Research Article

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Production and characterization of psychrophilic α -amylase from a psychrophilic bacterium, *Shewanella* sp. ISTPL2

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Abstract: A psychrophilic and halophilic bacterial isolate, *Shewanella* sp. ISTPL2, procured from the pristine Pangong Lake, Ladakh, Jammu and Kashmir, India, was used for the production and characterization of the psychrophilic and alkalophilic α -amylase enzyme. The α -amylase is a critical enzyme that catalyses the hydrolysis of α -1,4-glycosidic bonds of starch molecules and is predominately utilized in biotechnological applications. The highest enzyme activity of partially purified extracellular α -amylase was 10,064.20 U/mL after 12 h of incubation in a shake flask at pH 6.9 and 10 °C. Moreover, the maximum intracellular α -amylase enzyme activity (259.62 U/mL) was also observed at 6 h of incubation. The extracellular α -amylase was refined to the homogeneity with the specific enzyme activity of 36,690.47 U/mg protein corresponding to 6.87-fold purification. The optimized pH and temperature for the α -amylase were found to be pH 8 and 4 °C, respectively, suggesting its stability at alkaline conditions and low or higher temperatures. The amylase activity was highly activated by Cu^{2+} , Fe^{2+} and Ca^{2+} , while inhibited by Cd^{2+} , Co^{2+} and Na^{2+} . As per our knowledge, the current study reports the highest activity of a psychrophilic α -amylase enzyme providing prominent biotechnological potential.

Keywords: α -amylase; psychrophilic; alkalophilic; *Shewanella* sp. ISTPL2.

Abbreviations

BSA, bovine serum albumin; CAZy, Carbohydrate-Active enZymes; CDW, cell dry weight; GH, glycoside hydrolase; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

1 Introduction

Enzymes are complex biocatalysts that accelerate the metabolic reactions of a biological system [1]. Due to their chemical stability, inexpensive cost, feasibility, productivity and environmental sustainability, microbial enzymes gain a lot of attention [2]. Approximately 85% of the Earth's surface is covered with cold biome, including poles, alpine, and ocean depths. Cold-tolerant microbial strains produce enzymes stable even at very low temperatures, save energy, and avoid undesirable reactions; and this characteristic is well utilized at the industrial scale [3,4]. Along with these, thermo-tolerant enzymes also find many useful applications.

Starch is the primary storage agent for carbohydrates in terrestrial plants and an integral component of global food consumption. In addition to its direct use as food, starch is often used as a raw material for various commercial processes, like ethanol processing, glues, high-fructose corn syrups, paper sizing agents, etc. [5]. It is a common polymer comprising two main α -glucans: amylopectin and amylose (99% of total dry weight). Amylose is a linear α -1,4-glucan, while amylopectin is a branched polymer with α -1,6-glucosidic linkages in the structure. The ratio of both constituents (amylose : amylopectin) depends on the origin of starch, while the general levels are always around 1:3 [6]. Due to its vast existence as a storage product, several enzymes are distributed across the whole biodiversity for starch hydrolysis (glycosidases) or alteration (transglycosidases).

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Amylase is an important category of industrial enzyme used for starch hydrolysis and makes up 65-70% of the enzyme market [7]. Two enzyme classes, hydrolases (Enzyme Commission 3) and transferases (Enzyme Commission 2) are used to classify amylases broadly. Hydrolases that divide them into exoamylases includes α -glucosidase (EC 3.2.1.20), β -amylase (EC 3.2.1.2) and glucoamylase (EC 3.2.1.3), whereas endoamylases cover α -amylase (EC 3.2.1.1) and debranching enzymes, i.e., isoamylase (EC 3.2.1.68) and pullulanase (EC 3.2.1.41) [8]. A well-known endoamylase is the α -amylase (α -1,4-glucan-4-glucanohydrolase). α -Amylase is, in general, an enzyme with a vast preference for substrate and product specificity. This is one of the most commonly occurring glycoside hydrolases (GHs) in the sequence-based classification system of all carbohydrate-active enzymes (CAZy) into GH families [9,10]. The α -amylase is the key representative of the family GH13, although it is also likely to be found in the families GH57 and GH119, and probably even in the GH126. The larger and former α -amylase family GH13 forms the GH-H clan along with families GH70 and GH77, which, however, do not contain the specificity of α -amylase [7,11]. The family GH13 was identified at a very early stage as a group of enzymes that together often share only very small sequence similarities [12]. As a result, to draw similarities between the sequence and properties of the enzymes, the α -amylase family has been the focus of numerous studies. α -Amylase is present in many microorganisms, which belong to both *Archaea* and *Bacteria*. With the progression of α -amylase action, variable-length oligosaccharides are generated as end products with α -configuration. Branched oligosaccharides are also produced comprising α -limit dextrins [10]. On the basis of the degree of substrate hydrolysis, α -amylases are categorized into two groups: liquefying and saccharifying α -amylases bearing the capability of hydrolysing 30-40% and 50-60% of the starch's glycosidic bonds, respectively. α -Amylase is mostly a metalloenzyme that requires divalent calcium ion for its structural integrity, activity, and stability [13]. α -Amylase is faster than β -amylase since it can act on the substrate anywhere. Owing to a broad activity spectrum, it is widely used in starch industries like textile, paper, starch liquefaction, brewery, detergent, baking, and distillery industry [4,7]. It also has biotechnological applications like biological-remediation, pharmaceuticals, and food processing.

Microbial enzymes are preferred over other sources because they can be easily purified, are environment- and cost-friendly, of better quality and can be cultured in a controlled environment [14]. Depending on their origin,

amylases can be archaeal, bacterial and eukaryotic. Every group differs from one another owing to a different evolutionary origin. Bacterial enzymes are ubiquitous and diverse in comparison to other groups [15]. Some parameters control the process of enzyme production, including temperature, concentration of metal ions and pH. The pH and temperature optima for bacterial α -amylases vary between 1-11.5 and 25-100 °C, respectively [15,16].

In the present study, *Shewanella* sp. ISTPL2, a psychrophilic bacterium isolated from the Pangong lake was employed for the production and characterization of a psychrophilic α -amylase. Pangong lake is an endorheic lake, 134 km in length, which is located 4,250 m above mean sea level in the Himalayas [17]. The lake is a collection of potent psychrophilic microbota capable of producing economically sustainable metabolic products and enzymes. The same can find commercial applications in different industrial sectors. To our knowledge, enzymes from microorganisms isolated from cold ecosystems are less explored, so this deserves our attention more. The enzyme production is affected by culture pH, temperature, the concentration of metal ions and was optimized accordingly.

2 Materials and methods

High purity chemicals and nutrient medium were procured from the Fisher Scientific or Sigma-Aldrich (Merck KGaA) or stated otherwise.

2.1 Isolation and screening of the bacterial strains for amylase activity

Sediment sampling was performed at the Pangong lake (33°43' 04.59"N:78°53' 48.48"E), Ladakh, J&K, India, to isolate psychrophilic and psychrotolerant bacterial strains as reported by Mishra et al. [18], Rathour et al. [19] and Khosla et al. [20]. During winters, this lake stays completely frozen [21]. The representative isolates were screened on the basis of plate assay for amylase activity. During the screening procedure, substrates consumption by bacterial strains was studied as an indication of amylolytic activity. The enzyme activity of the isolates was tested in starch-agar plates with a defined media (g/L): yeast extract - 5, tryptone - 10, NaCl - 10, agar - 20 with 2% soluble starch. After the inoculation, plates were incubated at 10 °C for 2 days. Over the period, starch hydrolysis was detected by pouring the iodine solution on the plates. The bacterial

isolate that developed a clear hydrolysis zone in the agar plate supplemented with starch was selected for further study.

2.2 Culture conditions of amylase-producing bacteria

The bacterial isolate showing maximum amylase activity was used for amylase production in a shake flask. A defined basal medium consisted of (% w/v): $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ - 0.25, KH_2PO_4 - 0.1, NaCl - 0.1, $(\text{NH}_4)_2\text{SO}_4$ - 0.2, CaCl_2 - 0.005, MgSO_4 - 0.005, tryptone - 0.2; and soluble starch - 1% was used for the amylase production. One mL of overnight bacterial inoculum was transferred to 100 mL basal medium and maintained at pH 6.9 (10 °C), with the agitation of 170 rpm for at least 48 h. The bacterial samples were procured at 6 h, 12 h, 24 h, 36 h and 48 h to check the amylase enzyme activity. The cell pellets were separated by 4 °C centrifugation (30 min at 14,000×g). The cell dry weight (CDW) of collected cell pellets was determined after drying them at 105 °C until a constant weight was achieved. The maintenance of cold temperature during the extraction procedure was essential for efficient enzyme activity and stability.

2.3 Total protein concentration and enzyme assay for amylase activity

After centrifugation, the collected cell pellet was washed with distilled water and lysed by sonication process after adding 10 mL of lysis buffer composed of 10 mM 1,4-dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 140 mM lysozyme, and 500 μL of 100 mM Tris-Cl [22]. Lysed cell pellets were centrifuged for 15 min at 10,000×g (4 °C) to separate cell debris from the crude lysate. The amount of total protein present in the crude lysate and cell-free supernatant was estimated by the Bradford protein estimation method, which employs a bovine serum albumin (BSA) standard [23]. To calculate the amount of soluble protein, a standard curve prepared from known BSA protein was used as a reference (mg protein per mL of the test sample).

2.4 Enzyme assay for amylase activity

The enzyme activity of extracellular (cell-free supernatant) and intracellular (crude lysate) α -amylase was determined spectrophotometrically using soluble starch as a substrate.

The procedure to assess the amylase activity was followed as detailed by Gomes et al. [24] with a slight modification, which uses 1% (w/v) soluble starch as a substrate in 0.05 M sodium phosphate buffer (pH 6.9). A reaction mixture was prepared with 0.5 mL of each substrate solution and diluted enzyme, respectively, which was incubated at 50 °C for 10 min to initiate the reaction, which was later halted with 1 mL dinitrosalicylic acid. Miller method [25] was used to estimate the reducing sugars spectrophotometrically by recording the absorbance at 540 nm (Varian Carry 100 Bio Spectrophotometer). The amount of enzyme utilized to release 1 μmol of reducing sugars as maltose per minute is defined as one unit (U) of amylase activity.

2.5 Purification of α -amylase

Bacterial culture incubated in amylase-producing medium with 1% soluble starch was harvested at 14,000×g (30 min, 4 °C) in a refrigerated centrifuge. Ammonium sulphate (0-80%) was used to saturate the supernatant with constant stirring at 4 °C, followed by a 20 min centrifugation (14,000×g) [20]. The fraction of ammonium sulphate in a dialysis bag was dialyzed against 0.05 M sodium phosphate buffer (pH 6.9, 4 °C) for 6 h. Thereafter, the concentrated enzyme was packed onto a column of DEAE-cellulose, stabilized with the same buffer. Enzyme elution took place with a similar buffer at a 1 mL/min flow rate with a linear gradient of 0.05-0.5 M NaCl. The fractions were accumulated and their protein concentration was measured at 280 nm. The fractions having the maximum protein concentration were thus tested for the α -amylase activity. Following this, the active fractions having the α -amylase enzyme were accumulated and concentrated against a sucrose gradient. A Sephadex G-100 column equilibrated with 50 mM phosphate buffer (pH 6.8) was set up to load the concentrated, partially purified enzyme. The enzyme elution took place at a flow rate of 1 mL/min, after which its molecular weight was calculated by column calibration with BSA (66.5 kDa), carbonic anhydrase (29 kDa), cytochrome C (12.4 kDa) and ovalbumin (42.7 kDa). The collected enzyme fractions (1 mL each) were tested for their protein content spectrophotometrically at 280 nm. Fractions with high protein content were assayed for amylase activity. The resultant purified enzyme was used for the portrayal of extracellular amylase. The purified amylase's molecular mass was verified through sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) by using polyacrylamide stacking gel (5%) and polyacrylamide resolving gel (12%), as reported by Khosla et al. [20].

2.6 Characterization of α -amylase

Essential parameters that govern the enzyme activity and stability, like temperature, pH and metal ions were explored for the present experiment [26,27].

2.6.1 Effect of temperature on the α -amylase activity

The optimum temperature for the activity of purified α -amylase was determined in 0.5 mL of enzyme solution by enzyme incubation at variable temperatures (4, 10, 20, 30, 40, 50, 60 and 70 °C) for 10 min. The remaining enzyme activity and protein concentration were estimated spectrophotometrically to check the thermostability.

2.6.2 Effect of pH on the α -amylase activity

The α -amylase enzyme activity was determined at various pHs (5, 6, 7, 8, 9 and 10) in sodium phosphate buffer. The 0.5 mL of buffer solution (pH 5-10) were added to 0.5 mL of enzyme solution for 10 min at 4 °C, and the remaining enzyme activity was assayed to check the optimum pH.

2.6.3 Effect of metal ions on the α -amylase activity

The metal ions effect on the amylase activity was calculated by incubating 0.5 mL of an enzyme with the salt of metal ions (Mg^{2+} , Ca^{2+} , Cd^{2+} , Co^{2+} , Fe^{2+} , Mn^{2+} , Na^{+} , Cu^{2+} and Zn^{2+}). The remaining enzyme activity was measured spectrophotometrically. The α -amylase activity without treatment served as a control.

3 Results and discussion

3.1 Selection of bacterial strain for amylase activity

Microbial α -amylases have several applications in novel biotechnological processes, like pharmaceuticals, food processing, textile industry, brewing and bioremediation [13]. Many thermophilic and mesophilic amylases have been reported, but there are a few studies on the microbial production of a psychrophilic amylase [28]. In our study, psychrophilic bacterial strains were procured from the sub-glacial Pangong lake of North-Western Indian Himalayas. A total of four bacterial isolates (P1, P2, P3

and P4) were obtained by incubating the agar plates at 4 °C. These isolates were screened for the amylase activity. Among these isolates, bacterial strain P2 showed a clear hydrolysis zone around the colonies on starch agar plates maintained at 10 °C. The hydrolysis zone around the colonies developed due to starch hydrolysis in the plate assay confirmed the amylase-producing activity of the strain in cold conditions. Based on the previous study, the strain P2 was identified as *Shewanella* sp. ISTPL2 [19].

Shewanella is a potent genus, which is capable of producing several metabolic products including extracellular enzymes, out of which amylase holds substantial industrial importance. Previous finding has shown that bacteria belonging to the *Shewanella* genus can provide enzyme activity even under harsh environmental conditions [29]. Their distinctive characteristics include unprecedented respiratory diversity and the potential to survive at low temperatures.

3.2 Production of psychrophilic α -amylase

Shewanella sp. ISTPL2 was employed for the production, optimization and characterization of the α -amylase enzyme. The amylase producing ISTPL2 strain was grown in amylase-producing basal media supplemented with 1% soluble starch at 10 °C for 48 h. The extracellular and intracellular α -amylase activity was determined in cell-free supernatant and crude lysate at different time intervals (6, 12, 24, 36 and 48 h). The extracellular α -amylase activity was highest at 12 h of incubation (10,064.20 U/mL) and bacterial growth (5.4 g/L) (Fig. 1). Further, with an increase in the incubation time, the enzyme activity was reduced. Whereas maximum intracellular α -amylase enzyme activity of 259.62 U/mL was obtained in crude lysate at 6 h, further enzyme activity was decreased till 36 h of incubation (Table 1). This showed the potential of the psychrophilic strain ISTPL2 to produce intracellular α -amylase enzyme at low temperature (10 °C). Kuddus and Ahmad [4] reported 5,870 U/mL and 4,746 U/mL of α -amylase in *Microbacterium foliorum* strain GA2 (20 °C and pH 9) and *Bacillus cereus* strain GA6 (20 °C and pH 10) after 120 h and 96 h of incubation, respectively, procured from the soil of Gangotri glacier, Western Himalaya. *Pseudoalteromonas arctica* strain GS230 was also reported to produce a cold-active amylase at 20 °C [30]. One of the most deeply studied psychrophilic α -amylases is represented, however, by the α -amylase from Antarctic bacterium *Alteromonas haloplaktis* [31,32], for which also the three-dimensional structure has been solved [33-36].

Figure 1: Production of extracellular α -amylase by *Shewanella* sp. ISTPL2 in basal media.

Table 1: Production of psychrophilic α -amylase from *Shewanella* sp. ISTPL2 at 10 °C in shake flask cultivation.^a

Time (h)	CDW (g/L)	Extracellular			Intracellular		
		Amylase (U/mL)	TP (mg/mL)	SA (U/mg)	Amylase (U/mL)	TP (mg/mL)	SA (U/mg)
6	5	6,765.14 ± 0.36	1.37 ± 0.01	4,942.07 ± 0.10	259.63 ± 0.10	1.22 ± 0.01	211.47 ± 0.15
12	5.4	10,064.2 ± 0.10	1.88 ± 0.01	5,340.68 ± 0.11	150.37 ± 0.17	5.35 ± 0.01	28.11 ± 0.10
24	4.8	8,796.45 ± 0.15	6.61 ± 0.01	1,330.11 ± 0.25	119.16 ± 0.15	5.63 ± 0.01	21.15 ± 0.11
36	5.9	6,660.36 ± 0.11	7.11 ± 0.01	936.61 ± 0.20	76.58 ± 0.12	3.49 ± 0.01	21.94 ± 0.16

^a Time, incubation time; CDW, cell dry weight; TP, total protein; SA, specific activity.

3.3 Purification of α -amylase

There exists a series of methods employed for purification of the crude enzyme, including ammonium sulphate precipitation, dialysis, DEAE-cellulose ion-exchange chromatography and Sephadex G-100 gel exclusion chromatography to remove undesired proteins. The enzyme purification results were summarized in Table 2. The established molecular weight of α -amylase calculated by Kav (a gel-phase distribution coefficient) versus the log of BSA, ovalbumin, carbonic anhydrase and cytochrome C was found to be 45 kDa [37,38]. After subjected to four purification steps, 6.87 purification fold was achieved

with a 747% yield and specific activity of 36,690.47 U/mg protein of purified α -amylase. Previous studies have reported the purification of different α -amylases by several folds with increased yield. In the report of Roohi et al. [38], the cold-active α -amylase (55 kDa) produced by psychro-halo-tolerant *Bacillus cereus* GA6 isolated from Gangotri glacier was purified to 79-fold with enzyme yield of 53.33% and 175.92 U/mg specific activity by purification through DEAE-cellulose ion-exchange chromatography. A thermostable α -amylase produced by *Anoxybacillus flavithermus* was purified to 5.2-fold with 65.8% recovery after purification using 70% $(\text{NH}_4)_2\text{SO}_4$ and ion-exchange chromatography [39]. In the study of Xie et al. [40], an

Table 2: Purification of α -amylase from *Shewanella* sp. ISTPL2.

Purification steps	Total activity (U)	Protein (mg)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude	10,064.20	1.88	5,340.68	1	100
Precipitation ^a	2,198.02	0.14	16,128.85	3.02	21.84
Dialysis	1,447.23	0.07	19,493.48	3.65	14.38
DEAE-cellulose	1,020.51	0.04	23,552.40	4.41	10.14
Sephadex G-100	751.80	0.02	36,690.47	6.87	7.47

^a Precipitation by ammonium sulphate.

Figure 2: SDS-PAGE profile of *Shewanella* sp. ISTPL2 α -amylase. Lane M, molecular weight marker; lane (1), crude α -amylase; lane (2), ammonium sulphate concentrated and dialysed protein sample; lane (3), partially purified protein sample after DEAE-cellulose ion exchange chromatography; lane (4), purified α -amylase after Sephadex G-100 chromatography.

α -amylase of *Bacillus methylotrophicus* strain P11-2 was purified with 13.1-fold having a yield of 7.0% and specific enzyme activity of 330.7 U/mg protein after series of purification. Thus, compared to previous studies, our results confirm high total enzyme activity and specific activity of purified α -amylase excreted from psychrophilic bacterial strain *Shewanella* sp. ISTPL2. The purified α -amylase was analysed by SDS-PAGE under reducing conditions that showed a single band on SDS-PAGE. Thus the size of *Shewanella* sp. ISTPL2 α -amylase determined by SDS-PAGE was found to be about 44 kDa, which is in

agreement with the size determined by Sephadex G-100 gel exclusion chromatography (Fig. 2). Similarly, the size of previously reported purified α -amylases was falls within the range of 43-68 kDa when isolated from different sources [38-40].

3.4 Characterization of psychrophilic α -amylase

The effects of temperature, pH and metal ions on the purified enzyme activity were tested. Various studies have reported a significant change in enzyme stability and activity in the presence of different variable parameters and it brings positive outcomes in terms of microbial biomass [41].

3.4.1 Effect of temperature on the α -amylase activity

The α -amylase enzyme showed high activity through a wide temperature range, i.e., 4 °C to 70 °C at a constant pH of 6.9. The enzyme was assigned to be psychrophilic as it exhibited the highest activity at 4 °C (Fig. 3a). There was a constant decrease in the activity from 10 °C to 30 °C, with a rise in temperature until it reached 40 °C. Further, with an increase in temperature, i.e., at 40 °C and 60 °C, the α -amylase exhibited 90% and 72% activity, respectively. Based on this, the enzyme was also found to be mesophilic and thermophilic, along with being a psychrophilic enzyme. Related results were reported by Liu et al. [37] in their study on cold-active amylase of *Wangia* sp. C52, which showed the enzyme activity and stability at cold temperature, i.e., 10 °C. According to a study conducted by Aygan et al. [42], *Shewanella* sp. strain produced amylase, which was active between 20-90 °C with an optimized activity at 50 °C.

Figure 3: Effects of temperature (a) and pH (b) on the activity of α -amylase by *Shewanella* sp. ISTPL2.

Similarly, *Bacillus subtilis* strain N8 reported by Arabaci and Arikan [43] demonstrated 98.2% retained enzyme activity at 10 °C, while 83% activity at 40 °C. Al-Dhabi et al. [44] also reported the highest amylase activity at 40 °C by *Streptomyces* strain. Temperature is also an essential parameter that substantially affects microbial growth and the production of metabolites [45,46]. Compared to other microorganisms, bacterial amylases are produced at a more extensive temperature range (35–80 °C) [47,48]. There were other *Shewanella* species reported to provide α -amylase at various temperature ranges [49,50].

3.4.2 Effect of pH on the α -amylase activity

The effect of pH on the enzyme activity was tested between the pH 5–10. The enzyme showed the highest activity at alkaline pH 8.0 at 4 °C and was able to retain its 60–67% activity at range of pH 7–10 (Fig. 3b). However,

Table 3: Effect of metal ions on α -amylase activity from *Shewanella* sp. ISTPL2.^a

Metal ion	Residual activity (%)
Control	100
Mg ²⁺	88.1
Ca ²⁺	76.05
Cd ²⁺	31.85
Co ²⁺	20.38
Fe ²⁺	95.48
Mn ²⁺	53.01
Na ²⁺	25.11
Cu ²⁺	165.31
Zn ²⁺	28.89

^a The enzyme was incubated with 1 mM metal ions at 4 °C for 10 min.

only 48% and 32% enzyme activity were maintained at acidic pH 6 and 5, respectively. The enzyme exhibited optimum activity at alkaline pH and its activity was gradually reduced under acidic conditions showing the sensitivity of enzyme to low pH. Similar results were also reported by previous studies of Roohi et al. [38] and Xie et al. [40]. Thus, the α -amylase produced by *Shewanella* sp. ISTPL2 was considered as alkalophilic showing maximum enzymatic activity towards alkaline conditions.

Previous studies have shown that enzyme secretion is prominently controlled by the pH of the growth medium among the physical parameters. The stability of cold-active amylase from *Nocardiopsis* sp. strain 7326 was optimal at pH 8 [51]. The pH observed during the microbial growth also influences product stability [45]. A similar experiment has demonstrated an optimized pH between 6–7 (neutral range) for bacterial growth and production of enzymes [27]. Arabaci and Arikan [43] also observed that enzymes get inactive in an acidic medium.

3.4.3 Effect of metal ions on the α -amylase activity

A vast majority of amylases are dependent on metal ions, mainly divalent ions like Mg²⁺, Cu²⁺, Zn²⁺, Mn²⁺, Ca²⁺, Fe²⁺, etc. [15]. It was stated [27] that Ca²⁺ has a better affinity than other metal ions and α -amylase always contains at least one Ca²⁺ ion. The influence of metal ions on enzyme activity was examined at a concentration of 1 mM; the results being summarized in Table 3. The present study observed that the α -amylase activity was strongly

inhibited by Mn^{2+} , Cd^{2+} , Zn^{2+} , Na^{2+} and Co^{2+} , as only 53.01%, 31.85%, 28.89%, 25.11% and 20.38% enzyme activity was retained, respectively, when incubated with these metal ions [4]. However, 95.48%, 88.15% and 76.05% enzyme activity remained in the presence of Fe^{2+} , Mg^{2+} and Ca^{2+} ions, respectively, suggesting no or fewer effects of these metals ions on the α -amylase activity [38]. It was also concluded that this enzyme needs Cu^{2+} for its efficient activity as it demonstrated the highest activity (165.31%) with Cu^{2+} metal ion. Still, many researchers have reported Cu^{2+} ion as an inhibitor of an amylase [40]. In contrast, our study suggested that the α -amylase activation in the presence of Cu^{2+} ion might be correlated to its unusual structure [52,53].

4 Conclusions

With our current investigation, we conclude the psychrophilic bacterial strain *Shewanella* sp. ISTPL2 is a potential source of a psychrophilic and alkalophilic extracellular α -amylase enzyme. Moreover, the strain has excellent tolerance towards metal ions. Our research with this bacterial strain shows the high stability of α -amylase at low temperatures and alkaline conditions. The remarkable attributes suggest that the enzyme can be further explored to commercialize its industrial production.

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