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Extracellular Highly Thermostable α-Amylase from a Strain of *Lactobacillus fermentum*: Production and Partial Characterization

Frédéric Tavea[1], Bertrand Tatsinkou Fossi[2,*], Nchanji Gordon Takop[2], Robert Ndjouenkeu[3]

[1]Department of Biochemistry, University of Douala, Cameroon
[2]Department of Microbiology and Parasitology, University of Buea, Buea, Cameroon
[3]Department of Food Science and Nutrition, ENSAI, University of Ngaoundéré, Ngaoundéré, Cameroon

Abstract Lactic acid bacteria are potential source of enzymes that can be used in food biotechnology because they are generally regarded as safe. A highly thermostable α-amylase producing lactic acid bacterium, *Lactobacillus fermentum* 04BBA19 isolated from a soil of the western region of Cameroon was characterized for its starch degrading activity and biochemical properties. The bacterium exhibited maximal production of the enzyme at temperature 45°C, and within pH range of 4.0 to 5.0. The main environmental conditions affecting enzyme productivity were the nature of substrate, the nitrogen source, the minerals content and the presence of surfactants. The enzyme was identified as an α-amylase fragment exhibiting maximum activity and stability in temperature and pH ranges of 60-70°C and 4.0-7.0 respectively, with a very high thermostability revealed by the retention of 100% of original activity after pre-incubation for 30 min at 80°C. The stability improved considerably with addition of 0.1% (w/v) $CaCl_2.2H_2O$; the half live of the enzyme under the above conditions was 6 h at 80°C.

Keywords Lactic acid bacteria, Starch hydrolysis, Thermostable α-amylase, Fermentation

1. Introduction

Thermostability is one of the main features of many enzymes sold for bulk industrial usage. Thermostable α-amylases are of interest because of their potential industrial applications. They have extensive commercial applications in starch liquefaction, brewing, sizing in textile industries, paper and detergent manufacturing processes. [1-3]

Several thermostable α-amylases have been purified from *Bacillus* sp. and the factors influencing their thermostability have been investigated, but the thermostability of amylases from lactic acid bacteria has attracted very few scientific attentions. *Lactobacillus amylovorus, Lactobacillus plantarum, Lactobacillus manihotivorans, and Lactobacillus fermentum* are some of the lactic acid bacteria exhibiting amylolytic activity which have been studied [4-9]. However, most of α-amylases from these bacteria presented weak thermostability compared to those of genus *Bacillus*.

Owing to the important acidification of fermentation medium by most lactic acid bacteria, the production of thermostable amylase by a lactic acid bacterium under submerged or solid-state fermentation can help to reduce the risk of contamination caused by undesirable micro-organisms during the fermentation. [10, 11]. Another advantage is the non-pathogen character of the genus *Lactobacillus* that allows their utilization in food fermentation processes. The present study deals with the production and characterization of thermostable α-amylase from a lactic acid bacterium, *Lactobacillus fermentum* 04BBA19, isolated from starchy soil of the western region of Cameroon

* Corresponding author:
tatsinkoufossi@yahoo.fr (Bertrand Tatsinkou Fossi)
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2. Materials and Methods

2.1. Origin and Nature of the Microorganism

The starch degrading amylolytic lactic acid bacterial strains were isolated from the soil of a flour market in Bafoussam, a city of the western region of Cameroon. [12]. One of the isolates designated 04BBA19 was selected and identified using API 50 CH test kit (bioMerieux France) as *Lactobacillus fermentum* and used for this study.

2.2. Growth and Enzyme Production

The micro-organism was propagated at 40°C for 48 hours in 50 ml of a basal medium containing 1% (w/v) of soluble

starch, 0.5% (w/v) of yeast extract placed in 100 ml Erlenmeyer flask with shaking at 150 oscillations per minute in an alternative shaker (Kotterman, Germany). The initial pH of the basal medium was adjusted to 6.5 using 0.1 M HCl or 0.1 M NaOH. Cell growth was evaluated by reading the absorbance of culture medium at 600 nm, followed by enumeration on plate counting agar. In order to evaluate the capacity of micro-organism to acidify the culture medium, the pH of the fermented broth was measured using an electronic pH meter (Mettler Seven S20, Japan). After removal of cells by centrifugation (8000xg, 30 min, 4°C) (Heraeus, Germany), the supernatant was considered as the crude enzyme solution.

The amylase production was optimized by studying the effect of cultural and environmental variables (carbohydrate and nitrogen sources, metals, surfactants) individually and simultaneously. The carbohydrate sources tested were: glucose, fructose, maltose, amylose, amylopectine, cassava raw starch and soluble starch at concentration of 1% (w/v)), nitrogen sources (soya bean meal, yeast extract, peptone, ammonium sulphate and urea at concentration of 1.5% (w/v)), metal salts ($CaCl_2.2H_2O$, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, NaCl at concentration of 0.1% (w/v)) and surfactant (Tween 80 and Tween 40 at concentration of 1.5% (v/v)). All media were autoclaved at 121°C for 20 min, while for the medium containing raw cassava; starch powder was *sterilized* at 120°C for 2 h in a hot oven and used as *raw cassava starch* for enzyme production.

The effect of carbon sources was studied by replacing soluble starch with different carbohydrates.

2.3. Enzyme Purification and Assay

The culture supernatant was supplemented with solid ammonium sulphate to 65% (w/v) final concentration, with mechanical stirring at 4°C. The suspension was retained for 1 h at 4°C, and centrifuged at 8000 g for 30 min at the same temperature. The resultant supernatant was brought to 70% w/v ammonium sulphate saturation at 4°C. 50-70% (w/v) ammonium sulphate precipitate was recovered, dissolved in 0.1 M phosphate buffer and dialysed with Spectra/PorR, VWR 2003 dialysis membrane overnight against the same buffer at 4°C.

Affinity chromatography was carried out by the modified technique described by Zang *et al*. (1994) [13]. The modification was using of insoluble cassava starch instead of insoluble potato starch as support for the affinity column chromatograph. Before packing on the column (ϕ1,5x15cm), the cassava starch (25%) was swollen in 30 % $(NH_4)_2SO_4$ at 70°C. The concentrated supernatant was applied directly to column at 4°C and washed with 0.5M NaCl to remove impurities. The amylase was eluted with 0.05 M Na_2CO_3 at elution rate of 3 ml x $20.min^{-1}$ at room temperature (25°C).

The activity of a-amylase was assayed using modified method of Giraud et al. (1993) [14]. In a typical run, 5mL of 1% (w/v) soluble starch solution and 2mL of 0.1 mol/ L phosphate buffer (pH 6.0) were mixed and maintained at 60°C for 10 min, then 0.5mL of appropriately diluted enzyme solution was added. After 30 min the enzyme reaction was stopped by rapidly adding 1mL of 1 mol/L HCl into the reaction mixture. For the determination of residual starch, 1mL of the reaction mixture was added to 2.4mL of an iodine solution containing 3% (w/v) KI, 0.3% (w/v) I2 diluted to 4% (v/v) and its optical density was read at 620nm using a Secomam Prim Visible Light Spectrophotometer, 230 VAC. One unit of α-amylase activity (U) was defined as the amount of enzyme able to hydrolyze 1 g of soluble starch in 60 min under the experimental conditions. All the values presented are means of three replicates.

An aliquot of 0.5 ml of purified enzyme preparation was subjected to react with a specific substrate (2ml containing 1g/l of blocked p- nitrophenyle maltoheptaoside). This substrate known to be hydrolysed only by α-amylase [15], a positive reaction, was characterized by the appearance of yellow compound with maximum absorption at 530 nm.

In order to determine the specific activity and purification fold, protein was estimated using the method of Bradford (1976) [16] with pure casein as the standard.

2.4. Measurement of Active Component and Molecular Weight of Amylase

To determine the homogeneity and molecular weight, the enzyme preparation and known molecular markers (Phosphorylase b: 94000 Da, Catalase: 60000 Da, Pepsine: 34700 Da, Casein: 25000 Da, Trypsin: 24000 Da) were subjected to PAGE-SDS electrophoresis using homogenized 10% (w/v) acrylamide gel. After electrophoresis, the gel was stained for 4 h with 0.25% (w/v) coomasie blue R250 dye in methanol acetic acid-water solution (50/5/45, by volume), and destained in methanol acetic-water solution (80/10/10, by volume) without dye. The SDS was then removed by washing the gel successively with distilled water and with 50 mM phosphate buffer (pH 6.0). After washing, the gel was incubated at room temperature in 50 mM phosphate buffer solution pH 6.0 for 24 h, followed by a second incubation at room temperature in 0.5% (w/v) soluble starch solution for 24 h. The amylase activity was revealed by staining in 4% (v/v) diluted iodine solution (I_2, 1g/l; KI, 30 g/l).

2.5. Effect of Temperature and pH on Activity and Stability

The optimal temperature for amylase activity was determined by assaying activity between 30 and 100°C for 30 min in 50 mM phosphate buffer.

Measurement of optimum pH for amylase activity was carried out under the assay conditions for pH range of 3.0-10.0, using 50mM of three buffer solutions: Tris-HCl (pH 3.0), Na_2HPO_4-Citrate (pH 4.0 – 6.0), and Glycine-NaOH (pH 7.0-10.0).

The temperature stability was determined by incubating the purified enzyme solution in water bath for temperature range of 30-100°C for 30, 60, 90, 120, 180 min and then

cooled with tap water. The remaining α-amylase activity was measured as described above. The first order inactivation rate constants, k_i were calculated from the equation: $lnA = lnA_0 - k_i t$, where A_0 is the initial value of amylase activity and A the value of activity after a time t (min).

For the determination of pH stability, the enzyme was incubated in a water bath at 60°C at varying pH for 30 min. The residual activity was detected under the same conditions and expressed as the percentage of the activity of untreated control taken as 100%.

2.6. Effect of Metal Salts and Chelating Agent

The effect of metal salts and EDTA on amylase activity was determined by adding 0.05 to 0.1% (w/v) of metal salts ($CaCl_2.2H_2O$, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, NaCl, $FeCl_3$, $CuSO_4.5H_2O$) and EDTA to the standard assay. The effect of metal salt and chelating agent on amylase activity was evaluated by pre-incubating the enzyme in the presence of the above effectors for 30 min at 70°C. The remaining activity was determined as described above.

2.7. Examination of the Effect of Enzyme on Raw Starch Granules

In order to visualize the effect of enzyme on raw starch granules, 1ml of raw cassava starch suspension (0.5% w/v) was stained with iodine solution and examined by light microscopy (Olympus microscope BH-2). After treatment with 0.1 ml of purified amylase solution from *Lb. fermentum* 04BBA19, the examination of treated starch was carried out under the same conditions by microscopy.

3. Results and Discussion

Enzyme production

In the presence of starch as carbon source at 40°C, *Lb. fermentum* 04BBA19 strain grew and released amylolytic activity in the culture medium. Growth and amylase synthesis curves show the same profile (Figure 1). The level of amylase production increased during the exponential phase of growth. The amylase production pattern in *Lb. fermemtum* 04BBA19 indicates that the induction of amylase took place during the lag phase in the presence of starch. Cell growth and amylase production reached maxima values at the same time (40 h. of fermentation). The values of those maxima were $1.1x10^8$ cfu/ml and 107.3±0.5 U/ml for cell growth and amylase activity respectively. Such coincidence shows that amylase production by *Lb. fermentum* 04BBA19 was tightly linked to cell growth. This phenomenon is generally attributed to the nutritional activity of the bacterium, which needs glucose for its growth. Since the extracellular medium contains starch which cannot cross the cell membrane for bacterium nutrition, cells secrete amylase for breaking down starch molecules and releasing oligosaccharides such as glucose which can then enter into the cell for the bacterium nutrition.

This relationship between cell growth and amylase production was confirmed by soluble protein synthesis during fermentation (Fig. 1). Soluble protein production increased during fermentation, the highest values (0.72±0.04 mg/ml) occurred at the same time with maximum of growth and amylase activity.

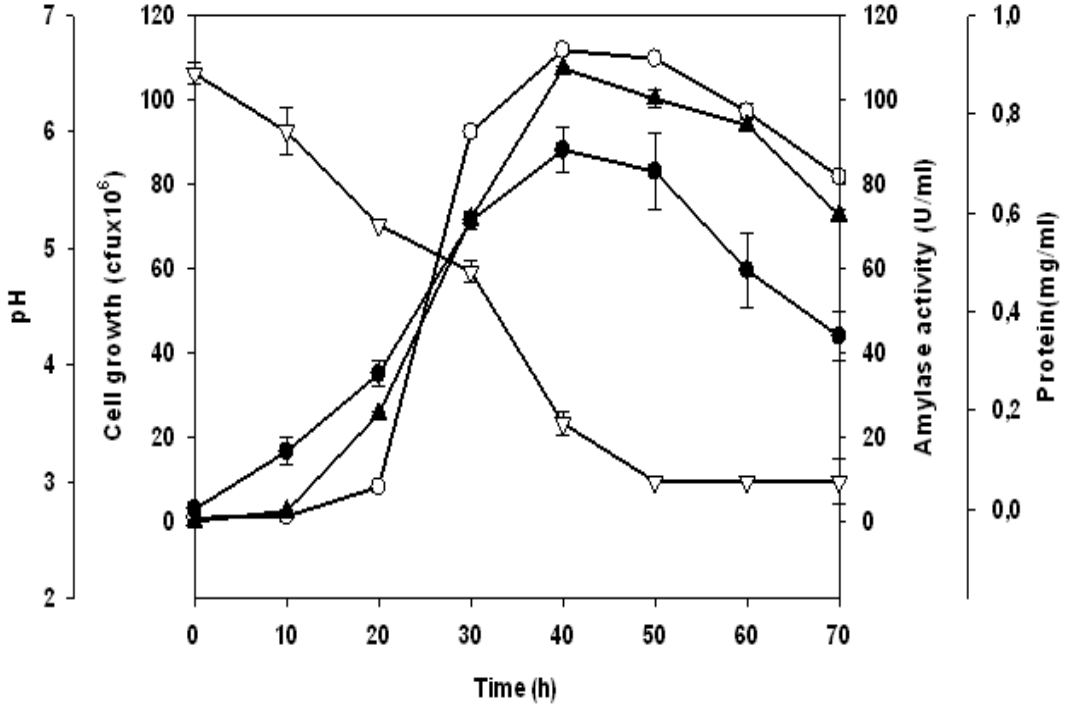

Figure 1. Time course of growth (▲), pH (▽), amylase (○) and soluble protein (●) production by *Lactobacillus fermentum* 04BBA19 in 1% (w/w) soluble starch medium at 40°C, pH 6.0. The data shown are averages of triplicates assays within 10% of the mean value

The decline of cell growth and amylase production after the peak can be attributed to the rise of glucose concentration resulting from the degradation of starch, or to the rise of protease levels [17]. The decline of cell growth is also attributed to high acidification of the medium; *Lactobacillus* genus as many lactic acid bacteria are known as lactic acid producers [10]. This acidification was expressed by the decrease of initial pH of culture broth (Fig 1). The pH of culture broth declined considerably and reached value of 3.0 around 50 h of incubation and still constant.

The study of cell growth and amylase production as a function of temperature (Fig. 2) showed that *Lb. fermentum* 04BBA19 is a thermophile micro-organism, since its maximum growth temperature 45°C, coincides with optimum temperature of amylase production. In addition, cell growth and amylase synthesis as a function of temperature, presented the same profile, confirming thus the strong relationships between the two phenomenons. Many other investigators reported that maximum amylase production occurred at the optimum growth temperature [18, 19]. These results are contrary to the findings of Chandra *et al*. (1980) [20] who studied the growth and amylase production of *Bacillus licheniformis* CUM 305. They have observed that this micro-organism grew very well at 30°C, but did not produce α-amylase at that temperature. In addition, Saito and Yamamoto (1975) [21] found α-amylase production at 50°C and cell growth at a temperature lower than 45°C for another strain of *B. licheniformis*.

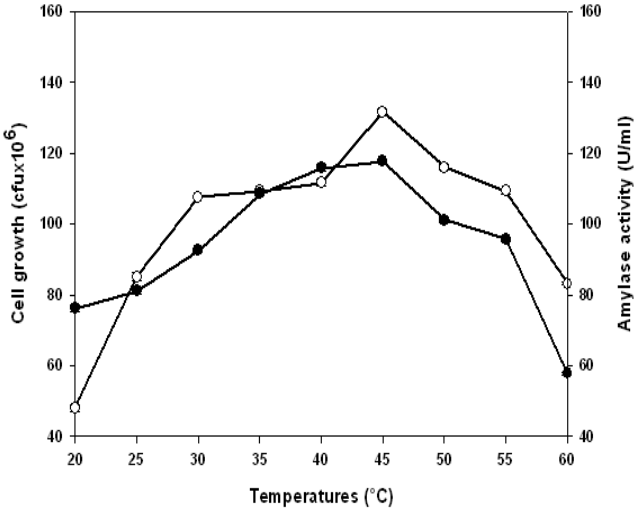

Figure 2. Effect of temperature on growth (●) and α-amylase production (○) by *Lb. fermentum* 04BBA19 grown in 1 % (w/w) soluble starch medium, pH 6.0. The data shown are averages of triplicate assays with SD within 10% of mean value

Amylase production is known to be induced by a variety of carbohydrate, nitrogen compounds and minerals [22, 23]. In order to achieve high enzyme yield, attempts are been made to develop a suitable medium for proper growth and maximum secretion of enzyme, using an adequate combination of carbohydrates, nitrogen and minerals [19]. In this respect, from the use of different carbon sources in the present study, soluble starch proved to be the best inducer of amylase production (Table 1). In the presence of soluble starch at concentration of 1% (w/v), the enzyme yield reached 107.0±1.2 U/ml after 48 hours of fermentation, while in the presence of raw cassava starch at the same concentration, the enzyme yield was 67.1±0.5 U/ml. This imply that amylase from *Lb. fermentum* 04BBA19 is able to hydrolyze raw starch, although at lower extend than soluble starch. Since amylase yield is higher with amylose (92.3 U/ml) as carbohydrate source than with amylopectin (50.1 U/ml), the *Lb. fermentum* 04BBA19 amylase is more efficient for hydrolysis of alpha-1,4 linkages than for hydrolysis of alpha-1,6 ones.

Table 1. Effect of different carbon sources on amylase production by *Lb. fermentum* 04BBA19 fermentation at 45°C

Carbon source (1% w/v)	Enzyme yield (U/ml)
Glucose	0.0±0.0[a*]
Fructose	0.0±0.0[a]
Maltose	0.0±0.0[a]
Amylose	92,3±3.1[b]
Amylopectin	50.1±0.5[c]
Cassava starch	67.1±0.5[d]
Soluble starch (Basal medium)	180.5±0.3

The amylase production increased with the soluble starch concentration (Fig. 3), the optimum production attained at the concentration range of 8-12% (w/v), with an enzyme yield of 180.5 ± 0.3 U/ml. The optimization of this basal medium by supplementation using different sources of nitrogen, mineral and surfactants showed that, when these supplements are used individually with soluble starch, they improve the enzyme production, except $CuSO_4.5H_2O$. Soya bean, $CaCl_2.2H_2O$, and Tween-80 appeared to be the best nitrogen, mineral and surfactant sources respectively for amylase production (Table 2).

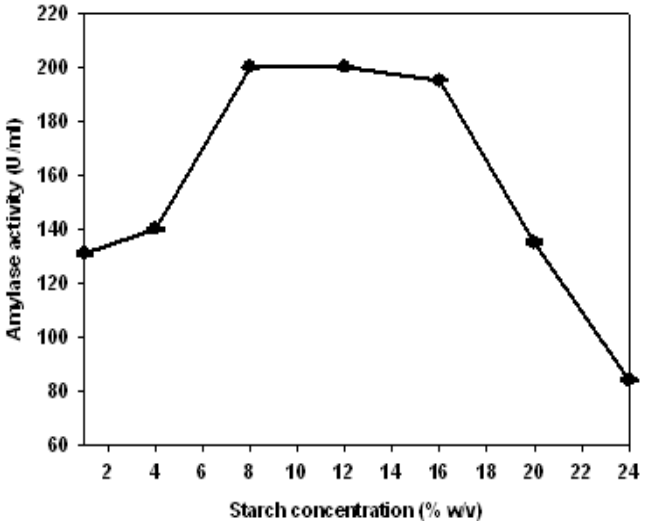

Figure 3. The effect of starch concentration on enzyme production by *Lb. fermentum* 04BBA19. The data shown are averages of triplicate assays with SD within 10% of mean value

Table 2. Effect of different supplements on α-amylase production by *Lb. fermentum* 04BBA19 in submerged state fermentation at 45°C

Media	Enzyme yield (U/ml)
Basal medium (Soluble starch 8 % w/v)	180.5±0.3^{ef*}
Nitrogen source (1.5% w/v)	
Soya bean meal	498.5±1.8^{b}
Yeast extract	250.2±2.3^{d}
Peptone	200.3±1.7^{f}
Ammonium sulphate	198.4±1.5^{f}
Urea	196.5±0.3^{f}
Minerals (0.1% w/v)	
$CaCl_2$. $2H_2O$	494.1±0.6^{b}
$MgSO_4$. $7H_2O$	355.1±0.4^{c}
FeSO4. $7H_2O$	237.3±0.7^{d}
NaCl	315.2±0.9^{c}
Surfactants (1,5% w/v)	
Tween-40	209.5±0.4^{e}
Tween-80	215.1±0.3^{e}
Optimized medium	**687.3±0.4^{a}**

The data shown are averages of triplicate assays with SD within 10% of mean value. *Means with different superscripts within columns are significantly different ($p<0.05$).

Though all nitrogen sources are positively influencing enzyme production by *Lb. fermentum* 04BBA19, an inverse behaviour has been observed with other bacterial strains. For instance, Tanyildizi *et al*. (2005) [24] reported zero effect of yeast extract on amylase production by *Bacillus* sp.

Enzyme properties

The amylase extract after ammonium sulphate fractionation and dialysis, binds to a column of starch, displaying two protein peaks (Fig. 4). The protein constituting the first peak did not present any amylase activity, while the protein of the second peak was active. This protein represented 55.4% of the original enzyme, with a specific activity of 1253.4 U/mg and 41.2 purification fold (Table 3). The SDS-PAGE analysis of the protein displayed a single band, confirming the purity of the enzyme. The zymogram obtained from incubation of the gel in the presence of starch, followed by staining using 4% (v/v) iodine ($KI+I_2$) solution showed a clear zone around the band, confirming amylolytic activity of the protein. On the other hand, the enzyme was able to hydrolyze blocked p-nitro phenyl methyl heptaoside, releasing a yellow compound (p-nitro phenol) with maximum absorption at 530 nm. This result was a proof that amylase from *Lb. fermentum* 04BBA19 is an endo acting amylase (α-amylase) because the blocked p-nitro phenyl methyl heptaoside is known to be hydrolysed only by endo-acting amylases [15].

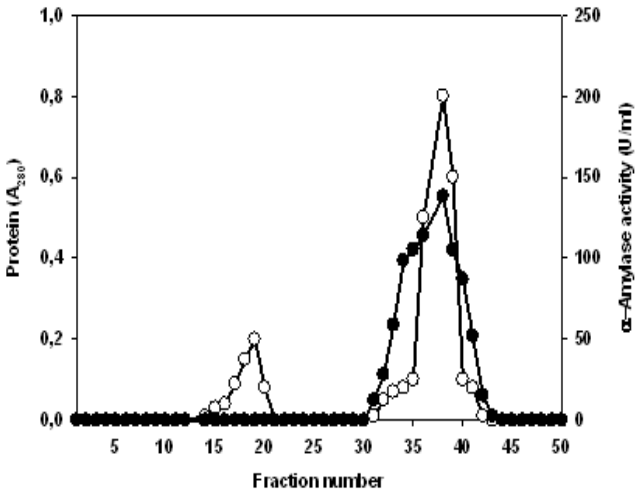

Figure 4. Elution pattern of starch column chromatograph (ϕ1.5x15cm), of *Lb. fermentum* 04BBA19 α-amylase; absorbance at 280 nm (●), α-amylase activity (○). Column (ϕ1.5x15 cm) was washed with 0.25 M NaCl to remove impurities; α-amylase from *Lb. fermentum* 04BBA19 was eluted with 0.05 M Na_2CO_3 at the flow rate of 3ml/20 min

The estimated molecular weight of this α-amylase was 95,000 Da (Fig.5). This molecular weight is near to the mean value of 100,000 Da of the majority of amylases from lactic acid bacteria. Talamond *et al*. (2002) [15] found the value of 106,000 Da for α-amylase from *Lb. fermemtum* Ogi E1., 100,000 Da for α-amylase from *Lb. manihotivorans* and 99500 Da for α-amylase from *Lb. plantarum*. Sidhu *et al.* (1997) [25] attributed these differences of the molecular weights of α-amylases to the gene corresponding to organism. In addition, determination techniques of molecular weight can also explain these differences [25].

Table 3. Purification and overall recovery of *Lb. fermentum* 04BBA19

Purification stage	Volume (ml)	Total α-amylase activity (U)	Protein (mg)	Specific activity (U/mg)	Yield (%)	Purification fold
Original broth	150.0	160502.0	527.3	30.4	100.0	1.0
Supernatant	112.3	12016.1	210.7	57.0	74.8	1.9
Ammonium sulphate precipitation and Dialysis	45.4	9889.9	102.4	96.5	61.6	3.2
Starch column chromatography	30.1	8899.7	7.1	1253.4	55.4	41.2

The data shown are averages of duplicate assays with SD within 10% of mean value.

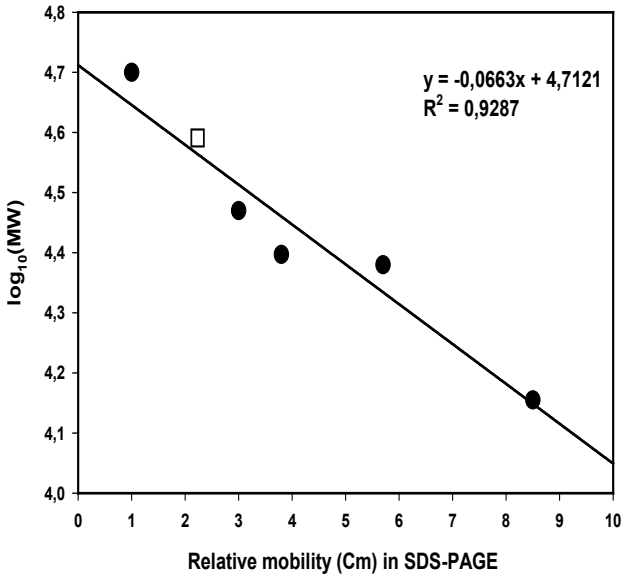

Figure 5. Determination of relative molecular weight of α-amylase from *Lb fermentum* 04BBA19 in SDS-PAGE electrophoresis, (●): standard proteins, (□):α-amylase from *Lb fermentum* 04BBA19

The enzyme exhibited maximum activity at 60-70°C and maintained 100% of its initial activity up to 80°C during 30 min of heat treatment (Fig 6). When the enzyme was treated, for the same time (30 min.) at 90°C and 100°C, the remaining activities were 90 and 87% respectively. These results showed the thermophilic character and very high thermostability of α-amylase from *Lb. fermentum* 04BBA19. In general, lactic acid bacteria do not produce amylases. However, this property have been observed in some genus of lactic acid bacteria, especially in *Lb. plantarum* [4], *Lb. amylovorus* [4], *Lb. manihotivorans* [27, 5], *Lb. fermentum* OGI E1 [15]. But amylases produced by these strains are not thermostable. Traditionally high thermostable and thermophiles amylases are found in *Bacillus* and *Thermococcus* genus: *B. amyloliquefaciens* [28]; *B. licheniformis* [29]; *B. stearothermophilus* [30]; *B. subtilis* [31], *T. aggreganes* [32], *T. profundus* [33], *B. cohnii* US147 [3].

Fig 7 shows the thermostability pattern of α-amylase from *Lb. fermentum* 04BBA19 at 80°C, 90°C and 100°C when the time of heat treatment is beyond 30 min. Table 4 presents the thermal inactivation rate constant (k_i) and half-life (T) at these temperatures. The half-life of this enzyme is higher than that of α-amylase from *B. licheniformis*: 120 min at 70°C [36]. The thermal stability was considerably improved by addition of 0.1% (w/v) $CaCl_2.2H_2O$. Goyal *et al.* (2005) [19] obtained at 80°C a half-life value of 3.5 h with α-amylase from *Bacillus* sp.I-3 in the presence of 0.1% (w/v) calcium chloride, while in the same conditions; α-amylase from *Lb. fermentum* 04BBA19 displayed a half-life value of 364.8 min (6.1 h).

Due to its high thermostability, α-amylase from *Lb. fermentum* 04BBA19 will be highly competitive in industrial bioconversion reactions, as compared to α-amylase from *Bacillus*. In addition, this competitiveness is enhanced by the fact that lactic acid bacteria, due to their non-pathogenic character [9], are easily used in food industry.

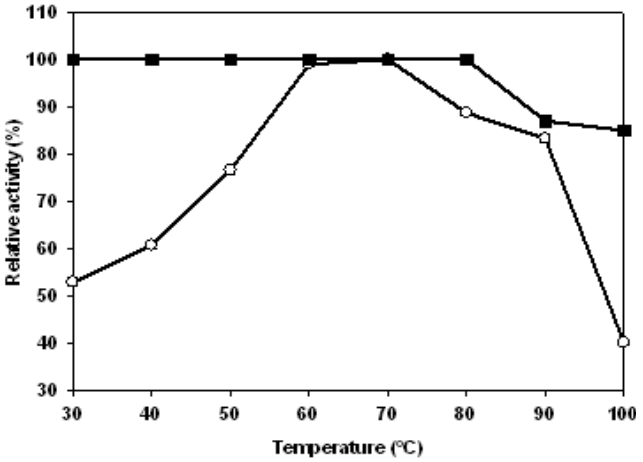

Figure 6. Effect of temperature on the activity (○) and thermal stability (■) of *Lb. fermentum* 04BBA19 α-amylase. The reaction mixture contained 5 ml substrate (1% w/v soluble starch in 100 mM; phosphate buffer, pH 6.0) and 0.5 ml partial purified enzyme solution. The mixture was incubated for 30 min at various temperatures (30-100°C) under standard enzyme assay condition. The enzyme displayed maximal activity at (60-70°C). For determination of the thermostability of amylase, the enzyme was pre-incubated at optimum pH, for 30 min at temperatures range of 30-100°C. The remaining activity was determined incubating the enzyme at optimum temperature, 60°C for 30 min. The data shown are averages of triplicate assays with SD within 10% of mean value

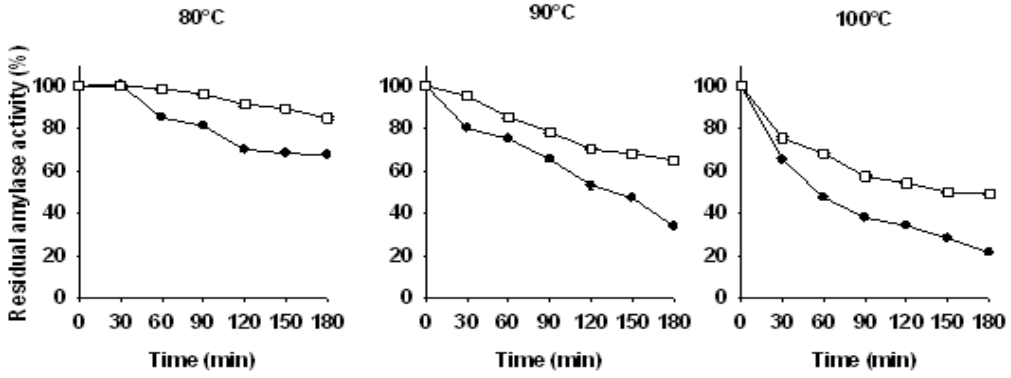

Figure 7. Thermostability pattern of α-amylase from *Lb. fermentum* 04BBA19, at 80, 90, 100°C without $CaCl_2.2H_2O$ (■) and with 0.1% w/v $CaCl_2.2H_2O$ (□) at different temperatures (80, 90, 100°C). The data shown are averages of triplicate assays with SD within 10% of mean value

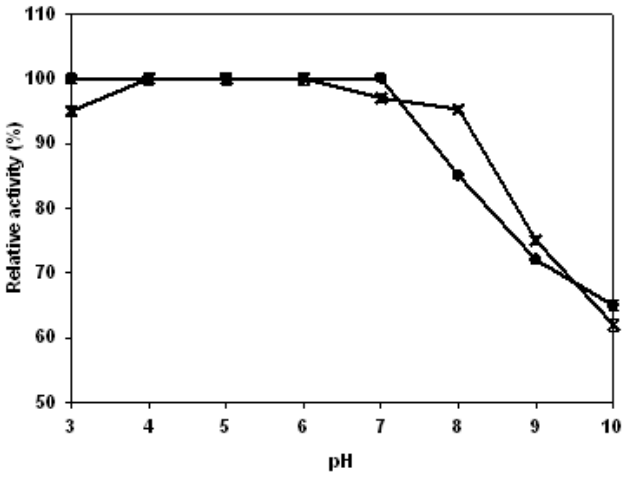

Figure 8. Effect of pH on the activity (**x**) and stability (■) of *Lb. fermentum* 04BBA19 α-amylase. The data shown are averages of triplicate assays with SD within 10% of mean value

The *Lb. fermentum* 04BBA19 α-amylase is active and stable in pH range of 4.0 – 7.0 (Fig. 8), which is the pH range of many foods. In this respect, this amylase could be used in starch hydrolysis, brewing and baking.

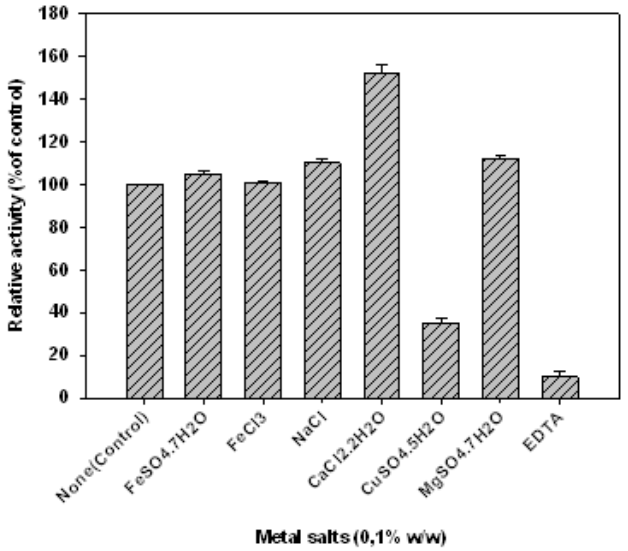

Figure 9. Effect of metal salts and EDTA on the activity of α-amylase from *Lb. fermentum* 04BBA19. The data shown are averages of triplicate assays with SD within 10% of mean value

The metal salts generally act on activity of enzyme through their cation, thus enzyme activity was increased by Ca^{2+}, while Fe^{2+}, Fe^{3+}, Na^{+} and Mg^{2+} have less significant effect. On the contrary Cu^{2+} and EDTA were inhibitors (Fig. 9). The behaviour of the enzyme towards metal ions, particularly calcium, indicates its metalloenzyme nature, which is confirmed by the action of EDTA.

The ability of α-amylase from *Lb. fermentum* 04BBA19 to hydrolyse raw starch was confirmed by microscopic observation of untreated and treated cassava starch granules. The photonic micrograph (Fig 10) indicates that cassava starch granules were damaged after treatment with α-amylase from *Lb. fermentum* 04BBA19. Similar results were observed by Goyal *et al* (2005) [19] for α-amylase from *Bacillus* sp I-3 with potato starch granules using scanning electron microscopy.

α-Amylase from *Lb. fermentum* 04BBA19 which is a soil isolate is more thermostable than those of other known lactic acid bacteria. Due to its properties, this enzyme is a good candidate for starch hydrolysis at high temperature. An economical process could be attained through the use of this enzyme at the liquefaction stage at high temperatures.

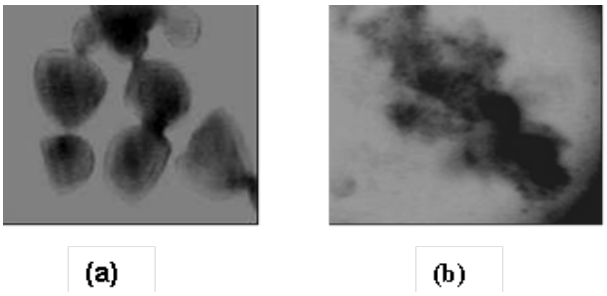

Figure 10. Photonic micrograph (x600) of untreated raw cassava starch granules (a) and deformed granules (b) after been treated with α-amylase from *Lb. fermentum* 04BBA19

4. Conclusions

The lactic acid bacterium tested in this study produced amylase with high thermostability, which is not common in lactic bacteria group. The use of this strain in bioprocessing involving amylase production will be advantageous. Because lactic acid bacteria are generally regarded as safe.

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