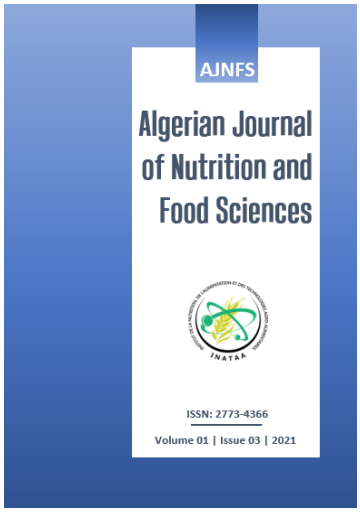

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Original Article

Isolation of thermophilic bacteria producing exopolysaccharides from the geothermal spring of Hammam Grouz (Mila, Algeria)

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Abstract *The synthesis of exopolysaccharides (EPS) has been reported in thermophilic microorganisms. These organisms are not pathogenic and their exopolysaccharides offer properties of resistance to the presence of salts, high temperatures and extreme pH. Thermophiles also allow short fermentation processes, a decrease in the viscosity of the fermentation broth in comparison with milder temperature conditions, and their EPS show a high molecular weight which generates very stable emulsions. Moreover, the optimization of the physico-chemical conditions for the production of EPS and for the growth of these microorganisms on inexpensive culture media can improve the economic profitability of their exploitation. The objective of this work is to isolate and characterize thermophilic bacteria from the hot spring of Hammam Grouz (province of Mila, Algeria) then to prospect their production of EPS on different solid and liquid media. Nine strains were purified and characterized using a phenotypic approach, and by studying the effect of culture medium conditions and composition on their growth and their EPS production. Thus, the strains present Gram-positive or Gram-negative rod cells and are able to form endospores. They are mildly thermophilic, strictly aerobic, and can grow at up to 5% (w/v) salinity and over a wide pH range. All strains are able to ferment different sugars and use them as their sole source of carbon and energy. The EPS production of eight strains was confirmed by India ink staining and the assay of EPS production of five strains was estimated by quantification of total sugars on two different liquid media after 72 hours of incubation at 60 °C. The results revealed a better production on galactose medium. This work should be continued in order to characterize the produced EPS and to estimate their potential.*

Keywords *Thermophilic bacteria, Hammam Grouz, Exopolysaccharides, Characterization, Prospecting*

Résumé *La synthèse d'exopolysaccharides (EPS) a été rapportée chez les microorganismes thermophiles. Ces organismes ne sont pas pathogènes et leurs exopolysaccharides offrent des propriétés de résistance à la présence de sels, aux températures élevées et aux pH extrêmes. Les thermophiles permettent également des processus de fermentation courts, une diminution de la viscosité du bouillon de fermentation par rapport à des conditions de température plus douces, et leurs EPS présentent un poids moléculaire élevé qui génère des émulsions très stables. De plus, l'optimisation des conditions physico-chimiques pour la production d'EPS et pour la croissance de ces microorganismes sur des milieux de culture peu coûteux peut améliorer la rentabilité économique de leur exploitation. L'objectif de ce travail est d'isoler et de caractériser des bactéries thermophiles de la source chaude de Hammam Grouz (province de Mila, Algérie) puis de prospecter leur production d'EPS sur différents milieux solides et liquides. Neuf souches ont été purifiées et caractérisées par une approche phénotypique, et en étudiant l'effet des conditions et de la composition du milieu de culture sur leur croissance et leur production d'EPS. Ainsi, les souches présentent des cellules en bâtonnets Gram positif ou négatif et sont capables de former des endospores. Elles sont légèrement thermophiles, strictement aérobies, et peuvent se développer à une salinité allant jusqu'à 5% (p/v) et sur gamme de pH acide à neutre. Toutes les souches sont capables de fermenter différents sucres et de les utiliser comme unique source de carbone et d'énergie. La production d'EPS de huit souches a été confirmée par coloration à l'encre de Chine et le dosage de la production d'EPS de cinq souches a été estimé par quantification des sucres totaux sur deux milieux liquides différents après 72 heures d'incubation à 60 °C. Les résultats ont révélé une meilleure production sur milieu galactosé. Ce travail doit être poursuivi afin de caractériser les EPS produits puis d'estimer leur potentiel.*

Mots clés *Bactéries thermophiles, Hammam Grouz, Exopolysaccharides, Caractérisation, Prospection*

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Introduction

Microbial polysaccharides have proven to be useful industrial ingredients that compete with plant and algal polysaccharides, although plant polysaccharides still dominate the carbohydrate market. Since the discovery of the first microbial polysaccharide, dextran, in 1880, the continuous search for new exopolysaccharides has led to the description of many new polymers, some of which are commercially accepted and others which are at different stages of development (Radchenkova *et al.*, 2011; Özcan and Öner, 2015).

Indeed, there is always a need for better bioactive principles in the food industry, including more efficient, cheaper and more cost-effective, environmentally friendly food additives that do not pose health risks to the consumer or alternatives that respect the religious beliefs and restrictions of certain populations (e.g. Halal or Kosher substances). EPS can therefore be used as food additives to improve texture. Exopolysaccharides (EPS) are water-soluble polymers synthesized by many microorganisms and can be easily obtained by short fermentation processes. They possess different chemical structures and therefore offer a number of new properties and applications (Nicolaus *et al.*, 2010). Microbial EPS have been produced by several groups as algae, pathogenic bacteria and lactic bacteria. However, the potential of EPS from other microbial groups such as extremophiles has yet to be thoroughly studied (Kambourova *et al.*, 2016).

Thermophiles are organisms that require optimal growth temperatures above 50 °C. They are found in many extreme terrestrial and marine environments. In particular in terrestrial hot springs or in deep hydrothermal vents of seas and oceans. They can survive under extreme conditions of temperature, pH and pressure by producing biomolecules (enzymes, compatible solutes, biopolymers, etc.) adapted to these conditions (Eswari *et al.*, 2019; Rakesh *et al.*, 2021). Algeria inhabits a great ecologically and geologically diversity of environments. Some of these are extreme ecosystems such as sabkhas, desert soils and, above all, hot springs, which are exploited for their benefits, particularly therapeutic (Bouanane-Darenfed *et al.*, 2011; Bouacem *et al.*, 2016). The interest in the isolation and characterization of bacterial strains indigenous to these environments and their biomolecules is increasing in the perspective of their industrial, biotechnological and ecological applications (Benammar *et al.*, 2020).

In this context, the synthesis of extracellular polysaccharides has been reported for some thermophilic microorganisms as one of their survival mechanisms at high temperature (Radchenkova *et al.*, 2013). The particularity of these microorganisms is that they are not pathogenic unlike some of the mesophilic microorganisms producers, and their EPS offer properties of resistance to the presence of salts, high temperatures and extreme pH values. Thermophilic producers also allow shorter fermentation processes, reduced fermentation broth viscosity, high molecular weight polymers and more stable emulsions. In addition, the optimization of physico-chemical conditions for the production of EPS and for the growth of these microorganisms on inexpensive culture media can further improve the economic profitability of their exploitation (Kambourova *et al.*, 2016).

In this work, we aim to isolate and select a set of thermophilic bacteria producing EPS from the hot spring of *Hammam* Grouz (wilaya of Mila, Algeria), to characterize phenotypically these isolates and to estimate the production of EPS by the most interesting strains on liquid media.

Materials and methods

Studied site

The choice was made on the thermal spring of *Hammam* Grouz (36° 15' 4.5" N, 6° 17' 6.036" E, altitude 717 m), located in the province (*wilaya*) of Mila (fig. 1). Water of the spring is used for therapeutic purposes. Its chemical composition is of calcic sulfate chemical facies (Bahri *et al.*, 2011).

Sampling

Sampling was carried out on February 2021. Water and sediment samples were collected using a disinfected dipper. The recovered samples were transferred into sterile bottles of 50 mL. Two liters of spring water were also collected from the same point and filtered in the laboratory. This water was used for physicochemical measurements and for the preparation of isolation media. The samples were transported in a cooled isothermal bag and stored at 4 °C.

Measurement of physico-chemical characteristics of samples

In situ measurements of water and ambient air temperature were performed using a total immersion rod mercury thermometer (-10 °C to 150 °C). At laboratory, both water and sediment samples were tested for pH values using a CRISON model GLP 21 pH meter. Total Dissolved Solids (TDS) and conductivity were measured by Jenway 430 pH/conductivity system. All measurements were done in duplicates.

Isolation

To induce the growth of aerobic thermophilic bacteria producers of EPS, two methods were adopted in order to vary the initial concentrations of microbial cells. For water samples, we performed a surface spreading of 0.5 mL of decimal dilutions (10^{-1}, 10^{-2}, 10^{-3}) on two different culture media (C1: 17.5 g/L dehydrated Plate Count Agar medium (PCA); 10 g/L glucose; 20 g/L agar, and C2: 30 g/L agar, 10 g/L glucose, 4 g/L peptone, 4 g/L yeast extract). In the case of sediments,

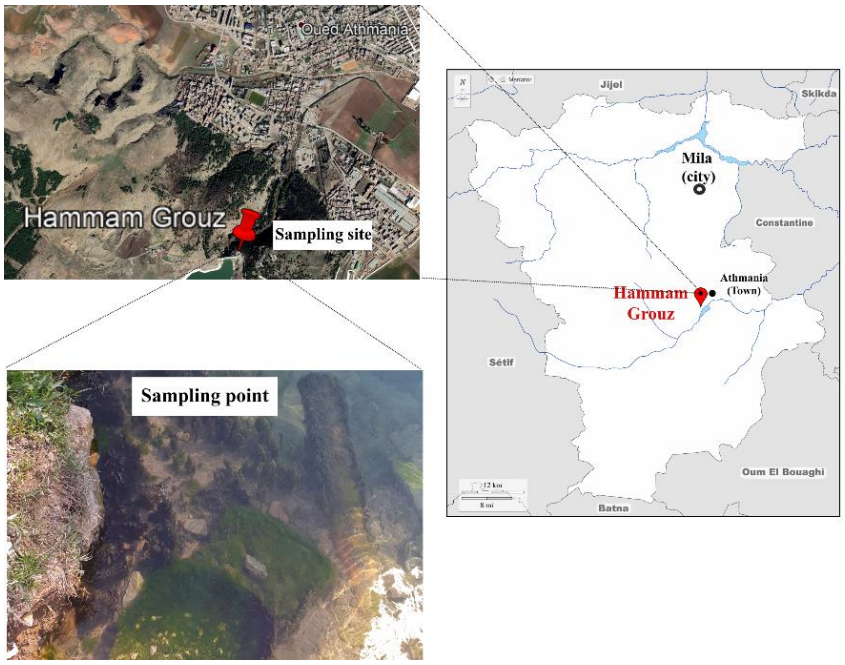

Figure 1. Localization of the sampling point.

we inoculated 9 mL of enrichment broth (C3: 5 g/L glucose, 4 g/L peptone, 4 g/L yeast extract) with 1 g of sediments followed by incubation for 24 h at 60 °C. Then, 0.5 mL of the enrichment broth was spread on the surface of C1 and C2 media. All Petri dishes were incubated at 60 °C for 18 to 48 hours (Atlas, 2005; Gomri and Kharroub, 2019).

Purification

The first isolates were purified by selecting 2 to 3 colonies from each Petri dish based on their mucoid aspect and were then subcultured at least 2 times on the C4 conservation medium (17.5 g/L dehydrated PCA, 20 g/L agar).

Phenotypic characterization of isolates

Morphological characterization of isolates

- Macroscopic characterization: The macroscopic characters of the colonies (size, shape, opacity, consistency, pigmentation) were visually appreciated. Strains were cultured on C4 medium and observed for different morphological aspects after 18 to 48 h incubation at 60 °C.
- Microscopic characterization: Microscopic characters of the isolates were determined by Gram and endospore staining following the protocols of Gram (1884) and Schaeffer-Fulton (1930), respectively (Harley, 2002). Observations were made using a motic type light microscope using an 100x objective under immersion.

Screening of EPS production

The isolates were screened for extracellular polysaccharides production on a basic medium containing 30 g/L agar, 2 g/L peptone, 2 g/L yeast extract, 2 g/L NaCl, 1 g/L $CaCl_2.2H_2O$, 1 g/L $MgSO_4$ $7H_2O$ supplemented by 20 g/L of one of eight different sugars: glucose, galactose, fructose, lactose, maltose, sucrose and dextrin. The isolates were incubated at 60°C for 24 h to 48 h. The search for the viscous/mucoid phenotype is done by touching the colony with the platinum loop, long viscous filaments of more than 5 mm are formed in the case of polymer production. All experiments were done in duplicate. EPS production was confirmed for strains that showed a positive result on solid medium using India ink staining for visualization of capsular polymers around bacterial cells under light microscope (Prescott *et al.*, 2002).

Physiological characterization

The influence of temperature, pH and NaCl concentration on growth were determined on solid medium by varying one of the parameters while the other two ones were held constant. Indeed, all the isolates were incubated at different temperatures (10-80 °C), different pH levels (4.0-9.0) and variable NaCl concentrations (up to 7.5%, w/v). Growth was periodically supervised between 24 and 72 hours of incubation. For all tests, the medium was adjusted with a phosphate buffer, shaken and autoclaved for 15 minutes at 105°C before use.

The use of sugars as a unique source of carbon and energy was further tested on a solid culture medium containing NH_4Cl (0.5 g/L), Na_2HPO_4 (0.005 g/L), agar (30 g/L) and the test sugar (10 g/L): D (+)-glucose, D (+)-galactose, D (+)-fructose, D (+)-lactose, D (+)-dextrin, D (+)-sucrose, D (+)- maltose and D (+)-mannose.

Determination of respiratory type and use of citrate were also performed according to Harley (2002) protocols.

Biochemical characterization

The isolated strains were subjected to different biochemical tests.

Respiratory enzyme tests (Cytochrome oxidase and Catalase) and search for tryptophanase were performed according to Joffin and Leyral (2006a). Methyl red reaction (MR), Voges Proskauer reaction (VP), the use of sugars on Triple Sugar Iron (TSI) medium and fermentation of sugars on liquid medium were performed according to Joffin and Leyral (2006b). Finally, nitrate reduction test was conducted following the protocol mentioned in Bergey's Manuel of Systematics (Logan *et al.*, 2009).

Estimation of the production of EPS by the determination of total sugars

The strains that showed a positive result for the screening step are selected for the production of their EPS on liquid medium containing the most suitable sugars (Wang *et al.*, 2021). The assay of EPS production is then determined by assaying total sugars according to the phenol-sulfuric acid method (DuBois *et al.*, 1956).

The preculture was prepared by inoculating 3 mL of enrichment broth (10 g/L glucose, 4 g/L peptone, 4 g/L yeast extract, 2 g/L NaCl, pH 7.0 ± 0.2) with the selected strains and then incubated at 60 °C for 24 hours. After that, EPS production was run in 20 mL of production medium containing 20 g/L sugar of interest; 4 g/L peptone, 2 g/L yeast extract, 2 g/L NaCl, 2 g/L Na_2HPO_4, 0.1 g/L $CaCl_2.2H_2O$, and 0.1 g/L $MgSO_4.7H_2O$ (pH 7.0 ± 0.2) and incubated for 72 hours at 60 °C with continuous shaking. The production medium was after that centrifuged at 4000 g for 15 minutes. The supernatant was collected and considered as the EPS extract. To quantify the EPS, the supernatant was mixed with an equal volume of iced absolute ethanol and added dropwise under stirring in an ice bath. The resulting alcohol solution was then kept at -20 °C overnight and centrifuged at 4000 rpm for 40 minutes. The precipitate was dried at 60 °C for 1 hour, then recovered by dissolving it in 5 mL of sterile distilled water. The total sugar content of the resulted solution was determined by the phenol-sulfuric acid method. All the tests were performed in triplicates.

Results and discussion

Sampling

Two water samples and two sediment samples were taken from the geothermal spring of Hammam Grouz. At the time of sampling, the spring water was clear and transparent, with no particular odor and no apparent signs of pollution. In addition, the presence of some vegetation and macro-algae around the site was observed.

Measurement of the physico-chemical characteristics of the samples

The results of physicochemical analyses of water and sediment are shown in Table 1. The temperature of the water at the time of sampling was 44°C. The values recorded for the spring water does not contradict those described by Bahri et al. (2011) and Saibi (2009). The pH values of the samples in this study are neutral for the water and alkaline for the sediments. The electrical conductivity and TDS of the water have relatively high values. These results are very similar to those described for the majority of thermal springs in North-East Algeria (Ouali and Hadjiat, 2018).

Table 1. Average values of physico-chemical parameters of water and sediments of the studied site

	T (°C)	pH	TDS (mg/L)	Cond (dS/m)
Water	44 ± 0	7.16 ± 0.02	1056.0 ± 12.8	1.65 ± 0.01
Sediments	-	8.71 ± 0.02	-	-

T: temperature of the water at emergence or temperature of the surrounding air at the time of sampling; TDS: Total Dissolved Solids; Cond: conductivity; -: not determined

Isolation and purification of isolates

The first colonies appeared after 24 to 48 hours of incubation. However, there was little diversity in colony morphology. In total, nine isolates were selected based on the mucoid aspect of their colonies, mostly from water samples on C1 medium.

Morphological characters of the isolates

The macroscopic and microscopic characters of the selection strains were determined on C4 solid medium and are presented in Table 2.

Macroscopic characters

Macroscopic observation of colonies on C4 medium revealed two different colony patterns: Smooth type colonies: this cultural type is the most common (8 strains), characterized by umbilical or flat colonies; with cream consistency; opaque, translucent or transparent creamy pigmentation. Their size varies between 0.3 mm and 18 mm; and Mucous type colonies: only one strain had this type of culture (AT5). It had an umbilicate shape, mucous; transparent colonies varying in size between 1 and 11 mm.

Microscopic characters

Strains were Gram positive or negative, rod-shaped, grouped in chains for 8 of the 9 isolates. Endospores were observed in all cases, in subterminal or in terminal position.

Table 2. Macroscopic and microscopic characters of the studied strains.

Strain	Macroscopic characters					Microscopic characters			
	Size (mm)	Aspect	Opacity	Consistency	Pigmentation	Gram	Cell shape	Cell arrangement	Endospore
AT1	1-13	Smooth	Transparent	Creamy	Cream	-	Rod	Isolated	Terminal
AT2	1-15	Smooth	Translucent	Creamy	Cream	-	Rod	In chain	Subterminal
AT3	1-18	Smooth	Translucent	Mucus	Cream	+	Rod	In chain	Terminal
AT4	0,3-5	Smooth	Translucent	Creamy	Cream	-	Rod	In chain	Subterminal
AT5	1-11	Mucous	Translucent	Creamy	Transparent	+	Rod	In chain	Subterminal
AT6	3-10	Smooth	Translucent	Creamy	Cream	+	Rod	In chain	Terminal
AT7	1-5	Smooth	Transparent	Creamy	Cream	+	Rod	In chain	Terminal
AT8	4-15	Smooth	Translucent	Creamy	Cream	+	Rod	In chain	Terminal
AT9	1-15	Smooth	Translucent	Creamy	Cream	-	Rod	In chain	Subterminal

+: Gram positive ; -: Gram negative

Physiological characters

Tolerance to temperature, pH and salinity

Results of the physiological limiting conditions study are presented in Table 3. Growth was observed in the majority of strains between 50 °C and 60 °C, and less frequently at 70 °C and at 30 °C. Therefore, it is likely that they are moderate thermophiles (Itoh and Iino, 2013).

Almost all the strains can grow at a pH range between 5 and 7. The strains in the study can also grow in the absence of NaCl and are able to grow at 5% (w/v) NaCl. This might indicate that the strains of the present study fall within the range of halotolerant neutrophilic bacteria.

Use of sugars as unique source of carbon and respiratory type

The growth results on different sugars as the sole source of carbon and energy are presented in Table 4. All the tested sugars were assimilated while citrate was not used by the isolates. All isolates were strict aerobes.

Biochemical characters

Results of the biochemical characters of the isolates are listed in Table 5. All the bacterial strains were able to produce catalase and/or oxidase.

Most aerobic microorganisms possess these enzymes including thermophilic bacteria (Counts et al., 2017). This confirms bacteria in this study are strict aerobes.

The results of the sugar fermentation test indicated that the strains reacted positively on lactose and glucose media except strains AT5 and AT6, on Galactose (exception to strains AT1, AT3, AT4, AT9) and on sucrose (exception to strains AT2, AT3, AT5, AT8). They tested positively in the majority of cases to the MR test and negatively to VP test. These results suggest that the strains present a facultatively anaerobic metabolic type characterized by the production of organic acids.

All strains do not possess the tryptophanase enzyme. Some strains produce nitrate reductase at the NO_2^- stage, others at the nitrogen stage. This could be interpreted as the presence of anaerobic respiration metabolism pathways.

Screening of EPS production

All nine isolates were screened for EPS production. The EPS producing character for most strains was expressed only on media containing galactose, sucrose or dextrin (Table 6). For example, strain AT9 presented on galactose medium a viscous aspect of the colonies when in contact with a pipette (fig. 2). In contrast, the strains did not show the characteristic appearance of EPS-producing colonies on media containing glucose, fructose, lactose and maltose.

Table 3. Physiological characters of the isolates (part I)

Strain	T (°C)							pH					NaCl % (w/v)					
	10	30	37	50	60	70	80	5	6	7	8	9	0	1	2	3	5	7.5
AT1	-	-	-	+	+	-	-	+	+	+	-	-	+	+	+	+	+	-
AT2	-	-	-	+	+	-	-	+	+	+	-	-	+	+	+	+	+	-
AT3	-	+	+	+	+	-	-	+	+	+	-	-	+	+	+	+	+	-
AT4	-	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
AT5	-	-	-	+	+	+	-	+	+	+	-	-	+	+	+	+	+	-
AT6	-	-	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	-
AT7	-	-	-	+	+	-	-	+	+	+	-	-	+	+	+	+	+	-
AT8	-	-	-	+	+	-	-	+	+	+	-	-	+	+	+	+	+	-
AT9	-	-	-	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-

+: presence of bacterial growth; -: absence of bacterial growth

Table 4. Physiological characters of the isolates (part II)

Strain	Use of substrate as the sole source of carbon									Respiratory type
	Glu	Gal	Fruc	Lac	Dex	Suc	Mal	Man	Cit	
AT1	+	+	+	+	+	+	+	+	-	Strictly aerobic
AT2	+	+	+	+	+	+	+	+	-	Strictly aerobic
AT3	+	+	+	+	+	+	+	+	-	Strictly aerobic
AT4	+	+	+	+	+	+	+	+	-	Strictly aerobic
AT5	+	+	+	+	+	+	+	+	-	Strictly aerobic
AT6	+	+	+	+	+	+	+	+	-	Strictly aerobic
AT7	+	+	+	+	+	+	+	+	-	Strictly aerobic
AT8	+	+	+	+	+	+	+	+	-	Strictly aerobic
AT9	+	+	+	+	+	+	+	+	-	Strictly aerobic

+: presence of bacterial growth, -: absence of bacterial growth, Glu: glucose, Gal:galactose, Fru: fructose, Lac: lactose, Dex : dextrin, Suc : sucrose, Mal : maltose, Man : Mannose, Cit : citrate.

Table 5. Biochemical characters of the isolates

Strain	TSI medium				Nit	Oxi	Cat	Try	MR	VP	Fermentation of							
	Sac/lac	Glu	H_2S	Gaz							Glu	Fru	Gal	Suc	Lac	Mal	Man	Dex
AT1	-	-	-	-	-	-	+	-	+	+	+	+	-	+	+	+	+	+
AT2	-	-	-	-	-	+	-	-	+	-	+	+	+	-	+	+	+	+
AT3	-	-	-	-	+	-	+	-	+	-	+	+	-	-	+	+	+	+
AT4	-	-	-	-	-	-	+	-	-	-	+	+	-	+	+	+	+	+
AT5	-	-	-	-	-	+	-	-	+	-	-	+	+	-	-	+	+	+
AT6	-	-	-	-	+	+	-	-	+	-	-	+	+	+	-	+	+	+
AT7	-	-	-	-	+	+	+	-	+	-	+	+	+	+	+	+	+	+
AT8	-	-	-	-	+	+	-	-	+	-	+	+	+	-	+	+	+	+
AT9	-	-	-	-	-	+	+	-	+	-	+	+	-	+	+	+	+	+

+: positive result, -: negative result, Nit: nitrate, Oxi: oxidase, Cat: catalase, Try : tryptophanase MR : methyl red, VP : Voges Prausker, Glu: glucose, Gal: galactose, Fru: fructose, Lac: lactose, Dex : dextrin, Suc : sucrose, Mal : maltose, Man : Mannose.

Table 6. Results of the screening for EPS production on solid media

Strain	Glucose	Fructose	Galactose	Lactose	Maltose	Sucrose	Dextrin
AT1	-	-	+	-	-	-	+
AT2	-	-	+	-	-	+	-
AT3	-	-	+	-	-	+	+
AT4	-	-	+	+	-	+	+
AT5	-	-	-	-	-	+	+
AT6	-	-	-	-	-	-	-
AT7	-	-	+	-	-	+	+
AT8	-	+	-	-	-	-	+
AT9	+	+	+	-	-	+	+

Colonies of strains that tested positive on sucrose, galactose and dextrin media were observed under the microscope after staining with India ink for confirmation. Microscopic observation detected in all strains the presence of capsular substances surrounding the bacterial cells.

The latter can be associated with the production of capsular or free EPS, as the excreted form depends mainly on the surrounding conditions (Sánchez et al., 2006; Pachekrepapol et al., 2017).

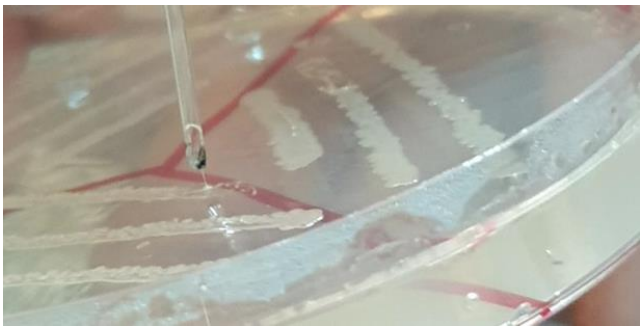

Figure 2. Viscous aspect of the AT9 strain

Production of EPS on liquid media

Strains which showed a positive result in the solid medium test, confirmed by Indian ink staining were selected for the estimation of their EPS production on sucrose and galactose liquid media by measuring total sugars. The results of the production rate of EPS after 72 hours at 60°C are shown in Figure 3. The highest production rates were obtained on galactose medium for strains AT2 (43.423 ± 5.313 µg/mL) and AT9 (43.423 ± 1.808 µg/mL). On the sucrose medium, the rate of EPS production was lower for all the five tested strains (between 30.119 ± 2.148 µg/mL for strain AT2 and 38.351 ± 3.195 µg/mL for strain AT9).

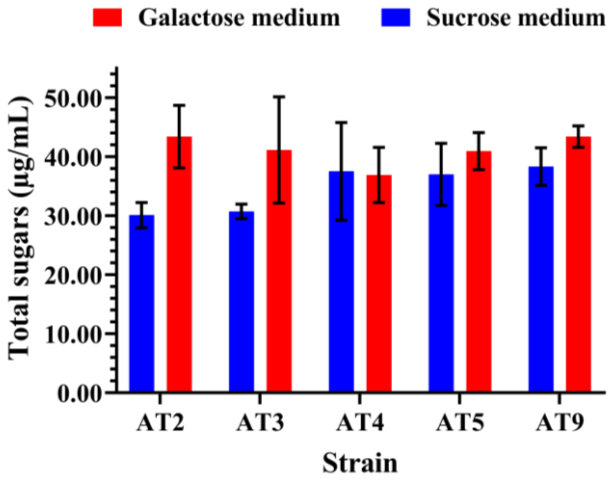

Figure 3. Total sugar rates on galactose and sucrose EPS production media

Grobben et al. (1996) reported that the type of carbon source has a great influence on the productivity of EPS and can also affect its composition. The work of Yuksekdag and Aslim (2008), also showed that the production of EPS depends on the carbon source and its concentration in the environment. Similarly, Grobben et al. (1996) reported that the type of carbon source has a great influence on the productivity of EPS and can also affect the composition of the latter.

The observed levels of EPS production by thermophilic producers are lower than those reported for mesophilic producers and usually varied between 50 µg/mL and 200 µg/mL (Manca et al., 1996; Nicolaus et al., 2003; Kambourova et al., 2009). Our results are close to these observations.

A higher amount of EPS (366 µg/mL) has been reported for the moderate thermophile *Bacillus licheniformis* (Spanò et al., 2013) for 48 h cultivation at temperature of 50 °C in a complex medium. Highest production (863 µg/mL) in comparison with other thermophiles was reported for the moderate thermophilic strain *Brevibacillus thermoruber* 423 (Yasar Yildiz et al., 2014).

Conclusion

In this work, we tried to isolate and phenotypically characterize thermophilic bacteria from the hot spring of *Hammam* Grouz, (wilaya of Mila, Algeria), and to investigate their production of EPS on different solid and liquid media.

The studied spring presented a temperature of 44°C, its waters have a neutral pH and are characterized by important deposits of minerals at the level of the sedimentary layers.

Nine bacterial strains were isolated and selected from water and sediment samples of this spring. A morphological, physiological and biochemical characterization of these strains was then performed. They presented rod cells, Gram positive or negative, and were able to form endospores in terminal or subterminal position. These strains were moderate thermophiles, strict aerobes, and could grow up to 5% (w/v) NaCl concentrations, and over an acid to neutral pH range. All strains are able to use lactose, galactose, sucrose, maltose, mannose and dextrin as the sole source of carbon and energy and can also ferment all sugars, except strain AT6 on galactose and maltose medium.

The screening of mucoid cultural aspect indicative of the production of EPS on solid media containing different sugars indicated its presence for 8 strains in galactose, sucrose and dextrin media. This result was confirmed by the detection of capsular substances for these same strains after staining with Indian ink. The assay of EPS production from selected bacterial strains was estimated by quantifying total sugars on two different liquid media after 72 hours of incubation at 60 °C. The results revealed a better production on the galactose medium for strains AT9 and AT2.

Our study is not exhaustive and should be completed by:

- a deeper phenotypical and molecular characterization of the strains;
- the optimization of the conditions of EPS production by the most promising strains, in particular AT2 and AT9, by determining the physico-chemical conditions of their growth and those of the production of their polymers;
- the purification and characterization of the EPS produced and the study of their biological and rheological properties;
- the incorporation of the most interesting EPS in appropriate food matrices and the study of their texturizing properties.

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