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Optimization of alkaline protease production by *Streptomyces* sp. strain isolated from saltpan environment

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Proteolytic activity of a *Streptomyces* sp. strain isolated from Ezzemoul saltpans (Algeria) was studied on agar milk at three concentrations. The phenotypic and phylogenetic studies of this strain show that it represents probably new specie. The fermentation is carried out on two different media, prepared at three pH values. The results showed the presence of an alkaline protease with optimal pH and temperature of 8 and 40°C, respectively. The enzyme is stable up to 90°C, having a residual activity of 79% after 90 min. The enzyme production media are optimized according to statistical methods while using two plans of experiences. The first corresponds to the matrixes of Plackett and Burman in N=16 experiences and N-1 factors, twelve are real and three errors. The second is the central composite design of Box and Wilson. The analysis of the results allowed the selection of two factors having a significant effect on the production of the enzyme (fructose and malt extract), then defining theirs optima (7 g/l of fructose and 12 g/l of malt extract).

Key words: Protease, *streptomyces*, identification, fermentation, optimization.

INTRODUCTION

Proteases are among the most important industrial enzymes, accounting for nearly 60% of total industrial enzymes in the market. These enzymes play an important role in biotechnology and are widely used in the tanning industry, in the manufacturing of biological detergents, meat tenderization, peptide synthesis, food industry, pharmaceutical industry and in bioremediation processes (Bhaskar et al., 2007; Jellouli et al., 2009; Deng et al., 2010). Proteases are ubiquitous, they are found, in plants, animals and microorganisms. Microorganisms are the most interesting source of proteases due to their broad biochemical diversity and bioengineering potentiality. Microbial proteases account for approximately 40% of the total worldwide enzyme

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sales (García-Gómez et al., 2009).

Streptomyces spp. (Family: Streptomycetaceae, order Streptomycetales, Class: Actinobacteria) (Whitman et al., 2012) are the most important group of the Actinobacteria with high G+C content of the DNA. Members of the group produce aerial hyphae bearing chains of conidiospores on their tips when growing on agar (Kieser et al., 2000). Several species of the *Streptomyces* are among the most important industrial microorganisms because of their capacity to produce numerous bioactive molecules, particularly antibiotics. *Streptomyces* species are heterotrophic feeders, which can utilize both complex and simple molecules as nutrients. In addition to antibiotics, *Streptomyces* species liberate several extra cellular enzymes (Gupta et al., 1995). In view of potential applications of protease enzyme and economy of production, an attempt was made to study and optimize the protease production from *Streptomyces* spp. The producer strain is isolated from a water sample of Ezzemoul saltpan, located in Ain M'lila (East of Algeria). The present work aims at (i) morphological, physiological, biochemical and molecular studies of the producer strain, (ii) determination of some properties of this protease and also (iii) optimization of media composition, using statistical methods.

MATERIALS AND METHODS

Microorganism

Streptomyces sp. LS strain originally isolated from Ezzemoul saltpan's water (Algeria) was inoculated onto 10, 20 and 30% skimmed milk agar plates (Harrigan and Mc Cance, 1976) and incubated at 30°C for 7 days. Appearance of clearing zone formed by hydrolysis of skimmed milk was used as indication of a protease producer.

Identification of producer strain

Traditional identification

LS strain colonies were characterized morphologically on different media (ISP_2, ISP_3, ISP_4, ISP_5, Starch casein agar, Glucose asparagine agar, Hickey and Tresner gar) following the directions given by the International *Streptomyces* Project (Shirling and Gottlieb, 1966). Cultural characteristics such as growth importance, aerial and substrate mycelium color and diffusible pigment production, were recorded after incubation for 7, 14 and 21 days at 28°C.

Micro morphological observations were carried out with a light microscope using two different methods: slide culture technique (Zaitlin et al., 2003) and inclined coverslips technique (Williams and Cross, 1971; Holt et al., 1994). The physiological and biochemical characteristics were determined according to the methods of Shirling and Gottlieb (1966), Crawford et al. (1993), Chaphalkar and Dey (1996), and Singleton (1999).

Molecular identification

DNA of protease producing strain was extracted using DNA extraction kit «Ultraclean Microbial DNA Isolation (Mo Bio)» and then 16S rRNA genomic regions were analyzed using 63F (5'-CAGGCCTAACACATGCAAGTC-3') and 1387R (5'-GGGCGGWGTGTACAAGGC-3') as forward and reverse primers, respectively. Amplification is carried out in « AB Applied Biosystems Veriti 96 well» thermal cycler, using Taq DNA polymerase. Polymerase chain reaction (PCR) program was 95°C/10 min for initial denaturing, 95°C/45 s, 56°C/45 s, 72°C/1 min for 36 cycles and 72°C/ 10 min for final extension.

PCR products were electrophorized on agarose gel with du Tris Borate EDTA in « Embi Tec Runone ^{TM} » electrophoresis cell, under 100 volts. The gel is finally photographed on a UV table «Pharmacia Biotech. Imager Master® VDS» Purified products were subjected to 16S rRNA sequencing by automated Sanger method (Sanger et al., 1977) using 1387R primer, in «GATC Biotech AG, Germany» laboratory. The sequences are finally corrected using the software «Sequencher v. 4.1.4 (Gene Codes)».

Sequences were analyzed by MEGA 5 software (Tamura et al., 2011) and nBLAST tool at NCBI. The 16S rRNA nucleotide sequence of the isolate was aligned with homologous regions from various actinomycetes, and the phylogenetic tree was constructed by neighbor-joining method (Saitou and Nei, 1987). A bootstrap confidence analysis was performed on 1000 replicates to determine the reliability of the distance tree topologies obtained.

Fermentation protocol and enzyme assay

Two fermentation media are used: M1 medium composed of: peptone 5 g/l, meat extract 3 g/l, gelatin 4 g/l, and M2 medium composed of: yeast extract 4 g/l, malt extract 10 g/l, dextrose 4 g/l. Each medium is prepared at three pH values (4.5, 7 and 8.5). Fermentations were carried out in Erlenmeyer flasks (250 ml) containing 50 ml of the fermentation medium. The broth was inoculated with 10^6 spores/ ml and incubated at 30°C for 7 days with shaking at 100 rpm. After fermentation, supernatant was harvested by centrifugation at 11000 g for 20 min at 4°C. The clear supernatant was used as crude enzyme. LS strain protease activity was measured by of Lenoir and Auberger (1977) method modified by Mechakra et al. (1999).

Study of some protease properties

For determination of optimum pH of the enzyme, the reaction mixture buffer was varied over the pH range 2 to 14. Similarly, enzyme production was also monitored at temperature in the range of 20 to 90°C (in increment of 10°C). For the determination of the thermal stability, the enzyme was incubated at different temperature values (60, 70, 80 and 90°C) for 120 min. The samples were submitted to determination of protease activity every 15 min.

Statistical optimization of protease production medium

The protease production is influenced by various production parameters including nutritional and environmental parameters. For this, the media composition is optimized according to statistical methods while using two plans of experiences. The first corresponds to Plackett and Burman design (Plackett and Burman, 1946) for selection of most appropriate medium components. The second is the central composite design of Box and Wilson (1951) to determine the optimal values of components selected in the first plan.

According to the Plackett and Burman design, 15 independent variables (including 3 dummy variables) were organized in 16 combinations. Each variable was examined at a high level (coded as +1) and a low level (coded as -1).The experimental values of the coded levels are shown in the (Table 1). Plackett and Burman

Table 1. Experimental definition for the Plackett and Burman design.

Factors	Low level (-1)*	High level (+1) *
X_1 : glucose	0	3
X_2: starch	0	3
X_3: fructose	0	3
X_4: maltose	0	3
X_5: error	-	-
X_6: yeast extract	0	10
X_7: malt extract	0	10
X_8: peptone	0	10
X_9: casein	0	10
X_{10}:$NaNO_3$	0	10
X_{11}: error	-	-
X_{12}: Na Cl	0	5
X_{13}:$MgSO_4$	0	0.5
X_{14}: K_2HPO_4	0	0.5
X_{15}: error	-	-

* Concentrations in (g/l).

Table 2. Coded and real variables values of central composite design.

Factors	Levels				
	-α	-1	0	+1	+α
X_3 : fructose	0.172	1	3	5	5.828
X_7 : malt extract	5.172	6	8	10	10.828

design is based on the first order polynomial model:

$Y=\beta_0+\sum \beta_i X_i$

Where Y is the response (protease enzyme production), β_o is the models intercept, β_i is the linear coefficient and Xi is the level of the independent variable. All the experiments were carried out in triplicates.

The central composite design was employed in order to find the optimum levels of significant media components. The two selected factors (in Plackett and Burman design) are studied in central composite designs with 11 combinations. Each variable is studied in 5 levels: (-1) lower level (+1) higher level and a central point (0). (-α) and (+α): two levels determined according to α value which is function of the factors number: for 2 factors α = 1.414. The experimental α value is found according to the equation:

(+ /-) α = experimental α value–central level (0) ***/*** step.

The step is the value which separates the central point (0) from the levels (-1) and (+1). The coded and real variables levels are presented in the (Table 2). Analysis of variance allows estimating the significance of results obtained by centered composite design. And if the test is significant, the effect of the factors (X_1, X_2) on the production of the alkaline protease is expressed in form of a quadratic equation:

$Y = b_0+b_1X_1+b_2X_2+b_{11}X_1^2+b_{22}X_2^2+b_{12}X_1X_2$

Coefficients values (bij) are determined by a of multilinear regression programme. Partial derivatives with respect to 0 gives an equation system, the resolution of this equation gives coded optimal levels.

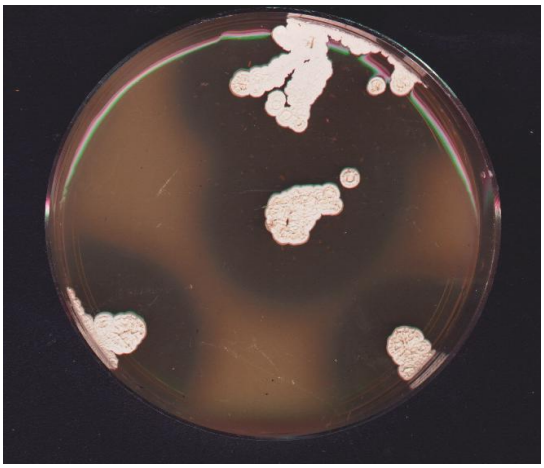

Figure 1. Proteolytic activity shown by LS strain on 20% skimmed milk agar.

RESULTS AND DISCUSSION

Screening for proteolytic activity

LS *Stretomyces* strain was selected for the production of protease on the basis of formation of clear zone near the vicinity of the colony (Figure 1). The dimension of clear zones increases with skimmed milk concentration.

Identification of LS *Streptomyces* strain

Cultural properties of LS strain are presented in the (Table 3). The *Streptomyces* strain grew well on Starch casein agar, ISP_4, ISP_6 and ISP_7. Our isolate produced cream, beige or yellow brown mycelia on all media with brown yellow to yellow diffusible pigment. While the mature spore mass was belonging to yellow series. By studying the micro morphological properties of LS strain, it was found that the aerial mycelia formed unfragmented, branched, straight hyphae bearing non motile cylindrical spores. Spores chains were related to Rectifexibiles (RF) category and contained up to 40 spores per chain.

The physiological and biochemical characteristics of our strain are summarized in the (Table 4). 16S rRNA gene sequence analysis has also been done to elucidate the taxonomic situation and relationship amongst closely related *Streptomyces* spp. LS strain16S rRNA gene sequence was submitted in the GenBank database with accession number KP342331 (2 January, 2015). Comparative analysis of this sequence with the corresponding sequences of other micro organismes obtained from the same database, confirmed the genus assignment and found only 8 most closely related

Table 3. Cultural characteristics of LS strain.

Time	Culture media	Growth characteristics	Pigmentation	
			Substrate mycelium	Aerial mycelium
7th day	ISP_2	Moderate	Brown yellow	Brown yellow(+pigment[1])
	ISP_3	Moderate	Light yellow	Bright cream
	ISP_4	Good	Dark grey	Powdery opaque cream
	ISP_5	Poor	Light yellow	Yellow cream(+pigment[1])
	ISP_6	Good	Beige	Beige
	ISP_7	Good	Light brown yellow	Light brown yellow
	GA	Moderate	Brown yellow	Brown yellow
	SC	Good	Greenish grey	Powdery cream
	HT	Moderate	Light pink	Beige
14th day	ISP_2	Moderate	Brown	Brown (+pigment[1])
	ISP_3	Moderate	Cream	Bright cream
	ISP_4	Good	Greenish grey	Powdery grayish cream
	ISP_5	Poor	Light brown yellow	Brown yellow(+pigment[1])
	ISP6	Good	Beige	Beige
	ISP_7	Good	Brown	Brown (+pigment[1])
	GA	Moderate	Orange yellow	Orange yellow
	SC	Good	Greenish yellow	Powdery cream
	HT	Moderate	Beige	Beige
21th day	**ISP_2**	Moderate	Brown	Brown (+pigment[1])
	ISP_3	Moderate	Cream	Bright cream
	ISP_4	Good	Greenish grey	Powdery grayish cream
	ISP_5	Poor	Light brown yellow	Brown yellow(+pigment[1])
	ISP_6	Good	Beige	Beige
	ISP_7	Good	Brown	Brown (+pigment[1])
	GA	Moderate	Orange yellow	Orange yellow
	SC	Good	Greenish grey	Powdery cream
	HT	Moderate	Beige	Beige

(1) Pigment color change from yellow in acid pH to pink in alkaline pH, GA: Glucose asparagine, SC: Starch casein, HK : Hickey and Tresner.

species with a high degree of relatedness (99%): *Streptomyces flaveus* NBRC 12345, *Streptomyces scabiei* NBRC 12914, *Streptomyces anulatus* NBRC 13369, *Streptomyces olivaceus* NBRC 3152, *Streptomyces tricolor* NBRC 15457, *Streptomyces cavourensis subsp. washingtonensis* NBRC 15391, *Streptomyces praecox* NBRC 13073, *Streptomyces chrysomallus* NBRC 12755.

The Neighbour - Joining phylogenetic tree revealed that the strain formed a monophyletic clade with *Streptomyces tricolor* NBRC15457, with 46% bootstrap support and high similitude degree (99.92%) (Figure 2). The phylogenetic study of Labeda et al. (2012) of almost all described species (615 taxa) within the family Streptomycetaceae, gives à new classification of 130 statistical clades defined at greater than 60% bootstrap support. *Streptomyces tricolor* strain was associated with Clade 116, a well-defined group formed by the strains of *Streptomyces roseodiastaticus*, *Streptomyces bangladeshensis* AAB - 4^T and *Streptomyces rameus* NBRC 15453^T with 99% bootstrap support. All these data suppose that it is probably the same species or closely related species (as *S. bangladeshensis* has different morphological characters of the other three).

Table 5 reports the characteristics of the four strains, it appears clearly that LS strain has different morphological characteristics of other strains with the exception of *S. bangladeshensis* which is the closest to our strain with a yellow color and rectiflexibile aerial mycelium form, however, differences are observed in the assimilation of rhamnose, sucrose, lactose and raffinose, as well as H_2S production, and other physiological characteristics. Therefore, LS strain is different from other species listed in the 116 group including *S. tricolor* (the closest at the phylogenetic tree) and *S. bangladeshensis* (the closest one regarding the phenotypic characters): our

Table 4. Physiological and biochemical characteristics of LS strain.

Test	Results
Degradation of :	
D - glucose	+
L - rhamnose	-
D - mannitol	+
Mannose	+
D - xylose	+
Dextrine	+
D - galactose	+
Sucrose	-
L - arabinose	+
D - fructose	+
Maltose	+
Lactose	+
Raffinose	-
i - inositol	+
Cellulose	+
Production of melanoide pigment on:	
ISP $_6$	- [(1)]
ISP $_7$	+
Hydrolysis of :	
Starch	+
Gelatin	+
Casein	+
Skimmed milk hydrolysis :	
Peptonisation	+
Coagulation	-
Nitrate reduction	+
NaCl tolerance	7 %
Growth at different temperature values :	(dry weight/40 ml)
4°C	-
22°C	+ (22.45 mg)
28°C	+ (50 mg)
37°C	+ (35 mg)
45°C	-
Growth at different pH values :	
5.5	+ [(2)]
6	++
6.5	+++
7	++
7.5	++
8	++
Citrate utilization	-
H_2S production	-
Indole production	-
Tryptophane desaminase test	-
Decarboxylase test:	
Arginine	-
Lysine	+
Ornithine	-
Urease test	+

[(1)]Fot the tests : (+): Strain growth or positive test reaction; (-): No growth or negative test reaction.[(2)] Growth at different pH values: (+++) : Good, (++) : moderate, (+) : poor.

Streptomyces strain is probably new specie. Indeed, the origin of LS strain (isolated from Saltpan) suggests that the species adapted to this extreme environment are eventually particular.

Protease activity assay

After primary screening, secondary screening of protease activity was done by quantitative method, LS strain showed proteolytic activity on two different broth media with better activity on M1 broth at pH 8 (Figure 3), supposing an alkaliphilic nature of the enzyme. However, no growth was observed at acidic pH on both culture media.

Study of enzyme characteristics

The study of LS strain protease characteristics, showed an optimum pH of 8 (Figure 4), an optimum temperature of 40°C (Figure 5) and especially significant thermal stability (Figure 6). In fact, this enzyme keeps 80% of its activity for 15 min at 90°C. After 120 min at the same temperature, the activity decreases to 39%.

Statistical optimization of protease production medium

Selection of the significant factors for protease production using Plackett and Burman design

Protease activities on different media of Plackett and Burman design are shown in Figure 7. Medium 6 has shown the best activity of alkaline protease, this medium is composed of 3 sugars (glucose, fructose and maltose), malt extract and 3 salts (NaCl, $MgSO_4$, K_2HPO_4). The lower proteolytic activity was observed on media 8 and 12. They contain glucose but no fructose or maltose in addition to the presence of $NaNO_3$ and NaCl.

Statistical analysis and modeling results were used to measure the effect of each factor and its significance level on the production of protease by strain LS. The results of this analysis shown in the (Table 6), showed a significant effect of X_3 and X_7 factors at 70% (with Students values of 1.35 and 1.26 respectively). However, a negative effect of X_9 and X_{10} factors is found with negative statistical effects, coefficients and Student values. Y response or protease production can be expressed by the following regression equation:

$$Y=239.5+6.3X_1+22.0X_2+43.2\ X_3+16.2\ X_4+21.8\ X_6+33.2\ X_7+16.3\ X_8-0.1\ X_9-13.8\ X_{10}+26.8\ X_{12}+11.2\ X_{13}+14.3\ X_{14}$$

Factors or culture media components, having a significant effect on alkaline protease production by LS

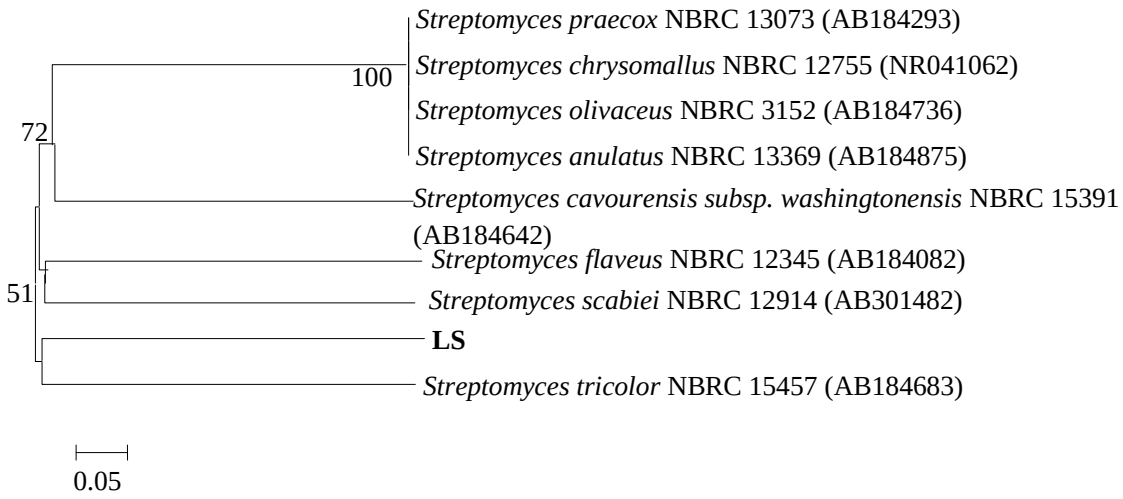

Figure 2. Neighbour-Joining phylogenetic tree based on 16S rRNA gene sequences showing LS strain and the nearest related taxa. (Numbers at nodes indicate percentages of 1000 bootstrap resamplings. Bar equals 0.05 nucleotide substitutions per site).

Table 5. Comparison of phenotypic characteristics of LS strain with related species of the group 11.

Phenotypic characteristics	**LS strain**	***Streptomyces tricolor*** **(Williams et al., 1989)**	***Streptomyces roseodiastaticus*** **(Williams et al., 1989)**	***Streptomyces rameus*** **(Williams et al., 1989)**	***Streptomyces bangladeshensis*** **(Al-Bari et al., 2005)**
Morphology:					
AM color	Cream, yellow to brown yellow	Grey	Grey	Grey	Yellow
SM color	Yellow to greenish grey (change to pink in basic medium)	Yellow, red or blue	nd	nd	Beige
DP	Brown yellow to brown (change to pink in basic medium)	Blue	No MP	No MP	Yellow. No MP
SCT	RF	S	S	S	RF
SNC	40	nd	nd	nd	8 to10
SF	cylindrical	nd	nd	nd	Nd
Hydrolysis of :					
D-glucose	+	nd	+	+	+
L-rhamnose	-	nd	+	-	+
D-mannitol	+	nd	nd	+	+
Mannose	+	nd	nd	nd	+
D-xylose	+	nd	+	+	+/-
Dextrine	+	nd	nd	nd	nd
D-galactose	+	nd	nd	+	+
Sucrose	-	nd	nd	+	+
L-arabinose	+	nd	+	+	+
D-fructose	+	nd	nd	+	+
Maltose	+	nd	nd	nd	+
Lactose	+	nd	nd	nd	-

Table 5. Contd.

Raffinose	-	nd	nd	+	+
i-inositol	+	nd	nd	-	+
Cellulose	+	nd	nd	nd	Nd
Other :					
H_2S production	-	nd	nd	nd	+
Growth temperature	28°C	nd	nd	nd	20 to 50°C
NaCl tolerance	7%	nd	nd	nd	2%

AM: aerial mycelium, SM: substrat mycelium, PD: diffusible pigment, MP: melanoide pigment, SCT: spore chaine type, SNC: spore number per chain, SF: spore form, RF: rectiflexibile, S: spiral, nd: not determined.

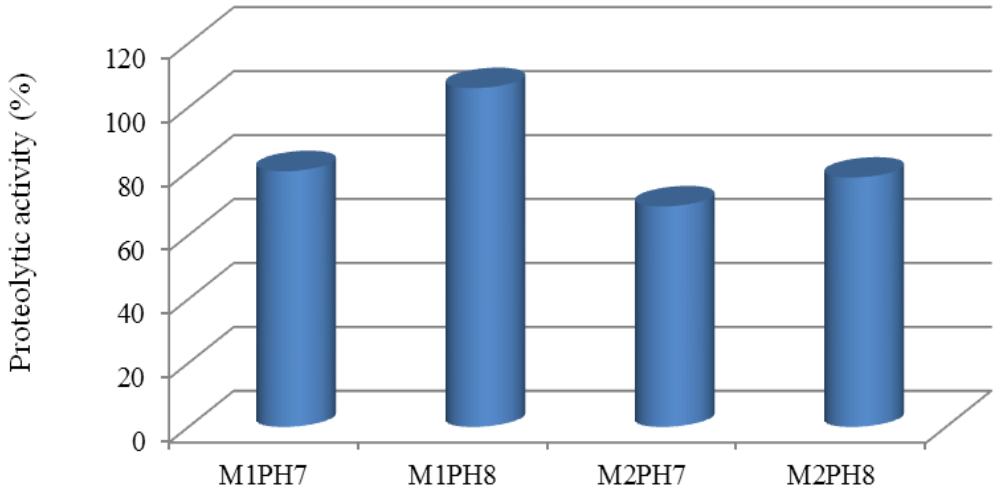

Figure 3. Proteolytic activity on two media broth M1 and M2.

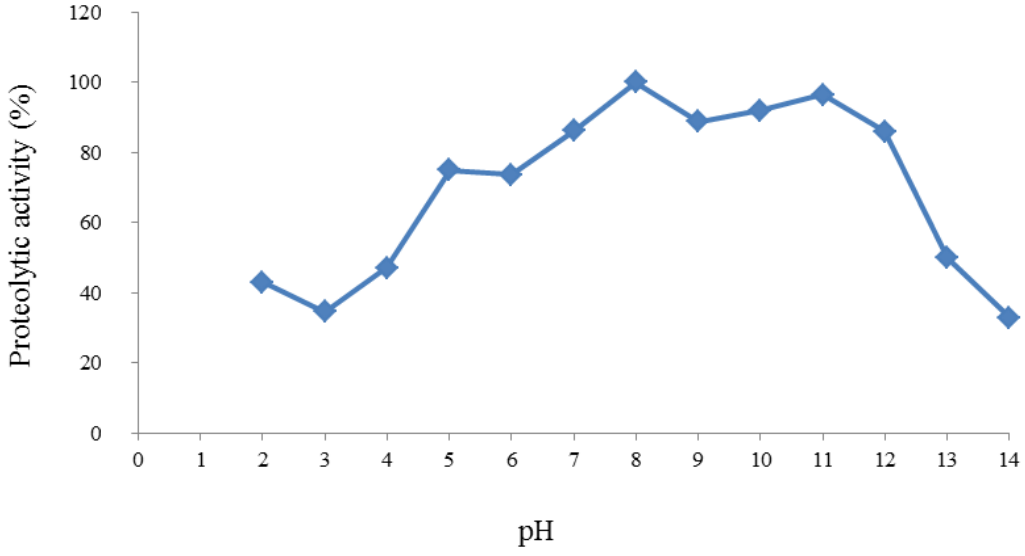

Figure 4. Effect of pH on protease activity.

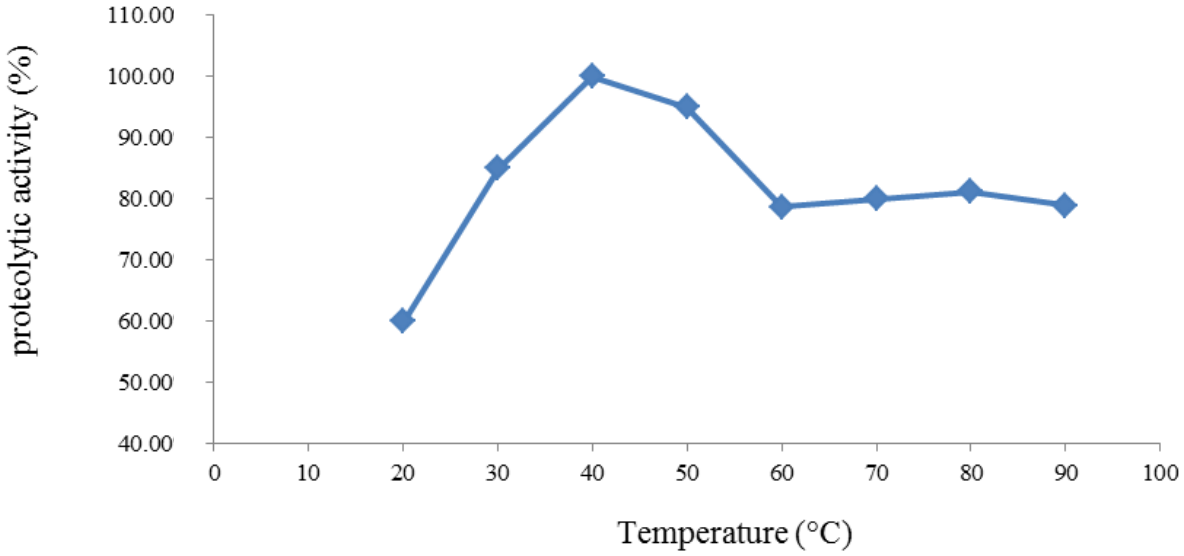

Figure 5. Effect of temperature on protease activity.

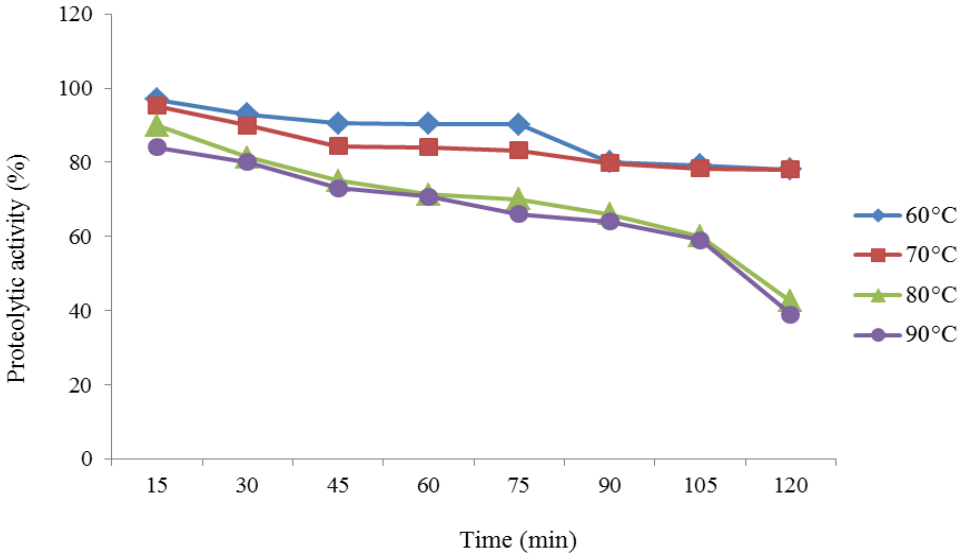

Figure 6. Protease thermal stability study.

strain are:

X_3:fructose.
X_7: malt extract.

(i)Fructose: is a simple sugar found naturally in fruits and honey but can be chemically synthesized. It is used in microbial culture media as a carbon source. Studies have shown that the addition of fructose to the culture medium increases significantly the protease production by *Alternaria* (Mandakini and Shastri, 1983). Others have shown a positive effect of fructose on acid protease production by *Aspergillus* sp. (Radha et al., 2012).
(ii) Malt extract: is obtained after a malted barley flour infusion; it contains, in addition to maltose, amino acids, vitamins and minerals. Several studies have demonstrated its positive effect on alkaline protease production from *Streptomyces* genus (Mostaf et al., 2012). Others found a negative effect of this compound (Elgammal et al., 2012). This is due to the high variability of the genus *Streptomyces*, in fact, their metabolism is different from specie to another, sub specie to another and even from one strain to another. Thus, each strain has its own conditions for growth and primary and

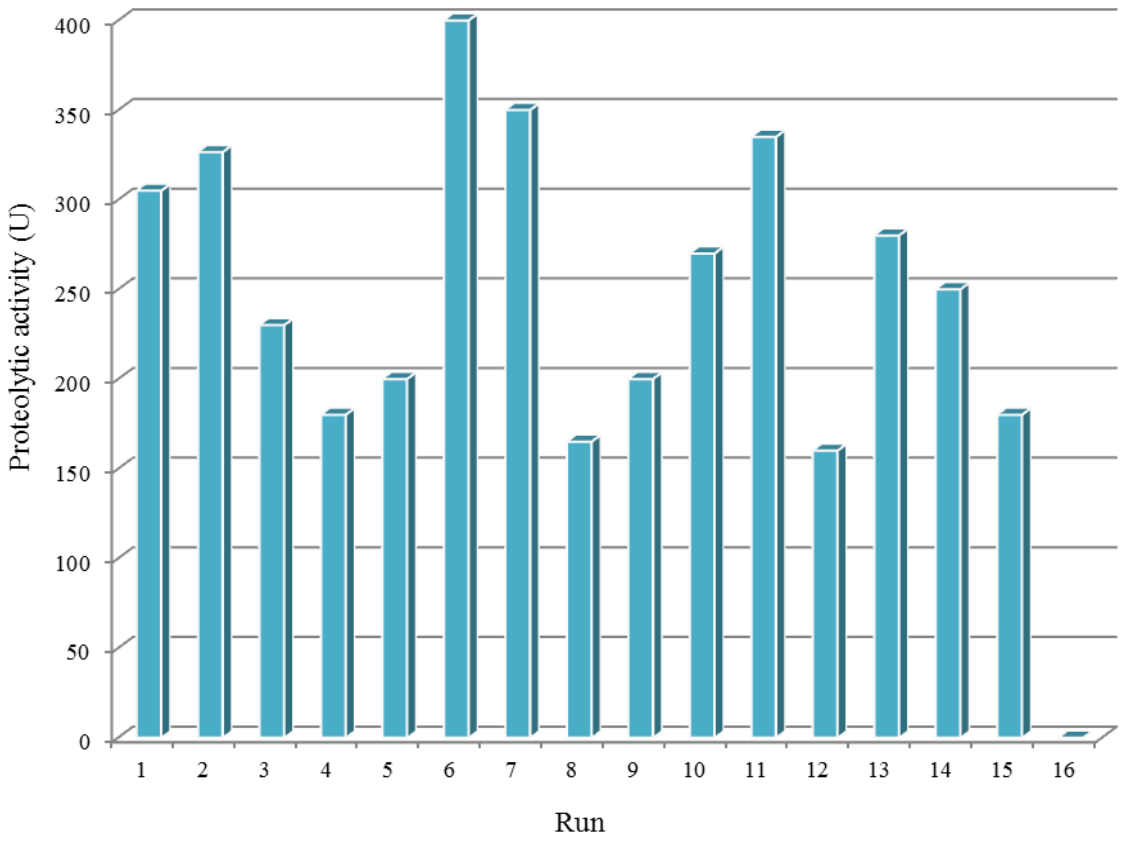

Figure 7. Protease activity according to Plackett and Burman design.

Table 6. Statistical parameters for Plackett and Burman design (Minitab17 software).

Term	Effect	Coefficient	T value	P value
Constante		239.5	7.46	0.005
X_1	12.7	6.3	0.20	0.856
X_2	43.9	22.0	0.68	0.543
X_3*	86.4	43.2	1.35	0.271
X_4	32.3	16.2	0.50	0.649
X_6	43.6	21.8	0.68	0.546
X_7*	66.4	33.2	1.26	0.299
X_8	32.7	16.3	0.51	0.646
X_9	-0.2	-0.1	-0.00	0.998
X_{10}	-27.7	-13.8	-0.43	0.695
X_{12}	53.6	26.8	0.83	0.465
X_{13}	22.3	11.2	0.35	0.751
X_{14}	28.6	14.3	0.45	0.686

T: Student test, p : probability, * : Statistically significant, R^2 = 65 %.

secondary metabolites production. On the other hand, malt extract is an organic nitrogen source which is an inducer of the production of a large amount of protease compared to inorganic sources (Wang et al., 2008).

A negative effect of casein on alkaline protease production by our strain was observed. Indeed, simple substrates such as casein and gelatin have a low solubility and give low enzyme activity yields in liquid medium. Many microbial protease production studies in liquid media have confirmed our results (Joo and Chang, 2005; Laxman et al., 2005; Tari et al., 2006; Chi et al., 2007; Abidi et al., 2008; Hajji et al., 2008 ; Abdelwahed et al., 2014).

In addition, Lazim et al. (2009) showed that the addition

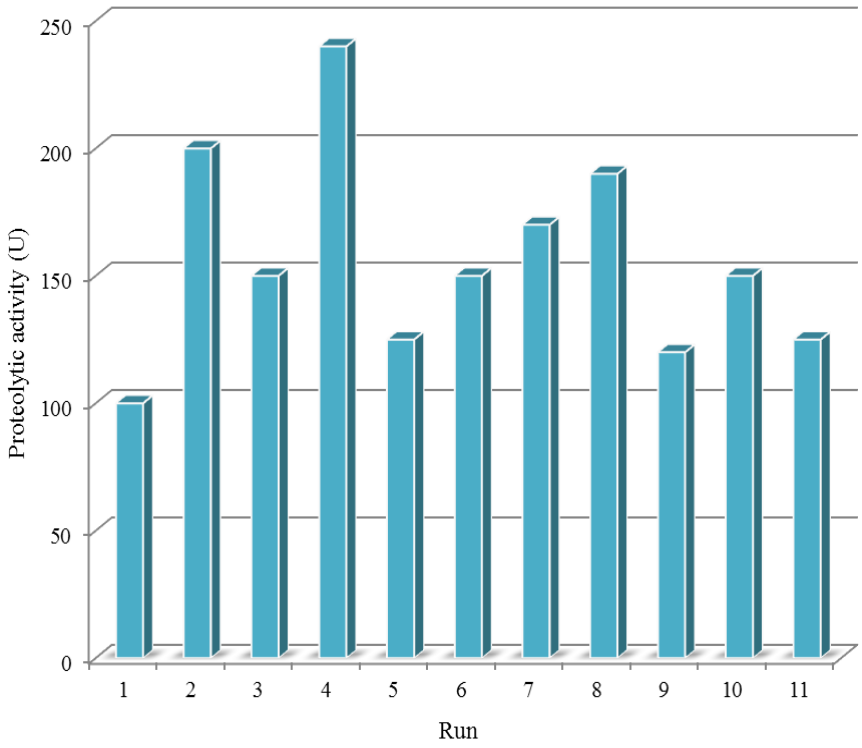

Figure 8. Protease activitiy according to central composite design.

Table 7. Statistical parameters for central composite design (Minitab17 software).

Term	Effect	Coeff	T value	P value
Constant	/	131.7	9.27	0.000
X_3*	63.42	31.71	3.64	0.015
X_7*	36.65	18.32	2.11	0.089
X_3X_3	22.7	11.4	1.10	0.323
X_7X_7*	45.2	22.6	2.18	0.081
X_3X_7	-5.0	-2.5	-0.20	0.847

Coeff : coefficient, T : Student, P : probability, * : Statistically significant.

of casein in the solid culture medium containing wheat bran did not increase the protease production by the *Streptomyces* sp.

Optimization of significant factors for protease production using centered composite design

LS strain proteolytic activities obtained from experimentally Box and Wilson design are shown in the (Figure 8). The best proteolytic activity is obtained on medium 4; it contains both X_3 and X_7 factors at their higher levels, confirming the positive effect of these factors on protease production. The statistical study conducted by Minitab 17 software is presented in Table 7, the Students values (3.64 for X_3 and 2.11 for X_7) confirms the significant effect of both X_3 and X_7 factors observed in Plackett and Burman design. The regression equation coefficients were calculated and the data was fit to a second-order polynomial equation. The response, protease production (Y) by LS *Streptomyces* strain can be expressed in terms of the following regression equation:

$$y = 131.7+31.71\ X_3+18.32\ X_7+11.4\ X_3^2+22.6\ X_7^2-2.5\ X_3X_7$$

The optimal coded values are obtained by calculation of partial derivatives of equation, which are converted into real values giving the optimal concentrations of selected factors (fructose and malt extract) (Table 8). Thus, the medium favoring better proteolytic activity of LS

Table 8. Coded and real values of X_3 and X_7 factors.

Factor	Coded values	Real values
X_3 : fructose	1.99940	7
X_7 : malt extract	1.99940	12

Streptomyces strain is composed of:

(i) Fructose (7 g/l)
(ii) Malt extract (12 g/l)

Conclusions and future perspectives

One of the strategies followed in the search for new metabolites is to isolate the actinomycete bacteria from unexploited ecosystems. Thus, Saltpans which are rare regions in the world seem to be promoter environments to isolate eventually new *Streptomyces* strains which can be sources of interesting or new molecules. The result of LS strain identification confirms this hypothesis. Indeed, the genotypic and phenotypic data show that strain LS forms probably a new specie within the genus *Streptomyces.*

Study of enzyme production and its properties show the high biotechnological potential of this *Streptomyces* strain for the production of the thermostable alkaline protease. Application of statistical methods has allowed the selection of two factors having a significant effect on the production of the enzyme (fructose and malt extract), then theirs optima (7 g/l of fructose and 12 g/l of malt extract). These results open the way to other works like the identification of the LS strain by DNA - DNA hybridization (with related species determined during 16S rRNA gene sequence analysis) and the development of enzyme purification protocol.

Conflict of Interests

The authors have not declared any conflict of interests.

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