 Φ80 δlacZ ΔM15 Δ (lacZYA-argF) U169 endA1 recA1 hsdR17* (r_k^-, m_k^+) *deoR thi-1* λ^- *gyrA96 relA1*] (Invitrogen, Carlsbad, CA, USA) was used as a host strain. All recombinant clones of *E. coli* were grown in Lysogeny-Broth (LB) media with the addition of ampicillin, isopropyl-thio-β-D-galactopyranoside (IPTG), and X-gal for screening. DNA electrophoresis, DNA purification, restriction, ligation, and transformation were all performed according to the method previously described by Sambrook et *al*. [13].

2.3.3.3. Sequencing of 16S rDNA

The nucleotide sequences of each cloned 16S rRNA gene were determined on both strands using BigDye Terminator Cycle Sequencing Ready Reaction kits and the automated DNA sequencer ABI PRISM® 3100-Avant Genetic Analyser (Applied Biosystems, Foster City, CA, USA). The obtained sequences were compared with sequences available in the public sequence databases and with the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/), a web-based tool for the identification of prokaryotes based on 16S rRNA gene sequences from type strains.

2.3.3.4. Phylogenetic analysis

Phylogenetic and molecular evolutionary genetic analyses were performed using the Molecular Evolutionary Genetics Analysis (MEGA) software v. 4.1. Distances and clustering were calculated using the neighbor-joining method. Bootstrap analysis was used to evaluate the tree topology of the neighbor-joining data by performing 100 re-samplings. Multiple nucleotide sequence alignment was performed using the BioEdit version 7.0.2 software.

2.4. Screening of hydrolytic activities

2.4.1. Amylases

Starch hydrolysis method was used to identify the amylolytic properties of strains. Each colony was streaked on a nutrient agar plate that contained 1% starch and incubated at 37°C for 48 h. After the incubation period, plates were flooded with Lugol's iodine to detect the presence of clear halos around those bacterial colonies capable of secreting amylase [14].

2.4.2. Caseinases

Caseinase activity was detected on nutrient agar containing 3% skimmed milk. Plates were streaked with test strains followed by incubation at 37°C for 48 h. The colonies with a clear zone formed by the hydrolysis of milk casein were evaluated as protease producers [15].

2.4.3. Chitinases

Chitinase activity was detected on nutrient agar supplemented with 1% colloidal chitin. Plates were streaked with test strains followed by incubation at 37°C for 48 h. The colonies with a clear zone formed by the hydrolysis of colloidal chitin were evaluated as chitinase producers [16].

3. Results and Discussion

3.1. Isolation, phenotypical, biochemical and physiological study

Samples were collected from soil of Lalla Khedidja (Tikjda), the highest summit of the Djurdjura Mountains in Kabylia, Algeria. Once samples had been brought back to the laboratory, bacterial strains were isolated as described in the section Materials and Methods. In total, thirty isolates were obtained after successive streak outs for purification.

The bacterial isolates showed varied colonies as well as cell morphologies (Fig.2). The colonies on the agar plates were circular, translucent or opaque. In general, the colony color ranged from white to beige (Table 1). According to phenotypic results, the strains appear rod-shaped cells, aerobic growth, endospore forming and Gram-positive (Table 1). Most strains occurred singly, others in pairs, or occasionally in short chains. All strains were motile, except KA7, KA11, KA14, LK-DZ6, LK-DZ10, LK-DZ13, and LK-DZ14 strains (Table 1).

API 50 CH carbohydrate fermentation test and 16S rRNA gene sequencing were carried out for the identification of the genus to which the strains KA15 and

LK-DZ15 belonged. These two strains were catalase and oxidase positive [10, 11]. In addition, biochemical profile obtained with API 50 CH gallery test, exhibited that these two isolates metabolize maltose, lactose, D-xylose, D-arabinose, D-tagatose, starch, galactose, rhamnose, and glucose besides other simple sugars (Table 2). Moreover, both strains were positive for esculin hydrolysis.

As shown in the table 3, out of 30 bacterial isolates, 13 could grow between 30 and 80 °C, 27 between 30 and 70°C. For most strains, growth occurred at pH values ranging from 5 to 9. Twelve isolates tolerate up to 3% NaCl, while KA15 and LK-DZ15 strains could tolerate a higher NaCl concentration (6%). All the results obtained concerning the physiological and biochemical properties of the isolate strongly indicated that the KA15 and LK-DZ15 strains belonged to the *Bacillu* and *Paenibacillus* genus, respectively.

3.2. Taxonomy identification and molecular phylogeny of the LK-DZ15 and KA15 strains

The KA15 and LK-DZ15 isolates were subjected to various morphological, biochemical, and physiological tests (Tables 1, 2, and 3). The pigmentation of KA15 and LK-DZ15 colonies were beige and white, respectively. These two strains were arranged singly or in sporulated, and rod-shaped bacterium. The API 50 CH profile revealed that the KA15 and LK-DZ15 isolates could utilize arabinose, esculin, and salicin. As shown in Table 3, KA15 and LK-DZ15 grew up to 60 °C and 80 °C, respectively. The pH range for growth of KA15 isolate is between 5 and 9. While, the pH range for growth of LK-DZ15 isolate is between 5 and 11. Thus, suggesting their alkali-tolerance property. The KA15 and LK-DZ15 isolates were able to grow in the presence of 1 to 6% NaCl.

In order to establish and identify further support of the novel KA15 and LK-DZ15 isolates, fragments of the 16S rRNA genes were amplified from the genomic DNA of each isolates, cloned in the pGEM-T Easy vectors, and sequenced on both strands. The nucleotide sequences of the two 16S rRNA genes, were aligned manually, then compared with others from different species. The related taxa were available from the latest versions of the Ribosomal Database Project (RDP) and GenBank databases. The subsequently constructed phylogenetic tree (Fig. 3) revealed that 16S rRNA gene sequences from KA15 and LK-DZ15 were nearly related to those of the *Bacillus* and *Paenibacillus* genus, respectively.

The nearest *Bacillus* and *Paenibacillus* strains identified by BLAST were imported into ARB software package and aligned. The phylogenetic tree was then constructed, using neighbour-joining methods and Jukes-Cantor distance matrices. The nucleotide sequences were imported into MEGA software package version 4.1 and aligned.

Phylogenetic tree was, afterwards, constructed and the findings further confirmed that the strains KA15 (accession n°.: **MK874318**) and LK-DZ15 (accession n°.: **MK734103**) were closely related to those of the *Bacillus altitudinis* and *Paenibacillus timonensis* strains respectively. The findings suggested that these isolates should be accounted for *Bacillus altitudinis* KA15 and *Paenibacillus timonensis* LK-DZ15 strains.

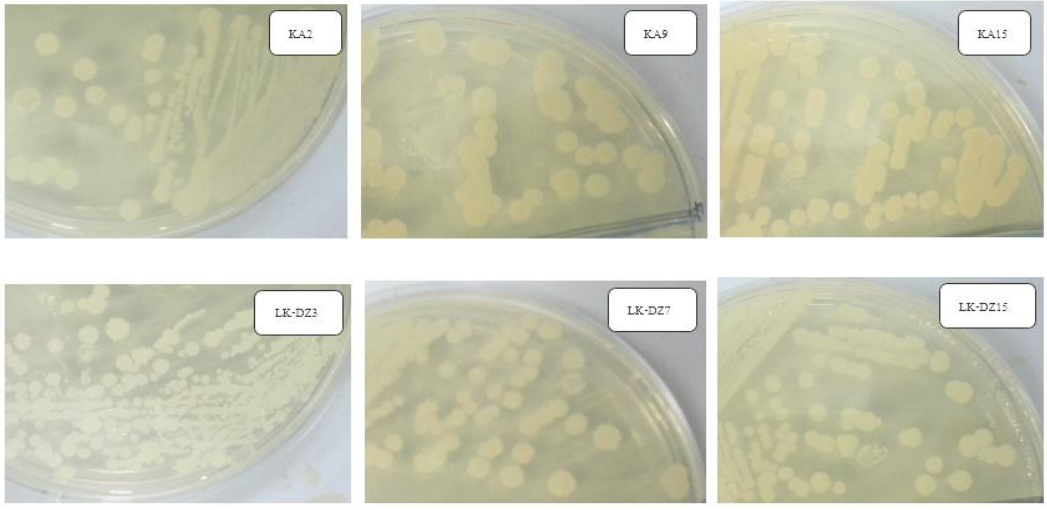

Fig. 2. Macroscopic appearance of some strains of *Bacillu*s isolated from the Djurdjura mountains.

Table 1. Phenotypic features of isolates of *Bacillu*s isolated from the Djurdjura mountains.

Strains	Phenotypic features							
	Colony morphology	**Colony density**	**Pigmentation**	**Cell shape**	**Cell arrangement**	**Spore**	**Motile**	**Gram**
KA1	Circular	Translucent	Beige	Rod	Single	+	+	+
KA2	Circular	Translucent	Beige	Rod	Short chains	+	+	+
KA3	Circular	Translucent	Beige	Rod	Single	+	+	+
KA4	Circular	Translucent	Beige	Rod	Short chains	+	+	+
KA5	Circular	Opaque	White	Rod	Single	+	+	+
KA6	Circular	Opaque	Beige	Rod	Single/Paired	+	+	+
KA7	Circular	Translucent	Yellow	Rod	Single/Paired	+	-	+
KA8	Circular	Translucent	Cream	Rod	Single	+	+	+
KA9	Circular	Opaque	Cream	Rod	Single	+	+	+
KA10	Circular	Opaque	Beige	Rod	Short chains	+	+	+
KA11	Circular	Opaque	Cream	Rod	Single	+	-	+
KA12	Circular	Opaque	Beige	Rod	Single	+	+	+
KA13	Circular	Opaque	Cream	Rod	Short chains	+	+	+
KA14	Circular	Opaque	Orange	Rod	Single	+	-	+
KA15	Circular	Translucent	Cream	Rod	Single	+	+	+
LK-DZ1	Circular	Translucent	Beige	Rod	Single	+	+	+
LK-DZ2	Circular	Opaque	Cream	Rod	Single	+	+	+
LK-DZ3	Circular	Opaque	White	Rod	Single	+	+	+
LK-DZ4	Circular	Opaque	Beige	Rod	Single	+	+	+
LK-DZ5	Circular	Opaque	Cream	Rod	Single	+	+	+
LK-DZ6	Circular	Opaque	Beige	Rod	Single	+	-	+
LK-DZ7	Circular	Opaque	Beige	Rod	Single	+	+	+
LK-DZ8	Circular	Translucent	Yellow	Rod	Single	+	+	+
LK-DZ9	Circular	Opaque	Cream	Rod	Short chains	+	+	+
LK-DZ10	Circular	Opaque	White	Rod	Single	+	-	+
LK-DZ11	Circular	Opaque	Beige	Rod	Single	+	+	+
LK-DZ12	Circular	Opaque	Cream	Rod	Single	+	+	+
LK-DZ13	Circular	Translucent	Beige	Rod	Single	+	-	+
LK-DZ14	Circular	Translucent	Beige	Rod	Single	+	-	+
LK-DZ15	Circular	Translucent	White	Rod	Single	+	+	+

3.3. Hydrolase activities

Biocatalysts are fascinating the scientists owing to their enormous potential of catalysis and eco-friendly nature. So, the isolates from Djurdjura mountains in Algeria were screened for amylase, caseinase, and chitinase activities at 37 °C (Fig. 4). As shown in Table 4, among the 30 strains selected in this study, LK-DZ15 and KA15 strains displayed amylase, caseinase, and chitinase activities. Six strains (KA6, KA15, LK-DZ5, LK-DZ12, LK-DZ13, and LK-DZ15) use starch, six strains (KA5, KA9, KA11, KA15, LK-DZ3, and LK-DZ15) degrade casein, and 14 strains (KA1, KA3, KA5, KA9, KA10, KA12, KA15, LK-DZ3, LK-DZ7, LK-DZ8, LK-DZ11, LK-DZ12, LK-DZ14, and LK-DZ15) hydrolyze colloidal chitin. The presence of a variety of biopolymer degrading hydrolases detected in bacteria from Djurdjura Mountains in Algeria suggest main involvement of these isolates to the hydrolysis of the major organic constituents (proteins and carbohydrates). In fact, microorganisms in mountains are adapted to surviving in ecological niches such as low temperatures. It produces novel organic compounds and stable biocatalysts that function under these conditions comparable to those prevailing in many industrial processes. Thanks to their ability to propagate under the conditions where other organisms either cannot grow or grow little, microorganisms living in the extreme environments, have always gained a significant attention from scientists. The literature indicates that these microorganisms are considered as an important source of enzymes with unconventional biochemical and molecular characteristics, and unique metabolic capabilities are the major points of attractions in biotechnological applications [3,9,11,12]. Hence, looking at the interest applications of extremo-enzymes in various biotechnological applications, the present work aimed to characterize the endospore-forming bacteria producing extracellular enzymes isolated from the Djurdjura Mountains in Algeria. That means, they could

contribute particularly to carbon and nitrogen cycling in the Mountains soil. Species belonging to the genus *Bacillus* and *Paenibacillus* are known for their interest production of hydrolases like chitinase, caseinase, amylase, cellulase, and xylanase representing scientific and biotechnological interest [17, 18, 19, 20].

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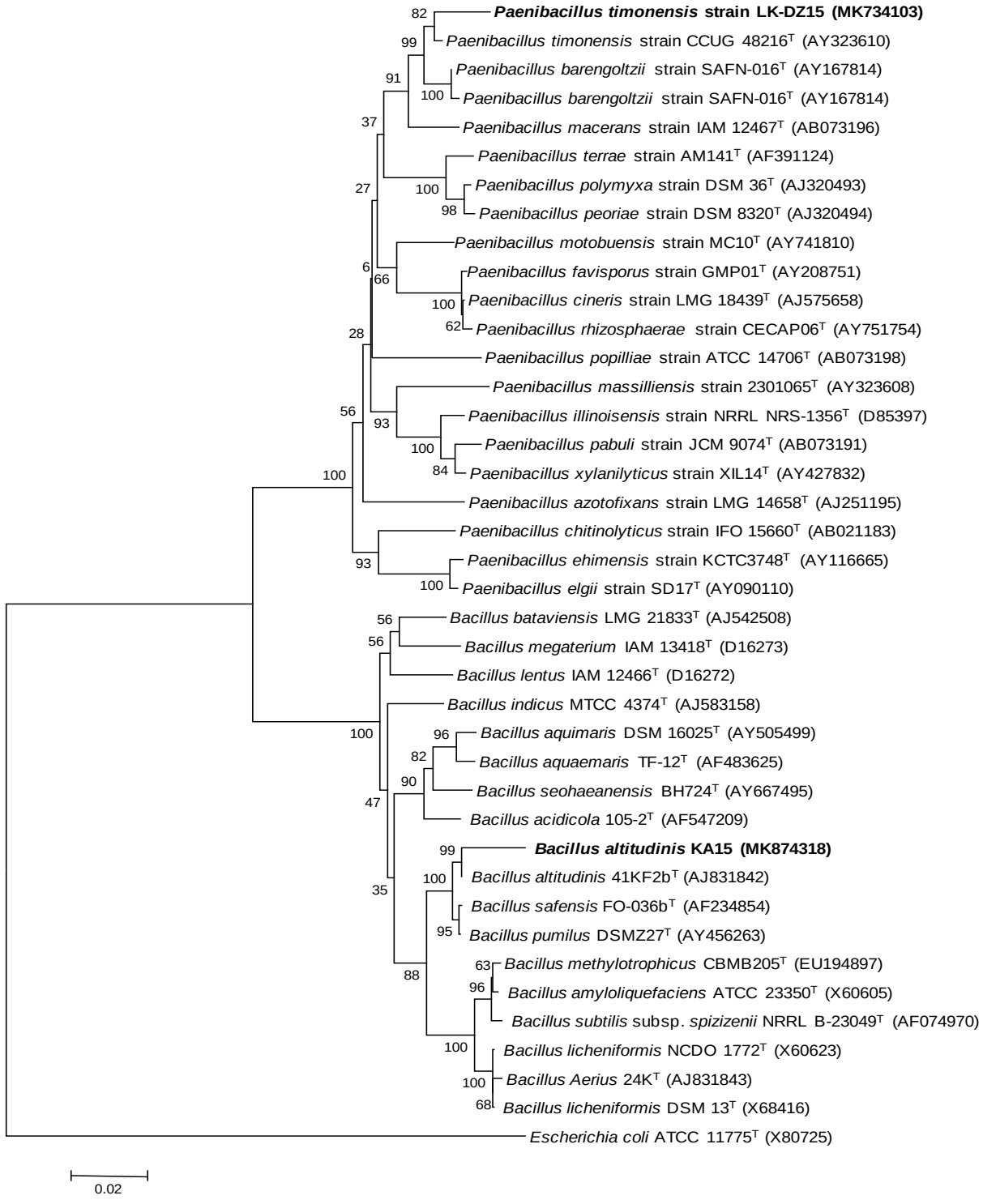

Fig. 3. Phylogenetic tree based on 16S rRNA gene sequences showing the position of strains LK-DZ15 and KA15 (GenBank accessions nos: **MK734103**and **MK874318**, respectively) within the genus *Paenibacillus* and *Bacillus*. The sequence of *E. coli* ATCC 11775^T (GenBank accession no.: X80725) was used as an outgroup.

Table 2. Biochemical characteristics of 2 isolates: KA15 and LK-DZ15 API Gallery 50 CHB.

Carbohydrate	Symbol	KA15	LK-DZ15	Carbohydrate	Symbol	KA15	LK-DZ15
-	0	-	-	Esculin	ESC	+	+
Glycerol	GLY	+	-	Salicin	SAL	+	+
Erythritol	ERY	-	-	D-cellobiose	CEL	+	-
D-arabinose	DARA	+	+	D-maltose	MAL	+	+
L-arabinose	LARA	-	+	D-lactose	LAC	+	+
D-ribose	RIB	-	-	D-melibiose	MEL	+	+
D-xylose	DXYL	+	+	D-saccharose	SAC	-	-
L-xylose	LXYL	-	+	D-trehalose	TRE	-	+
D-adonitol	ADO	-	-	Inulin	INU	+	-
Methyl-ßDXylopyranoside	MDX	-	-	D-melezitose	MLZ	-	-
D-galactose	GAL	+	+	D-raffinose	RAF	+	+
Glucose	GLU	+	+	Starch	AMD	+	+
D-fructose	FRU	+	+	Glycogen	GLYG	+	+
D-mannose	MNE	+	+	Xylitol	XLT	-	-
L-Sorbose	SBE	+	-	Gentiobiose	GEN	+	+
L-rhamnose	RHA	+	+	D-turanose	TUR	-	-
Dulitol	DUL	-	-	D-lyxose	LYX	-	-
Inositol	INO	+	-	D-tagatose	TAG	+	+
D-mannitol	MAN	-	-	D-fucose	DFUC	-	-
D-sorbitol	SOR	+	+	L-fucose	LFUC	+	+
α-Methyl-D-Mannoside	MDM	+	+	D-arabitol	DARL	+	+
α-Methyl-D-Glucoside	MDG	+	+	L-arabitol	LARL	-	-
N-Acetyl Glucosamine	NAG	+	-	Gluconate	GNT	+	+
Amygdalin	AMY	+	+	2- keto-gluconate	2KG	+	-
Arbutin	ARB	+	+	5- keto –gluconate	5KG	+	-

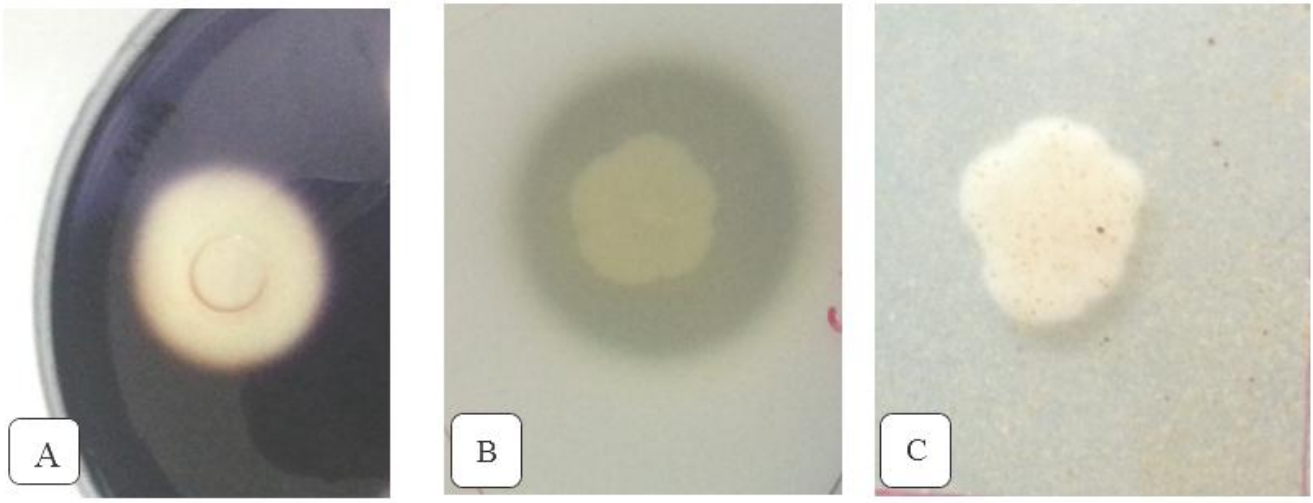

Fig. 4. Detection of some extracellular enzymatic activities. **A**. Amylase, **B**. Protease, **C.** Chitinase. The enzyme screening studies were experienced three times for each isolate.

Table 3. Physiological characteristics of isolates of *Bacillu*s isolated from the Djurdjura mountains.

Strains	Physiological characteristics																			
	Temperature (°C)						pH							Salinity (%)						
	30	40	50	60	70	80	4	5	6	7	9	11	12	1	2	3	4	5	6	7
KA1	+	+	+	+	-	-	-	+	+	+	+	-	-	+	+	+	+	+	+	-
KA2	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	+	-	-	-
KA3	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	-	-	-	-
KA4	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	-	-	-	-
KA5	+	+	+	+	-	-	-	-	+	+	+	-	-	+	+	+	-	-	-	-
KA6	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	-	-	-
KA7	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	-	-	-
KA8	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	-
KA9	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	-
KA10	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	-
KA11	+	+	+	+	-	-	-	-	+	+	+	-	-	+	+	+	+	+	+	-
KA12	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	-	-	-	-
KA13	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	-	-	-	-
KA14	+	+	+	+	+	-	-	-	+	+	+	-	-	+	+	-	-	-	-	-
KA15	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	-
LK-DZ1	+	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	-	-	-	-
LK-DZ2	+	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	-	-	-	-
LK-DZ3	+	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	-	-	-	-
LK-DZ4	+	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	-	-	-	-
LK-DZ5	+	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	-	-	-	-
LK-DZ6	+	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	-	-	-	-
LK-DZ7	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	-	-	-	-
LK-DZ8	+	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	-	-	-	-
LK-DZ9	+	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	-	-	-	-
LK-DZ10	+	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	-	-	-	-
LK-DZ11	+	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	-	-	-	-
LK-DZ12	+	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+	+	-	-
LK-DZ13	+	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	-	-	-	-
LK-DZ14	+	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+	-	-	-
LK-DZ15	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	-

Table 4. Enzymatic activities of isolates at 37 °C.

Strains	Hydrolytic activities		
	Amylase	Protease	Chitinase
KA1			
KA2			+
KA3			
KA4			+
KA5		+	+
KA6	+		
KA7			
KA8			
KA9		+	+
KA10			+
KA11		+	
KA12			+
KA13			
KA14			
KA15	+	+	+
LK-DZ1			
LK-DZ2			
LK-DZ3		+	+
LK-DZ4			
LK-DZ5	+		
LK-DZ6			
LK-DZ7			+
LK-DZ8			+
LK-DZ9			
LK-DZ10			
LK-DZ11			+
LK-DZ12	+		+
LK-DZ13	+		
LK-DZ14			+
LK-DZ15	+	+	+

4. Conclusions

In this study, thirty strains were isolated and analysed in relation to their ability to produce extracellular enzymes. Large numbers of recovered isolates have wide diversity for production of potential industrial enzymes (amylase, chitinase, and caseinase). The isolated bacteria were quite diverse in their environmental attributes, encompassing a temperature range from 30 to 80°C, pH ranges from 4 to 12, and salinity from 0 to 6 %. Because of these important properties, wide pH and temperature ranges, enzymes produced by these strains can be preferred in industrial applications. Efforts are on to purify and characterize these enzymes.

To our best knowledge, this is the first study available on characterization of enzymes produced by bacteria, isolated from the Djurdjura Mountains in Algeria.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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