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THERMOPHILIC ACTINOBACTERIA ISOLATED FROM TLEGHMA HOT SPRING: A POTENTIAL SOURCE OF THERMOSTABLE α-AMYLASE

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ABSTRACT

Thermostable α-amylase is a commercial enzyme that has found several biotechnological applications in recent years. This prompts researchers to check out the hot ecosystems the least explored to find producing microorganisms. Thermal waters are a poorly studied ecological niche compared to other ecosystems. Actinobacteria are known for their unique metabolic abilities to produce the most innovative bio-molecules. In this study, the isolation by conventional microbiological methods, allowed to obtain 13 thermophilic actinobacteria from a hot spring of TLEGHMA located in the eastern Algerian. All these isolates were characterized morphologically and by physiological methods. Molecular identification by sequencing of the rRNA16s gene, allowed to assign them to the genus *Streptomyces sp*. Among these isolates, 9 actinobacteria showed abilities to produce a thermostable α-amylase active at a temperature of 55°C. Two isolates named TA3 and TA4 are however, the most successful. They were assigned to *Streptomyces albidofalvus* and *Streptomyces cavourensis* respectively. they are able to produce a thermostable α-amylase at 55°C with an activity rate of 110.33U/ml by strain TA3 and 224U/ml by strain TA4 and with an optimum of activity in a pH equal to 9. These results show that these thermophilic *Streptomyces* from these hot waters, are a very important source of thermostable and alkalophilic α-amylase

1.Introduction

Hot springs are an environment with special physical, chemical and nutritional properties. These ecosystems are home to a large biodiversity of thermostable microorganisms (Chan et al., 2017; Msarah et al., 2018). This microbial population grows optimally at temperatures between 45-80°C (Alrumman et al., 2018). They also have the ability to acclimatize to many solvents and detergents and are resistant to acid and basic pH (Mohammad et al. 2017; Adiguzel et al., 2009). These microorganisms are an important source of several biotechnologically useful compounds, including antibiotics and enzymes. (Sayeh et al., 2010). These extreme ecosystems remain relatively unexploited compared to other ecological niches. Actinobacteria are an integral part of this microbial population (Zhaoqi et al., 2009; Medjemadj et al., 2020). These thermophilic bacteria are characterized by the presence of membrane lipids that contain more saturated, straight-chain fatty acids than their mesophilic analogues. This lipid specificity, allows these bacteria to grow at high temperatures by providing the right degree

of fluidity necessary for membrane function (Aditi and Nupur 2016). Research in the field of enzymes from thermophiles is more attractive than those using mesophilic microorganisms. The main reasons for this are their high stabilities and substrate specificities, The low risk of contamination by other bacteria in the fermentation process is greatly reduced due to the high temperature which stops the growth of mesophilic contaminants (Drejer et al., 2018). Several enzymes thermostable at high temperatures (50-65°C), have been discovered in recent years in various genera of actinobacteria, these are generally species belonging to the genus *Thermoactinomyces* that have been isolated from the sediments of thermal station (Aditi and Nupur. 2016). The works on the research of actinobacteria in the samples of thermal waters remain however very rare (Medjemadj et al., 2020; Bahamdain et al., 2020; Ruwini et al., 2022).

Amylase is an extracellular enzyme composed of three types: α, β and glucoamylase, which hydrolyzes mainly starch, dextrins and some small polymers into glucose units (Dash et al., 2015). Amylases are enzymes that have aroused the interest of industries for their wide applications especially in the food industry. This group of enzymes contributes to about 25% of the global enzyme market (Arikan, 2008; Kumar et al., 2014). The demand for α -amylase has increased due to multiple industrial applications because of its crucial role in starch hydrolysis processes used in the food, paper, brewing and textile industries (Hmidet et al., 2009).

Amylases are considered as food additives in the baking industry. The addition of a small amount of amylase to the flour provides simple sugars that are well assimilated by the yeast. This increases the production of carbon dioxide after fermentation and leads to an aerated bread (Pandey et al., 2000).

The use of alpha amylase "bread improver" has become a machine manufacturing practice in the bread industry, not only to improve bread quality but also to control the manufacturing process. This industry uses α-amylases for the regulation of diastatic activities of flours by hydrolysis of starch to maltose. This promotes the formation of soft crumb in baking and improves the texture of pastry cakes and cookies (Malhotra et al., 2002).

Sometimes, cane syrups can be disturbed by the presence of starch contaminations which also increase their viscosity and then affect the crystallization process. This drawback is eliminated by introducing a small amount of immobilized bacterial amylase at a temperature equal to or higher than 80°C (Reddy et al., 2003). In addition, α- amylase is used to facilitate sucrose extraction and refining operations from sugar beet or sugar cane to remove traces of starch interfering with purification. Nevertheless, fungal and bacterial α- amylases are widely used for the preparation of sweet syrups from corn starch and chocolate syrups (Martin et al., 2003).

According to (Tanyildizi et al. 2005), microbial amylases retain greater stability compared to animal and plant amylases.

The objective of this study is to isolate actinobacteria from the hot waters of a thermal spring located in eastern Algeria. It is also a question of searching for and identifying those thermophilic bacteria capable of producing the enzyme α-amylase. Finally, we plan to study some properties and the optimal conditions for the activity of this enzyme produced by the most efficient bacteria.

2. Materials and methods

2.1. Sampling site

Figure 1. Hammam Tleghma sampling site (Mila, Algeria) (© d-maps.com)

The thermal spring of Hammam Tleghma is located in the southeast of the wilaya of Mila

(Latitude: 36.1153, Longitude: 6.36417 36° 6′ 55″ North, 6° 21′ 51″ East) (Figure 1). The physicochemical characteristics of the thermal water of this station are grouped in Table 1.

Table 1.Physicochemical characteristics of the water of the thermal spring of Tleghma

Temperature	pH	Anions (mg/l)	Cations (mg/l)
58°C	7.16	Chlore: 190	Sodium: 120
		Bicarbonates: 274.5	Calcium:118.39
		Sulfates: 164	Potassium: 03
		Nitrates: 08	Magnesium: 31.54

2.2. Isolation of actinobacteria from the hot waters of Tleghma

Three selective isolation media for actinobacteria were used: AIA (Actinomycetes isolation agar), SCA (Starch casein agar) (Uzel, et al., 2009) and ISP5 (Shirling et Gottlieb, 1966).

These media were supplemented with an antifungal agent (fungizone) at a concentration of 50µg/ml. The plates were seeded and incubated at 30°C, 45°C, and 55°C for 20-30 days.

2.3. Phenotypic and physiological characterization of isolates

Fresh and Gram-stained microscopic observation was performed on all isolates. The slide culture technique described by Shirling and Gottlieb 1966, allows to describe the morphology of the aerial mycelium and that of the substrate of each isolate was also performed.

The scanning electronic microscopy (SEM) (ZEISS) was used to examine the morphology of spore chains of the isolates TA3 and TA4, it was performed in Scientific and Technical Research Center in Physico-Chemical Analysis in Algeria.

The production of melanoid pigments was tested on ISP7 medium (Shirling and Gottlieb, 1966). Growth of all isolates at different temperatures (4°C, 10°C, 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C), at different concentrations of NaCl (5, 7 and 10g/l) and at different pH (3, 4, 5, 6, 7, 8, 9, 10,11) was then carried out on ISP2 medium.

2.4. Molecular identification

2.4.1. DNA extraction of Actinobacteria isolates

Genomic DNA was extracted from the isolates using the Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan), following the manufacturer's instructions. Extracted DNAs were eluted in 50µL of elution buffer. The quantity and purity of DNA were evaluated by a Nanodrop (Thermo Scientific 2000c, USA). DNAs with an A280 / A260 value close to 1.80 were considered pure. The extract was used immediately or stored at -20° C for further use. The genomic DNA was quantitated and assessed for integrity by agarose gel electrophoresis.

2.4.2. 16s PCR for identification

All PCR reactions were performed with the Gene Amp® PCR system (Applied Biosystems). PCR reactions were performed in 50 µL mixtures containing 10 PCR buffer, 1.5 mM MgCl2, 0.4 mMdNTP, 0.2 mM primer, 1.25 U of Taq DNA polymerase (Amplitaq Gold, Applied Biosystems) and 50 to 500 ng of genomic DNA template. Theprimers used for the amplification of the 16S rDNA were FC27 (5'-AGAGTTTGATCCTGGCTCAG-3) and RC1492 (5'-TACGGCTACCTTGTTACGACTT-3'). PCR conditions for 16S rDNA reactionswere 1 cycle of denaturation for 5 min at 94 °C; 30amplification cycles consisting of denaturation (94 °Cfor 30s), primer annealing (49 °C for 30 s), and primer extension (72 °C for 90s); and a final extension of 7min at 72 °C. The PCR products were sequenced.

The similarity of sequences was determined using BLAST

(http://www.ncbi.nlm.nih.Gov/Blast). Multiple sequences alignment used the Neighbour-Joining (N-J) method to determine the closeness of the isolates, and the phylogenetic tree results were viewed using the Mega version 11 program.

2.4.3. Nucleotide sequence accession numbers

The 16S rRNA gene sequences of strains were deposited in NCBI GenBank database.

2.5. Amylase activity assay

Nutrient agar medium containing 1% soluble starch was inoculated with each actinobacteria isolate. After 07 days incubation at 55°C, the agar was covered with a solution of lugol. Hydrolysis is evidenced by the absence of coloration around the colonies on the contrary, the areas containing starch are colored in a brown color (Jeffrey, 2008). Isolates with a large clearing zone were used for α-amylase determination.

2.6. Amylolytic activity on liquid medium

2.6.1. inoculum Preparation

A suspension of each isolate was inoculated into nutrient broth with 1% starch and incubated at 55°C for 3 days until reaching an OD equal to 0.05 at 550 nm (Chaudhary and Prabhu, 2016).

2.6.2. Batch culture

1ml of the bacterial suspension is inoculated into a 100ml volume of Starch fermentation broth medium with the following composition (in g/l) (10g starch, 10g of nutrient broth, 15g agar) at pH 7. The cultures were then incubated under agitation (75 rpm) at 55°C for 5-6 days (Naif, et al.,2020).

2.6.3. Assay of α-amylase

A volume of 2 ml of the bacterial suspension of each batch cultured isolate is placed in an Eppendorf tube. This volume is then centrifuged at a speed of 3000 rpm for 20 min at a temperature of 4°C. A 0.5 ml volume of the supernatant is added with a 0.5 ml volume of a 0.1M phosphate buffer solution, containing starch at a concentration of 1g/l and adjusted to pH 7. The reaction mixture is then incubated at 55°C for 20 min. The reaction is then stopped by adding 1ml of DNSA reagent. The tubes are boiled for 5 minutes and then cooled to ambient temperature. The mixture is then completed to 5 ml by adding distilled water. The estimation of reducing sugar content is measured by reading the absorbance at a wavelength of 540 nm using a spectrophotometer type UV-1800A, Shimadzu, Japan (Bernfeld, 1955).

The results obtained are expressed as U/ml. One unit of amylase activity is defined as the amount of enzyme that produces one µmole of glucose (Ahmed et al., 2011).

2.7. Effect of temperature and pH on α-amylase activity

In this study, the variation of the two key parameters affecting the production of α-amylase was tested. These are temperature and pH. Starch fermentation broth liquid culture medium is inoculated with the bacterial suspension of the performing isolates and incubated at 4°C, 20°C, 30°C, 40°C, 55°C, 60°C and 70°C for 6 days. For pH, the same culture medium is adjusted to different pH using different buffers. We used acetate buffer (for pH 4), phosphate (for pH 7), Tris-amine methane buffer (for pH 9) and borate (for pH 10.2).

The incubation temperature is 30°C for all experiments. A volume of 1 ml is taken in Eppendorf from the 5 to 6 days old batch cultures.

The supernatant is recovered by centrifugation at a speed of 3000 rpm for 20 minutes. Then, the amylolytic activity is measured by the DNSA method with the variation of temperature and pH of the enzymatic reaction.

3.Results anddiscussions

3.1.Isolation and phenotypic characterization of actinobacteria isolates

The colonies obtained show the macros and micro-morphological characteristics of actinobacteria. After 30 days of incubation, all isolates show a typical macroscopic appearance of actinomycetes with different colors (white, green, gray) (Figure 2), (Table 2). This result is

confirmed by microscopic observation which shows the presence of filaments of different sizes and different fragmentations as well as the presence of spores in most isolates (Figure 3, 4and 5). All strains are Gram positive (Figure 3).A total of 13 isolates, 6 isolates from AIA medium, 2 from ISP5 medium and 5 from SCA medium, were selected after purification on ISP 2 medium. Generally, the isolation of actinobacteria pauses some difficulties, mainly because of their relatively long growth time. This feature allows time for other bacteria to contaminate the Petri dishes and occupy the environment especially by fungi and invasive bacteria (Williams et al., 1982; Crawford et al., 1993; Boudemagh and Bensouici, 2014). As such, we used three selective media for the isolation of these bacteria from the tested hot spring. These are Actinomycetes Isolation Agar (AIA) medium, containing sodium propionate and sodium caseinate which have an important antifungal role that prevent fungi from multiplying in the isolation medium. The presence of asparagine according to some researchers, favors the growth of actinobacteria over other microorganisms (Uzel et al., 2009, Akhagari et al., 2014). In the work of Medjemadj et al., 2020, this medium allowed the isolation of the greatest number of actinobacteria from thermal waters. The second medium used in this research is Starch Casein Agar (SCA), the presence of starch and casein in its composition, allows a selective growth of actinobacteria. This medium was successfully used in the work of Suzuki, 2001, where several rare genera such as *Actinomonospora*, *Actinopolyspara*, *Planomonospora* and *Planobispora*, could be isolated. *Streptomyces* and other genera were isolated (Medjemadj et al., 2020). Using ISP5 medium for selective isolation yielded only two actinobacterial isolates, despite its glycerol-rich composition, which is known to be favorable for actinobacteria (Jihani et al., 2012; Siddique et al., 2014).

3.2.Physiological characterisation of isolates

The results of the growth of the 13 isolates at different temperatures indicate that all 13 isolates show good growth between 30-55°C. However, the isolates (TA3 and TA4) are resistant to a temperature of 70°C.

According to the literature, thermophilic actinobacteria are generally classified into two groups. The first is represented by strictly thermophilic and moderately thermophilic actinobacteria, which grow between 37 and 65°C with an optimumbetween 55-60°C. The second category includes moderately thermophilic actinobacteria, which grow between 28-60°C with an optimum between 45-55°C (Jiang and Xu, 1993). Another group known as thermotolerant actinobacteria is also present. Its representatives can survive at temperatures up to 50°C (Lengeler et al., 1999).According to these two types of classifications, the isolates (TA3, TA4, TA5, TA6, TS1, TS2, TS3, TS4 and TS8) are moderately thermophilic actinobacteria. While the other isolates (TS4, TA1, TA10, TA12) are considered to be thermotolerant actinobacteria.

The actinobacteria isolated in our investigations show growth at temperatures ranging from 10 to 70°C, only two isolates (TA3 and TA4) in this group can survive at temperatures up to 70°C. This tolerance was considered by some researchers, is due to their molecular modification at the cellular and subcellular level, saturation of membrane lipids that reduces membrane fluidity at elevated temperatures, and the presence of histone-like in the DNA of hyperthermophiles that protects the DNA (Mrunmaya, 2013;Aditi Nupur, 2016).

The totality of the actinobacteria isolated from the thermal water, present a good growth in the pH interval going from 6 to 8. Among them, the isolates, TA1, TS3 and TS6 are acidotolerant bacteria, which can resist a pH equal to 4. However, the isolates (TA3, TA4, TA5, TA6, TA10, TA12 and TS8) are, according to the classification of Jiang and Xu, 1993, alkaline-tolerant and can resist a pH equal to 11 (Table 3). Our results are in perfect

agreement with other works where the majority of actinobacteria isolated from this kind of ecosystems, are able to live at temperatures ranging from mesophilic to thermophilic and at basic pH. The work of Mokrane, et al., 2016 succeeded in isolating and identifying a new strain *Thermoactinomyces khenchelensis* from the sediments of the thermal spring of khenchela in Algeria. This strain is able to grow in a temperature range of 37-55°C and a pH range of 7-9.Two strains *Nocardoides pakistanensis* and *Streptomyces caldifontis* isolated from Tatta Pani hot spring in Pakistan, are able to survive in a temperature range of 20-40°C and 18-40°C respectively at pH 6-9 (Archia, 2016). The *Saccharomonospora viridis SJ*-21 strain isolated from a hot spring in India is able to grow in temperatures between 35-60°C and pH between 7-10(Jani et al., 2012).

Regarding NaCl tolerance, 6 named isolates (TA1, TA3, TA4, TA5, TA6, and TA10) show good growth in 10g/l NaCl concentration. According to Larsen's 1986 classification, these actinobacteria are moderate halotolerants, whereas the other isolates (TA12, TS1, TS2, TS3, TS4, TS6, and TS8) are weak halotolerants. These results are in agreement with the studies of Perry and Staley in 1997which state that thermophilic actinomycetes are characterized by halophilia**.**

Table 2. Morphological characteristics of actinobacteria isolates

	Culture medium AIA						**Culture medium ISP5**		**Culture medium SCA**				
Caracteristics	**TA1**	**TA3**	**TA4**	**TA5**	**TA6**	**TA10**	**TA12**	**TS1**	**TS2**	**TS3**	**TS4**	**TS6**	**TS8**
Size (mm)	3	1.5	1	1.5	1	2	2	3	3	3	2	2	2
Color of MA	Whi	grey	Whi	grey	Whi	Whi	Whi	Gre	Gre	Gre	Whi	Whi	Whi
Color of MS	Whi	Bro	Whi	Bro	Whi	Whi	Whi	Bro	Bro	Whi	Whi	Yell	Whi
Melanoid pigments	-	-	+	-	-	-	-	-	-	-	-	-	-
Contour	Reg.	Reg.	Reg.	Reg.	Reg.	Reg.	Reg.	Reg.	Reg.	Reg.	Reg.	Reg.	Irrg.
Shape	Circ	Circ	Circ	Circ	Circ	Circ	Circ	Circ	Circ	Circ	Circ	Circ	Circ
Texture	Inla	Inla	Inla	Inla-pow	Inla-pow	Inla-pow	Inla	Inla-pow	Inla	Inla	Inla-pow	Inla-pow	Inla

Whi; White, Gre; Green, Bro; Brown, Yell; Yellow; Reg; Regular, Irrg; Irregular, Circ; Circular, Inla; Inlaid, Pow; Powdery. (+) : presence of melanoid pigments (-) : absence of melanoid pigments.

(a) **(b)** **(c)**

Figure 2. Macroscopic appearance of some isolates (a)TA6, (b) TA3, (c) TA4

(a) **(b)**

Figure 3. Microscopic appearance in the fresh state and after Gram staining of isolates (a) TA4 et (b) TA3

(a) **(b)**

Figure 4. Microscopic appearance by the slide technique (GX100).
(a) Aerial mycelium of TA6 isolate, (b) Substrate mycelium of TA6 isolate

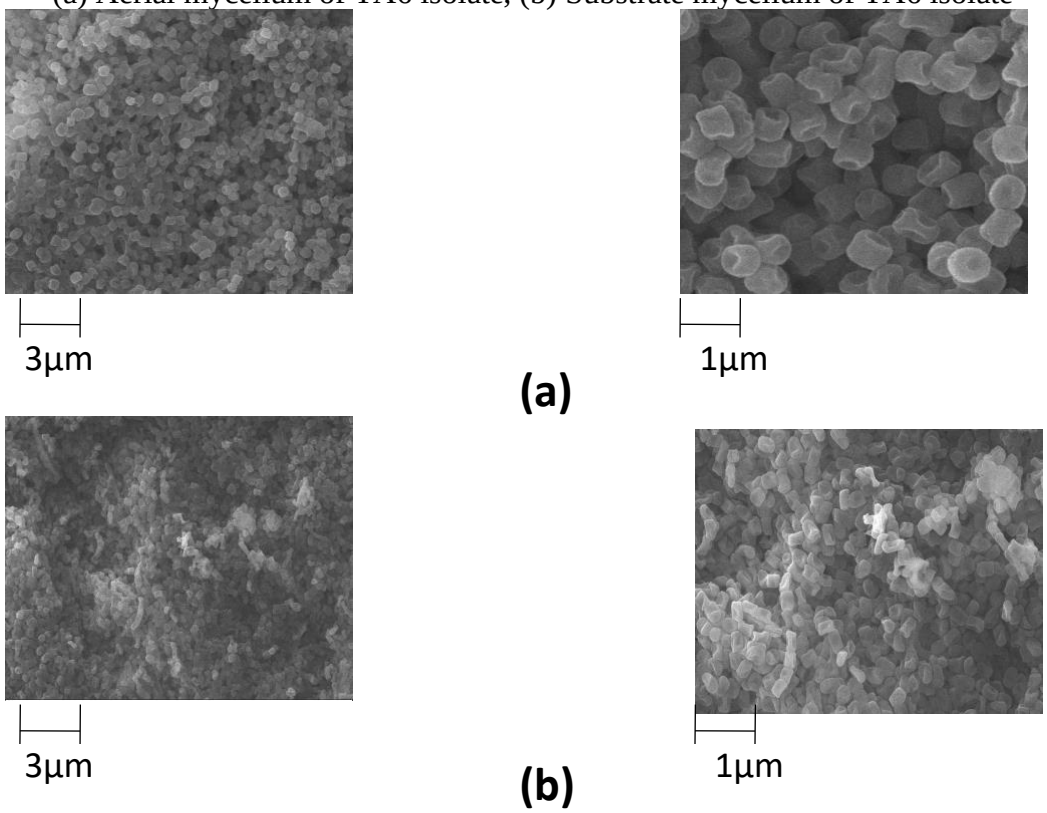


Figure 5. Scanning electron microscopy (SEM) (ZEISS) of two isolates.
(a) Isolate TA3. **(b)** Isolate TA4.

3.3.Molecular identification

Molecular identification indicates that all isolates belong to the genus *Streptomyces* (Table 4) (figure 6).This genus of actinobacteria has clear advantages in biotechnology (Hodgson, 2000; Manteca et al., 2008). The presence of *Streptomyces* in thermal springs is not uncommon, it has been proven in some works (Medjemadj et al., 2020; Akhgari et al. 2014; Zu et al., 1998). Also, the presence of actinobacteriain thermal springs has been demonstrated in some studies.

In the work of Medjemadj et al., 2020, three genera of actinobacteria were isolated from thermal waters of eastern Algeria. These are the genera *Rathayibacter, Streptomyces* and *Rhodococcus*. Arshia. 2016, successfully isolated a new species (*Nocardioides pakistanensis*) from the water samples of a hot spring located in Pakistan. *Planifilumy unnanense sp. Nov* is a new strain isolated from the hot spring in Yunnan Province, China. This thermophilic actinobacterium is able to grow in a temperature range of 50-75°C (Zhang, et al. 2007).From the sediments of a thermal spring in western Anatolia, Turkey, two actinobacteria: *Thermoactinomyces thalpophilus* and *T.saccharis*, which grow at 55°C, were isolated (Uzel et al., 2009).

The metagenomic approach in the work of Zhaoqi et al., 2009 showed a surprising diversity of culturable and non-culturable actinobacteria in three geographically distant hot springs.

It should be noted that in the literature, we found little research on the isolation of actinomycetes from thermal waters compared to those performed on sediments

Table3. Physiological characteristics of actinobacteria isolates

Characteristics	TA1	TA3	TA4	TA5	TA6	TA10	TA12	TS1	TS2	TS3	TS4	TS6	TS8
T°C range	20-55	10-70	20-70	20-60	10-65	20-55	20-55	20-60	10-60	20-65	20-55	20-65	20-65
Optimal growth	40	55-60	55-60	45-55	45	40	40	45	45	55	35	45	55
pH range	4-11	5-11	6-11	5-11	6-11	5-11	5-11	5-9	5-9	4-10	5-10	4-10	5-11
Optimal growth	7	9	10	8	8	7	7	6	7	6	7	6	8
NaCl (g/l)	5,7,10	5,7,10	5,7,10	5,7,10	5,7,10	5,7,10	5,7	5,7	5	5,7	5	5,7	5,7

Table 4. Molecular identification of actinobacteria isolated from the hot spring of Tleghma.

Isolate codes	Closest sequence match withBLAST (accession number)	Percentage of Similarity	Accession numbers
TA1	*Streptomyces rhizosphaericola* 1AS2c (NZ_SRZK01000437.1)	99.72¨%	OP456977
TA3	*Streptomyces albidoflavus* (NC_020990.1)	99,44%	MW301212
TA4	*Streptomyces cavourensis* strain 1AS2a (NZ_CP024957.1)	99,32%	MW301210
TA5	*Streptomyces albidoflavus* (NC_020990.1)	99.53%	OP003989
TA6	*Streptomyces rhizosphaericola* strain 1AS2c (NZ_SRZK01000437.1)	98.03%	OP003990
TA10	*Streptomyces albidoflavus* (NC_020990.1)	99.64%	OP003991
TA12	*Streptomyces rhizosphaericola* strain 1AS2c (NZ_SRZK01000437.1)	99.77%	OP003992
TS1	*Streptomyces griseoflavus* strain JCM 4479 (NZ_BMUC01000046.1)	98.51%	OP480010
TS2	*Streptomyces azureus*strain ATCC 14921 (NZ_DF968281.1)	99.14%	OP480011
TS3	*Streptomyces torulosis* strain NRRLB-3889 (NZ_LIRK01000400.1)	99.02%	OP003986
TS4	*Streptomtcesmelanogensis*strain JCM4398 (NZ_BMTS01000080.1)	99.39%	OP003987
TS6	*Streptomyces calvus* strain DSM 41452 (NZ_CP022310.10)	99.06%	OP480012
TS8	*Streptomtcesaurantiogriseus*strain JCM4346 (NZ_BMSX01000069.1)	98.39%	OP003988

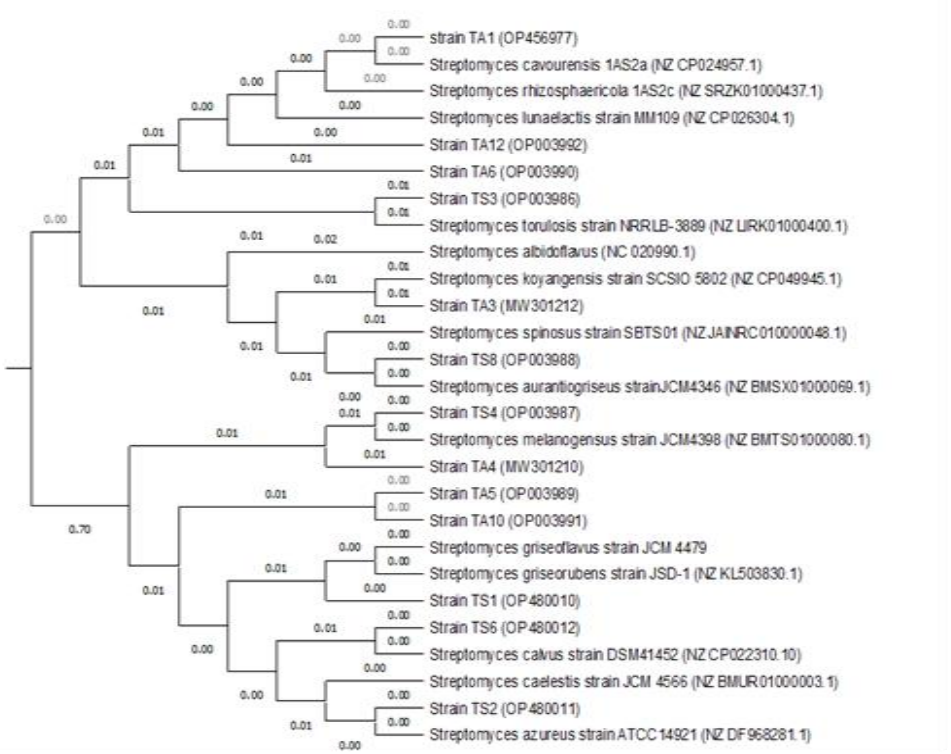


Figure 6. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences amplified from the hot spring isolates, showing the nearest neighbours of the isolated strains. GenBank accession numbers are given in parentheses.

3.4. Enzyme activity at 55°C

The amylolytic activity is calculated and shown in Table 5 and Figure 7. The results show that 9 isolates (TA3, TA4, TA5, TA6, TA10, TA12, TS3, TS4 and TS8) among the 13 isolated, are able to produce α-amylase in the temperature 55°C. Isolates TA3 and TA4 gave however, better enzymatic activity (Figure 7 and 8).

The hydrolysis of starch by enzymes of microbial origin has found wide applications in different industrial fields and has replaced tedious and very expensive chemical methods (Vidyalakshmi et al., 2009).

Among a wide range of microbial species capable of secreting α-amylase, bacteria are the most efficient. In addition, genetic engineering studies are easier to perform with bacteria and they are also very suitable for the production of recombinant enzymes (Nielsen et al., 2000)

Actinobacteria and especially the genus *Streptomyces* are characterized by the production of several enzymes (Hang et al., 1996). These bacteria are better candidates in the industrial field because of their ability to survive in harsh physicochemical conditions, such as temperature and pH (Al-Dhabi et al., 2016; Krishnasamy, 2017).

Thermostable α-amylase is widely used in biotechnology, as the main technological steps of starch processing, such as saccharification, gelatinization and liquefaction, all require high temperature. Therefore, thermostable α-amylase has a definite application in industrial sector (Gazali., 2018).

3.4.1. Effect of temperature and pH on α-amylase activity of TA3 and TA4 isolates

pH and temperature are two important parameters that control α-amylase production (Pandey et al., 2000).

In this study, the 13 isolates obtained were tested for their abilities of thermostable α-amylase production at a temperature of 55°C. The results show that the two isolates TA3 and

TA4 identified as *Streptomyces albidoflavus* and *Streptomyces cavourensis*, present the highest amylolytic activity equal to 110.33 and 224 U/ml respectively (figure 9 (a)) . The isolates (TA5, TA6, TA10, TA12, TS3, TS4 and TS8), also show amylolytic activity but less important ranging from 12-48 U/ml.Concerning the maximum production of α-amylase tested at temperature 30°C, it was observed at pH 9 for isolates TA3 and TA4, with values equal to 105U/ml and 208U/ml respectively (figure 9. (b)).

These important results are in perfect agreement with those of Gommez and Steiner, 2004 who showed that thermo-enzymes are generally not only thermostable, but they are also active at extreme pH.

According to the literature, thermophilic actinobacteria capable of producing thermostable α-amylase have been widely searched mainly in desert and semi-desert soils throughout the world. A thermostable α-amylase was obtained by a strain named *Streptomyces sp. SLBA-08* isolated from a semi-arid soil was reported (Edilla et al. in 2012). In other similar works, the *Streptomyces fragilis DA7-7* strain isolated from a desert soil in Riyadh province in Saudi Arabia, shows a very high amylolytic activity equal to 923.12 U/ml at a temperature of 28°C, which decreases with increasing temperature. Our results are more interesting because our two strains show high enzymatic activity at an optimal temperature of 55°C. (Krishnasamy., 2017).

In a more recent study, *Streptomyces sp. Al-Dhabi* strain isolated from the soil of Jazanen Saudi Arabia region is able to produce a thermostable α-amylase with maximum activity equal to 124± 12.1 U/ml in a temperature of 40°C, and at pH equal to 8 (Naif et al., 2020).

Studies that focus on the production of thermostable α-amylase by thermophilic *Streptomyces sp*. isolated from hot springs are very rare. This study is the first report on the production of this enzyme from thermal waters in Algeria. According to our knowledge, the few works done on the role of *Streptomyces* in the production of thermostable α-amylase are those of Sabita et al. In 1999, who state that the *Streptomyces megaspores* strain SD12 isolated from the thermal spring of Occidental Maharashtra, is able to show an optimal amylolytic activity equal to 52,534 U/ml in a pH of 6 and a temperature of 60°C. Chaudhary and Prabhu in 2016 also found amylolytic activity in two isolates of the thermophilic *Streptomyces* genus isolated from a hot spring located in Vajreshwari, India.

Table 5. Amylolytic activity at 55°C of actinobacteria isolates

Isolates	Amylolytic activity(U/ml)
TA1	-
TA3	110.33
TA4	224
TA5	20
TA6	12
TA10	16.5
TA12	23.5
TS1	-
TS2	-
TS3	13
TS4	48
TS6	-
TS8	15

(-) : Absence of enzymatic activity

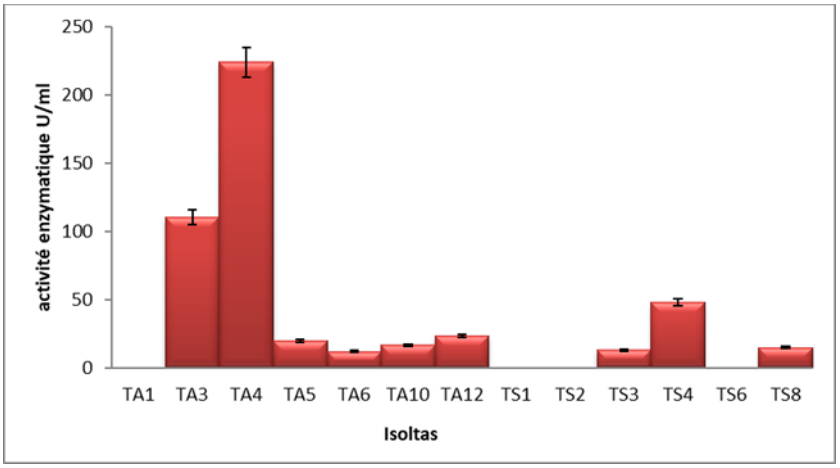
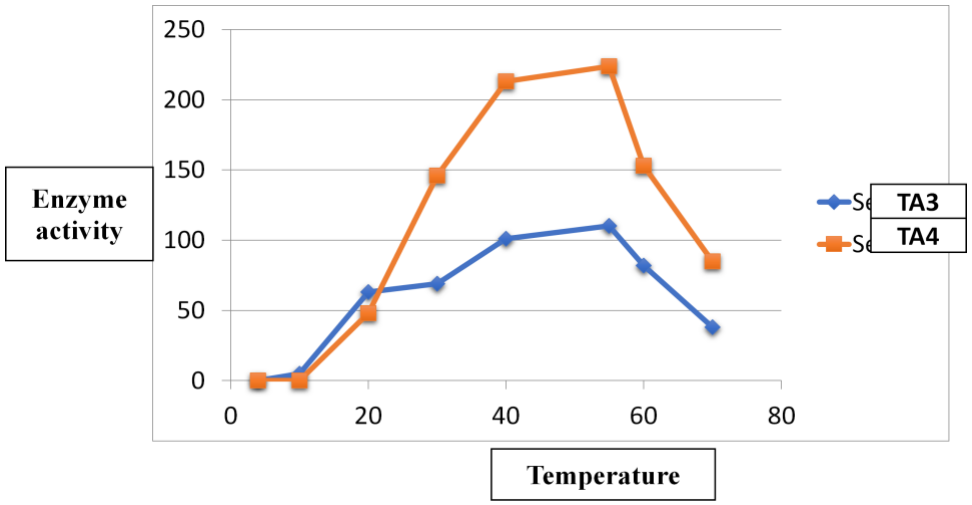
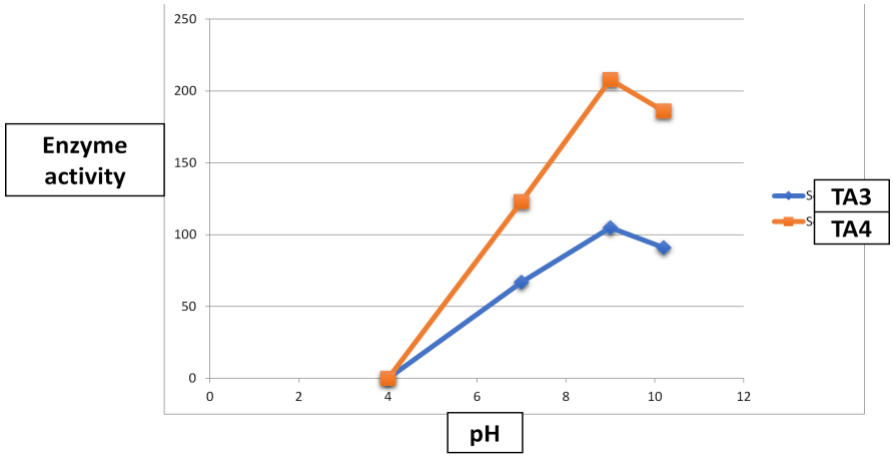


Figure 7. Enzymatic activity at 55°C of α-amylase produced by selected isolates

Figure 8. Starch hydrolysis zones of isolates (a) : TA3, (b) : TA5, (c) : TS4, (d) : TA4 isolates



(a)



(b)

Figure 9. Effect of temperature (a) and pH (b) on the activity of amylase produced by TA3 and TA4 isolats

4. Conclusions

In this study, we reveal that thermal waters are populated by thermophilic and thermotolerant actinobacteria. This result is very important and agrees with recent works, which affirm the presence of these bacteria in thermal waters. Molecular identificationby r16sDNA sequencing showed that all isolates belong to the genus *Streptomyces*. Two successful isolates assigned to *Streptomyces albidoflavus* and *Streptomyces cavourensis*, are capable of producing thermostable α-amylase with an optimum of 55°C. These two strains also present an alkalophilic character by producing the desired enzyme at a pH equal to 9. According to our knowledge, it is for the first time that these bacteria producing this enzyme, are isolated from hot springs in Algeria. These ecosystems constitute an inexhaustible reservoir of very promising bacteria from the biotechnological point of view.

5.References

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