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Identification and characterization of *Hydrogenophilus hirschii* strain KB-DZ44 isolated from Hammam Righa hot spring in Algeria

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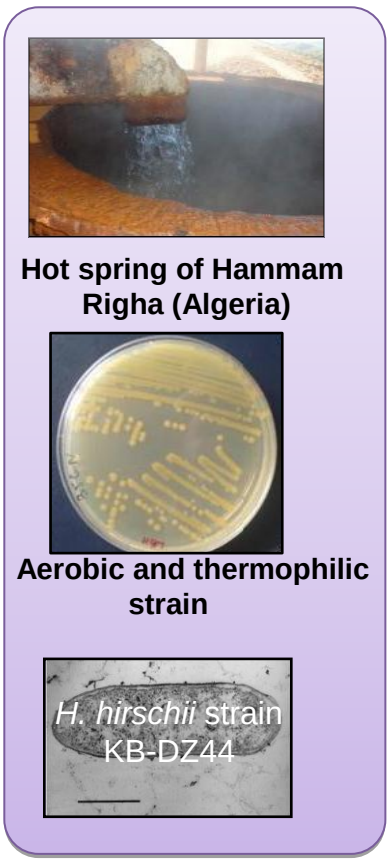
Graphical Abstract



Abstract

The search for thermo- and extremophilic microorganisms as talented sources for highly stable enzymes has gained growing attention in recent research. The literature indicates that several microorganisms that have the ability to live under extreme conditions, such as thermophilic hot springs, volcanic and geothermal regions, are endowed with unique features of considerable interest to various industrial applications and processes. In this context, a thermophilic bacteria *Hydrogenophilus hirschii* strain KB-DZ44 was isolated from Hammam Righa hot spring in Ain Defla (Algeria). Identification of the isolate was done according to morphological, physiological, and biochemical characteristics and *16S rDNA* sequence similarity as well. The temperature range for growth was 40–80 °C (opt. temp. 60 °C), pH range was 6–12 (opt. pH 6.5–7.5), and NaCl range of 0 to 4 g/L (opt.1- 1.5 g/L). *16S rDNA* sequence and blast analyses confirmed that the isolate belonging to the genus *Hydrogenophilus.* The sequence was submitted to GenBank with accession number (KY646164).

1. Introduction

Microorganisms, growing optimally above 50 °C, are naturally found in many geothermally heated parts of Earth (DeCastro et al., 2016). Hot springs are one of natural environments for thermophilic microorganisms. In the last decades, thermal environments and thermophiles have gained interest due to their scientific and biotechnological importance (Bouacem et al., 2014; Bouacem et al., 2018). The exploration of new microbes in hot springs continues to gain momentum owing to the intricate biogeochemistry and extreme conditions (Deep et al., 2013). Microorganisms that survive in these biotopes possess unique adaptations to high temperature and represent a significant bio-resource. Some investigations have revealed the presence of new species of bacteria from different hot springs.

Thermophilic bacteria have gained a significant attention to industrial scale as they possess enzymes that are active and stable at high temperatures. Thanks to their biochemical properties, they are relevant for specific industrial applications that determine the demand for tailor-made enzymes and shift the industrial interest towards biocatalysts from extremophiles including thermozymes. Based on promising performances and their broad applicability, optimally improved thermozymes represent the biocatalysts of choice for future green industries (Mechri et al., 2019). Hot springs are natural habitats of peculiar interest for searching such thermopiles (Bouacem, 2016; Bouacem et al., 2015).

Members of the genus *Hydrogenophilus* are straight rods, Gram-negative, non sporulating, and use the Calvin cycle to fix carbon dioxide. The genus comprises two moderately thermophilic species isolated from geothermal areas, *Hydrogenophilus thermoluteolus* (type strain NBRC 14978^T), isolated from a geothermal site in Japan, and *Hydrogenophilus hirschii* (type strain DSM 11420^T), isolated from Yellowstone National Park, USA (Bouacem et al., 2018). The current research was undertaken to isolate and identified a new strain named KB-DZ44 from the genus *Hydrogenophilus*.

2. Materials and Methods

2.1. Substrates

All of the other chemicals and reagents used were of analytical grade or the best grade commercially available, unless otherwise stated.

2.2. Isolation and cultivation of chitinase-producing microorganisms

Water samples were collected from Hammam Righa hot spring (GPS coordinates: 2°24’ East, 36°22’ 60’’ North) in Algeria using 1 L sterile thermal glass bottles. Samples were stored in the laboratory at room temperature. Enrichment cultures and isolation were performed in initial medium containing (in g/l): glucose, 3.6; NH_4Cl, 1; K_2HPO_4, 0.3; KH_2PO_4, 0.3; NaCl, 1; KCl 0.1; $CaCl_2{\cdot}2H_2O$, 0.1; $MgCl_2{\cdot}6H_2O$, 0.25; yeast extract, 1; and Biotrypcase, 2. pH was adjusted to 7.0. Enrichment cultures were sub-cultured several times under the same conditions. Submerged cultures were carried out in 250 mL shake flasks with 50 mL of medium. The flasks were inoculated and incubated in an orbital shaker at 60 °C and 150 rpm for 48 h. From each sample, 100 µL aliquot was plated by spreading on initial medium plates (at least five replicates) and incubated for 12, 24, and 48 h at 60 °C. Different colonies were selected and restreaked several times to obtain pure cultures which were stored in nutrient agar until used.

2.3. Identification of microorganism, DNA sequencing, and phylogenetic analysis

Analytical profiling index (API) strip tests and 16S rRNA gene sequence analysis (ribotyping) were carried out to identify the genus to which the KB-DZ44 strain belonged. API 20E and API 50 CHB strips (bioMérieux, SA, Marcy-l'Etoile, France) were used to investigate the biochemical characteristics of the strain in accordance with the manufacturer's instructions.

The morphological, cultural, physiological, and biochemical characteristics of the bacterium were investigated. The colony morphologies were determined using cultures grown aerobically on nutrient agar (NA). Cell morphology and motility were examined microscopically in exponentially growing liquid cultures after 18–24 h of incubation at 60 °C. The thermophilic isolate 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 20E and API 50 CHB as recommended by the manufacturer. The temperature range for growth was determined by incubating the isolate at 30, 40, 50, 60, 70, and 80 °C. The effect of NaCl on bacterial growth was studied in presence of 1 to 7% (w/v) NaCl. The pH dependence of growth was tested in the pH range of 4.0–12.0. All the physiological tests were determined in NA medium only exception of the pH dependence of growth at pH 4.0 and the temperature growth at 80 °C which were performed in nutriment broth.

The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using forward primer, F-S73, 5'-AGAGTTTGATCCTGGCTCAG-3', and reverse primer, R-S74, 5'-AAGGAGGTGATCCAAGCC-3'. The genomic DNA of strain KB-DZ44 was purified, amplified, and cloned as described by authors (Bouacem et al., 2018). DNA electrophoresis, DNA purification, restriction, ligation, and transformation were all performed according to the method previously described by Sambrook et al. (Sambrook et al., 1989).

3. Results and discussion

3.1. Taxonomy identification and molecular phylogeny of the microorganism

The KB-DZ44 isolate was subjected to various biochemical, microbiological, and physiological tests. The pigmentation of the colony was yellow. The isolate was identified as a Gram-negative, motile, and aerobic rod-shaped bacterium. As shown in Table 1, strain KB-DZ44 was catalase, oxidase, and nitrate reduction positive, and asporulated and showed negative results regarding Arginine Dihydrolases (ADH), Lysine Decarboxylase (LDC), Ornithine Decarboxylases (ODC), H_2S production, and urease. API 20E profile indicated that the KB-DZ44 isolate could utilize gelatin (GEL) and D-glucose (GLU). In addition, API 50 CH tests revealed that it could utilize salicine, cellobiose, D-saccharose, D-trehalose, gentiobiose, starch, D-tagatose, D-turanose, D-fucose, D-arabitol, D-mannose, L-ramnose, L-Methyl- D-mannoside, *N*-Acetyl glucosamine and amygdaline but not the others carbohydrates (data not shown). As shown in Table 1, this strain grew up to 80 ° C. The pH range for growth of this isolate is between 6.0 and 12.0 thus suggesting its alkali-tolerance. Salt tolerance of the isolates was tested by their ability to grow in NA medium containing 1, 2, 3, 4, 5, 6, and 7% NaCl (pH 7.0 and 60 °C). The KB-DZ44 isolate was able to grow in the presence of 1 and 2% NaCl but not at 7%.

Table 1

Phenotypic, physiologic, and biochemical characterization features of the KB-DZ44 isolate.

Characterization features of the strain KB-DZ44		
Phenotypic characteristics	Colony morphology	Circular
	Colony density	Translucent
	Pigmentation	Yellow
	Cell shape	Rod
	Cell arrangement	Single/Paired
	Motile	
	Gram	
Physiological characteristics	Temperature range (°C)	40–80
	pH range	6.0–12.0
	NaCl range (%)	1.0–4.0
Biochemical characteristics	Catalase	+
	Oxidase	+
	Nitrate reduction	+
	Sporulation	-
	ß-galactosidase	-
	Arginine Dihydrolase	-
	Lysine Decarboxylase	-
	Ornithine Decarboxylase	-
	Citrate	-
	H_2S	-
	Urease	-
	Tryptophane Desaminase	-
	Indol	-
	Voges-Proskauer	+
	Gelatin	+
	Glucose	+
	Mannitol	-
	Inositol	-
	Sorbitol	-
	Rhamnose	-
	Saccharose	-
	Melibiose	-
	Amygdaline	-
	Arabinose	-

In order to establish further support for the identification of the KB-DZ44 isolate, a ~ 1.5 kb fragment of the 16S rRNA gene was amplified from the genomic DNA of the isolate, cloned in the pGEM-T Easy vector, and sequenced (1515 bp) on both strands. The 16S rRNA gene sequence obtained was subjected to GenBank BLAST search analyses, which yielded a strong homology with those of several cultivated strains of *Hydrogenophilus*, reaching a maximal of 99% sequence identity. The nearest *Hydrogenophilus* strains identified by the BLAST analysis were the *Hydrogenophilus hirschii* strain DSM 11420^T (accession n°: FR749905). This sequence was imported into MEGA software package version 4.1 and aligned. Phylogenetic trees were then constructed (Fig. 1) and the findings further confirmed that the KB-DZ44 strain (accession n°: **KY646164**) was closely related to those of the *Hydrogenophilus* strains. In a nutshell, all the results obtained strongly suggested that this isolate should be assigned as *Hydrogenophilus hirschii* strain KB-DZ44.

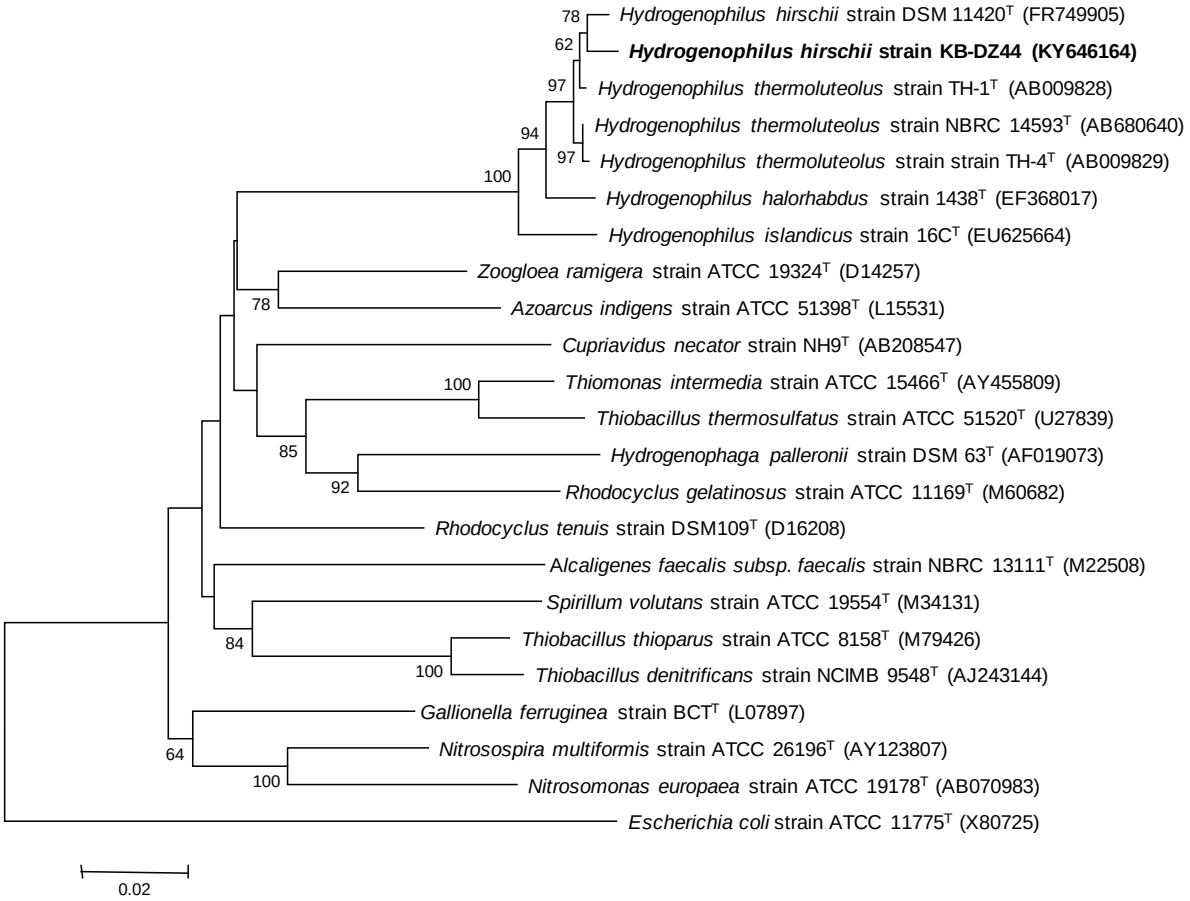


Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the position of strain KB-DZ44 within the radiation of the other bacterial genus.

4. Conclusion

Hot springs environment with elevated temperature than surrounding area is inhabited by a vast variety of microbial communities. From this study, a new strain with unique properties, *Hydrogenophilus hirschii* strain KB-DZ44, was isolated from Hammam Righa hot spring in Ain Defla (Algeria) and characterized.

5. References

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