Isolation and Characterization of Thermostable Amylase Producing Bacteria from Hot Springs of Bihar, India

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***Abstract*—Amylases are starch degrading enzyme with wide industrial applications. Microbial production of amylase is more simplified and economical than other sources. In this study, amylase producing bacteria were isolated from hot springs of Munger (Bihar, India). Water samples were collected from three different kunds, viz., Sita kund, Rishi kund and Bhimbandh kund with temperature 40-50 °C and pH 5.2-6.3. Amongst amylase positive isolates, RK6 was selected for amylase characterization, exhibiting enzyme activity at highest optimum temperature of 80 °C. On the basis of 16S rRNA gene sequence, it was identified as *Bacillus subtilis* RK6 (KX247637). Amylase of RK6 was characterized with optimum pH of 8.0 and was thermoactive, retaining its activity upto 90 °C and stability at 60-90 °C after preheating for 30 min. V_{max} and K_m for enzyme activity were 60.56 U/ml and 1.86 mg/ml respectively, showing an appreciable affinity for substrate. The enzyme was activated in the presence of Ca^{2+}, Mg^{2+}, and Fe^{2+}, while that of Zn^{2+} and Cu^{2+} resulted in its inhibition. The present finding indicates potential of thermo-alkaliphilic amylase of *B. subtilis* RK6 for various biotechnological applications.**

***Index Terms*—amylase, *Bacillus subtilis*, Munger hot spring, thermoactive**

I. Introduction

The recent upsurge of biotechnology has prompted studies on the isolation of various enzymes, with high industrial and economic values, as they are macromolecular biological catalysts, accelerating the rate of chemical reactions [1], [2]. The world industrial enzyme market in 2016 has been estimated to be valued at USD 6.30 Billion, and is projected to grow at a CAGR of 5.8% from 2017-2022 (http://www.marketsandmarkets.com/Market Reports/industrial-enzymes-market-237327836.html?gclid=CIL3wralt9ICFYihaAod64QGRA). Among industrially important enzymes, amylases sharing 25-30% of world enzyme market [3], are drawing more attention because of their wide commercial applications and economic benefits. They hydrolyze starch to generate maltose and maltotriose from amylose, while glucose, maltose and limit dextrin from amylopectin [4]. Unfortunately, application of amylase-catalyzed reactions has limitations for industrial processes carried out at high temperature, because of its poor stability. Discovery of thermostable amylases not only solves stability issue, but also accelerates reaction rate, reduces possible contaminations and lowers viscosity of medium, thus directly benefitting the starch-processing industries under high temperatures [5]. The prevailing scenario urges to search for probable source of thermostable organisms producing novel extracellular amylases, which could only be possible by exploration of the virgin ecological niches [6]. About 71 percent of the aquatic earth's surface, encompassing extreme environments of temperature, pH, salinity, high metal concentrations, oxygen tension and pressure have to be explored for novel and potentially robust enzymes that are better suited for industries [7]. Hot springs, where geothermally heated water emerges out from the earth's crust are reservoir of thermophilic organisms. Diversity analysis of hot springs has gained momentum as it provides opportunities to identify and isolate rare compounds and genes because of their diverse and unusual chemistry [8]. In the last two decades, a number of publications appeared on various facets of bacterial diversity of Indian hot springs: Manikaran, Tattapani-Himachal Pradesh [1], [4]; Taptapani, Deulajhari-Odhisha [9], [10]; Bakreshwar-West Bengal [11]; Suryakund-Jharkhand [12]; Tulsi Shyam-Gujrat [13]; Solhar and Suryakund-Uttarakhand [14]; Unkeshwar-Maharashtra [15], etc. The culturable bacterial diversity include *Bacillus subtilis*, *B. amyloliquefaciens*, *B. cereus*, *Brevibacillus* sps., *Paenibacillus* sps., *Acinetobacter* sps.,[1], [9], [16]-[18]. Fortunately, the land of Bihar is also bestowed with a number of hot and cold springs located along the Rajgir-Munger metasedimentary belt of Munger, Nalanda and Gaya districts. These sites are repository of microbial diversity and the most desired enzymes, which have not been systematically explored as per authors' information. Considering the above mentioned facts, the aim of present study is to isolate amylolytic bacterial diversity of Munger hot spring water, selection and characterization of most potent thermoactive amylase.

Manuscript received September 27, 2017; revised November 27, 2017.

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doi: 10.18178/ijpmbs.7.2.28-34

II. Materials and Methods

A. Site Survey and Sample Collection

The water samples were collected during winter season from three hot springs i.e., Sita kund, Rishi kund and Bhimbandh kund, located in Munger (Latitude- 25.38, Longitude- 86.47) and brought to Microbial Biodiversity Laboratory, Dept. of Botany, Patna University, under aseptic conditions. Temperature and pH of each sample was measured at the time of sample collection.

B. Isolation and Screening of Amylase Producing Bacteria

Isolation of bacteria was done by serial dilution and streak plate methods. The aliquots (0.1 ml) of 10^{-1} to 10^{-6} dilution were spread on Nutrient agar (NA) plates and incubated at 50±2 ℃ for 48 h. The isolated colonies were purified and maintained at 4 ℃ for further studies. The purified bacterial isolates were screened for amylolytic properties by starch hydrolysis test. The isolates were streaked on NA plates containing 1% starch and incubated at 50±2 ℃ for 24 h. Thereafter plates were flooded with Lugol's iodine solution [(w/v) 1% iodine in 2% potassium iodide] and the isolates showing clear halo zone were considered amylase positive.

C. Amylase Production and Assay

60 ml of production medium [(w/v) 0.6% peptone, 0.05% MgSO4, 0.05 KCl, 1% starch, pH 7] inoculated with 1% overnight (24 h) grown culture (approximately 2×10^6 CFU/ml) and incubated at 50±2 ℃ in triplicates. After incubation, fermented medium was centrifuged at 12,000 × g for 10 min at 4 ℃ and supernatant was used for estimation of enzyme activity. The reaction mixture containing 1 ml substrate (1% in 0.1M phosphate buffer, pH 6.5) and 1 ml crude enzyme was incubated at 50 ℃ for 10 min. The reaction was stopped by adding 2 ml of 3,5-dinitrosalicylic acid (DNS) reagent, followed by boiling for 5 min to develop color. The absorbance was measured at 540 nm using double beam UV/VIS spectrophotometer (Systronics, 119). The reducing sugar released was measured by the method of [19]. One enzyme unit (U/ml) is equivalent to the amount of enzyme needed to release 1μmol of reducing sugar per minute under assay condition.

D. Determination of Optimum Temperature and Thermal Stability

The effect of temperature on amylase activity was studied by incubating the reaction mixture (1 ml) and substrate (1 ml) at different temperatures (40-90 ℃) at interval of 10 ℃ for 10 min. Amylase with highest optimum temperature was selected for further study. For determination of thermal stability, enzyme was heated for 60 min without substrate at different temperatures i.e. 60, 70, 80 and 90 ℃. Thereafter, enzyme activity of heat treated enzyme was measured under standard assay conditions.

E. Determination of Optimum pH

The effect of pH on the activity of amylase was measured by using the following buffers; 0.1 M phosphate (pH 6.0-8.0), 0.1 M Glycine-NaOH buffer (pH 9.0-10.0).

F. Effect of Substrate Concentration

The effect of substrate concentration [S] on enzyme activity was studied by pre-incubating the crude amylase with different concentration of substrate (soluble starch) (0.5- 3%) at 50±1 ℃. The relative activity at each exposure was measured as per standard assay procedure. Both the V_{max} and K_m of the enzyme was calculated by using Lineweaver-Burke plot.

G. Effect of Metal Ions

The effect of various metal ions on enzyme activity was investigated by using $CaCl_2$, $FeSO_4$, $MgCl_2$, $CuSO_4$ and $ZnSO_4$ at the final concentration of 2 mM. The enzyme was pre-incubated with different metal salts at 50 ℃ for 30 min to study metal ion stability of the enzyme and assayed under standard conditions. The activity of enzyme assayed without any metal was considered as control and the activity was taken as 100%.

H. Statistical Analysis

Each experiment was performed in triplicate and graphically represented as the mean ± SE (n=3) using MS excel.

III. Results and Discussion

A. Site Survey, Isolation and Screening of Amylase Producing Bacteria

Water samples of three hot springs of Munger were analyzed to screen bacteria, capable of producing amylase. The temperature and pH of the samples, recorded at the time of sample collection are mentioned in Table I. Temperature of Sita kund was 50 ℃, while that of Rishi kund and Bhimbandh kund was 45 ℃. All the kunds were acidic with almost similar pH values of 6.3 and 6.2 for Sita kund and Bhimbandh kund, respectively, but that of Rishi kund was quite low i.e., 5.2. The variation in temperature and pH of hot spring water is governed by the depth from which, geothermal water is emerging out, physical condition, chemical composition, cultural and social activities associated with spiritual values. Ref. [20] has remarked that temperature is one of the most important factor followed by pH that governs the distribution of microbial diversity. A total of 15 bacterial colonies were isolated when incubated at 50±2 ℃ on NA plates among which only 07 (LC4, RK1, RK4, RK5, RK6, BH3A and BH3B) tested +ve for amylase on the basis of clear halo zone produced on starch agar plates (Table I). Some preliminary observations of this study are: (i) highest (07) no. of isolates from Rishi kund followed by Bhimbandh and Sita kund, (ii) highest (04) no. of amylase positive isolates appeared from Rishi kund and lowest (01) from Sita kund, (iii) From the observations it could be

concluded that temperature and pH of Rishi kund is more conducive for growth and population diversity of bacteria as compared to the other two hot springs.

TABLE I. MUNGER HOT SPRINGS: TEMPERATURE, PH & BACTERIAL ISOLATES

Sampling sites	Temperature (℃)*	pH*	Total no. of isolates	No. of amylase +ve isolates	Halo zone diameter (cm)
Sita kund	50	6.3	04	01	LC4 (0.9)
Rishi kund	45	5.2	07	04	RK1(0.65)
					RK4(0.6)
					RK5(0.7)
					RK6(0.6)
Bhimband kund	45	6.2	04	02	BH3A(1.2)
					BH3B(0.8)

*Values are mean of triplicate

TABLE II. MORPHOLOGICAL CHARACTERISTICS OF AMYLASE POSITIVE ISOLATES

Isolates	Form	Elevation	Margin	Color	Gram staining	Shape of vegetative cell	Spore formation	Motility
LC4	Regular	Flat	Regular	Pale yellow	+ ve	Rod	+ ve	Motile
RK1	Irregular	Raised	Undulate	Dull white	+ ve	Rod	+ ve	Motile
RK4	Round	Raised	Undulate	Off white	+ ve	Rod	+ ve	Motile
RK5	Irregular	Raised	Rough	Dull white	+ ve	Rod	+ ve	Motile
RK6	Irregular	Flat	Undulate	Dull white	+ ve	Rod	+ ve	Motile
BH3A	Round	Raised	Undulate	Yellowish	+ ve	Rod	+ ve	Motile
BH3B	Round	Flat	Serrate	White	+ ve	Rod	+ ve	Motile

B. Morphological Characterization

Ample variations in colony morphology among 07 amylase +ve isolates were seen. Isolates RK1, RK5 and RK6 were irregular, while, LC4, RK4, BH3A and BH3B were round. Elevation was flat in LC4, RK6, and BH3B, and raised in others. Margin was regular to undulate, while serrate in BH3B. Colony color varied from yellowish to white. However, all the 07 amylase +ve isolates were found to be gram positive spore bearing motile rods (Table II). Other studies on Indian hot springs revealed presence of different *Bacillus* sps. [1], [9], [16]-[18].

C. Determination of Optimum Temperature and Thermal Stability

All the 07 isolates exhibited amylase activity at the temperature range of 40-90 ℃ (Fig. 1). Amongst them, RK6 gave highest activity at 80 ℃ (31.4 U/ml), while RK5 (21.18 U/ml) had optima at 70 ℃. Isolates LC4 (43.32 U/ml), RK1 (38.58 U/ml), RK4 (39.74 U/ml) and BH3B (19 U/ml) showed highest activity at 60 ℃ and BH3A (38.2 U/ml) at 50 ℃ (Fig. 1 a & b). In majority of literatures, 50 ℃ has been reported as optimum for α-amylase activity while, [21] obtained 65 ℃ as optimum in *B. amyloliquefaciens* KCP2. However, in our study, optimum temperature ranged from >50-80 ℃. Ref. [5] reported optimum temperature between 45-80 ℃ in several bacterial strains, which confirms our findings. The isolate RK6, which gave highest amylase activity at 80 ℃ was selected for further characterization of its enzyme.

16S rRNA gene sequence of RK6 showed 99% homology and phylogenetic tree showed significant relation with *Bacillus subtilis* (Fig. 2) thus, identified as *Bacillus subtilis* RK6. The sequence was deposited in the GenBank of the NCBI (Accession no: KX247637).

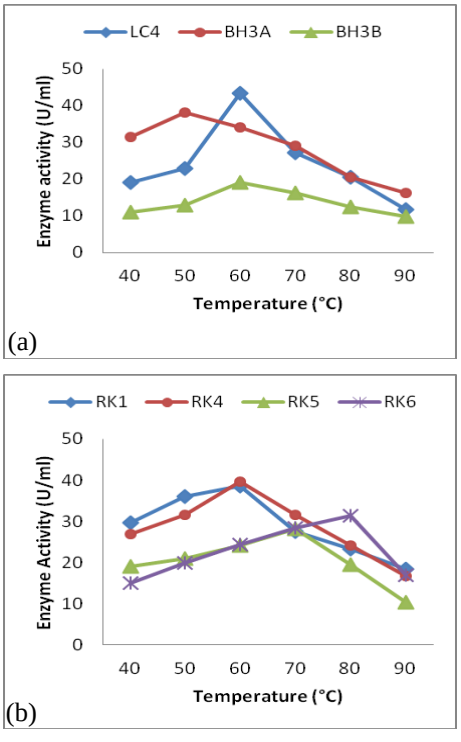

Figure 1. (a & b). Effect of temperature on amylase activity of amylase positive isolates.

In the present study, amylase of *B. subtilis* RK6 retained 94%, 82%, 60% and 51% of its activity after pre-heating for 30 min at 60, 70, 80 and 90 ℃, respectively (Fig. 2).

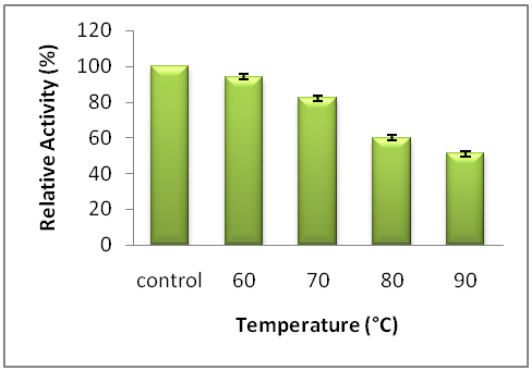

Figure 2. Effect of thermal stability of amylase of *B. subtilis* (KX247637).

From the observations, it is evident that even at higher temperature the crude enzyme is active, establishing its thermostable nature. Thermostability of enzyme most crucial for economy of industrial applications, especially liquid sugar industry requires thermostable α-amylase to maintain their activity longer at higher temperature [22]. Higher core hydrophobicity, shorter length loops, increased packing density, increased formation of hydrogen bonds, disulfide bonds and a greater number of ionic interactions are deciding factors for thermostability of enzymes [23], [24]. The work of [25] reveals that thermal stability of α- amylase has been disseminated from the constituent amino acids. According to them, the region I (Gln 178) and region II (255 to 270 residues) of *Bacillus licheniformis* amylase was identified to be essential for imparting thermostability. Deamidation of Asn/Gln residue for *Bacillus licheniformis* has been evolved as a cause of thermal inactivation.

D. Determination of Optimum pH

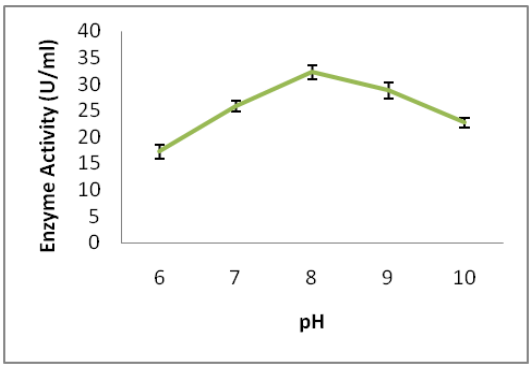

Figure 3. Effect of pH on amylase activity of *B. subtilis* (KX247637).

The effect of different pH on amylase activity and stability is shown in Fig. 3. *B. subtilis* RK6 showed highest (32.25 U/ml) amylase activity at alkaline pH 8.0. It's activity was quite reduced beyond and above its optimum pH. This may be due to change in the tertiary structure of the enzyme, caused by the change of the electrical charge of the side chains on the active site, thus influencing enzyme activity. The pH of 8.0 has been reported as optimum for amylase activity by *Bacillus subtilis* JS-2004 [26]. Enzyme activity inclined towards alkaline pH range, indicates alkaliphilic nature of the enzyme. Many of the reported amylases were either thermostable or alkaline active, but amylase from *B. subtilis* RK6 is both thermostable and alkaliphilic, which is industrially significant.

E. Effect of Substrate Concentration

K_m and V_{max} are significant coefficients in guiding scientific research and engineering design. Moreover, K_m is independent of enzyme concentration and is a true characteristic of the enzyme under defined conditions of temperature, pH, etc. [27]. To determine these parameters, reactions were carried out at different starch concentrations under optimized conditions. It was observed that amylase activity increased with increase in substrate concentration upto 2.0% and then gradually become constant. A Lineweaver –Burke plot (Fig. 4) indicates that this enzyme has apparent K_m and V_{max} values of 1.86 mg/ml and 60.56 mg/ml/ min, respectively for the hydrolysis of soluble starch. The results of K_m were in accordance with [28], where observed $_{Km}$ was 1.91 mg/ml in an alkaline chelator- resistant α-amylase from an alkaliphilic *Bacillus* sp. Isolate L1711. The K_m and V_{max} values of the α-amylase for soluble starch were 7.28 mg/ml and 13.07 mg/ml min, respectively from cold-adapted α-amylase isolated from *Pseudoalteromonas arctica* GS230 as reported by [29]. Concentration of substrate is a crucial and significant factor for determination of enzyme activity. Smaller the K_m value, more firmly the enzyme binds to substrate [5] and in the present study, K_m value is 1.86 mg/ml, exhibiting binding capacity of the secreted enzyme by *B. subtilis* RK6. Moreover this finding also observes that thermostable amylase isolated from hot spring has quiet less K_m value than cold adapted ones.

F. Effect of Metal Ions

Most of the amylases are known to be dependent on divalent metal ion such as, Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+} etc. [30]. Studies on influence of different metal ions on amylase activity, indicated that the enzyme was considerably activated in presence of Ca^{2+}, Mg^{2+} and Fe^{2+} but maximum, when $CaCl_2$ was used. Mn^{2+} and Ca^{2+} ions catalyzed the enzyme activity as well as stability. These metal ions may act as co-factor which is required to increase the enzyme activity. On the other hand, a strong inhibitory effect was observed in the presence of Zn^{2+} and Cu^{2+} ions (Fig. 5) on amylase activity, which may be due to competition between the exogenous cations and the protein-associated cations, resulting in decreased metalloenzyme activity [31]. Ca^{2+} ion is the most critical factor that affects the production and activity of amylase [32] and is also essential for the enzyme's thermostability [33]. Ref. [34] pointed out that α-amylase generally contain at least one Ca^{2+} ion and it has much stronger affinity for Ca^{2+} than that of other metal ions, which may be responsible for highest stimulatory effect (136%) of $CaCl_2$, as compared to other metal ions in this work.

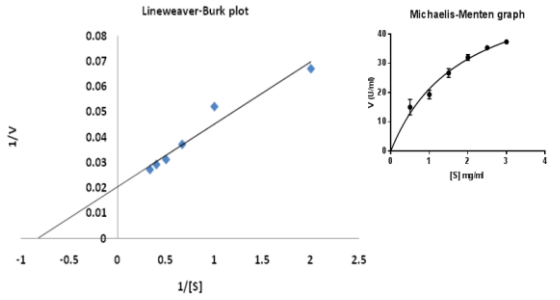

Figure 4. Lineweaver-Burk plot of amylase of *B. subtilis* (KX247637).

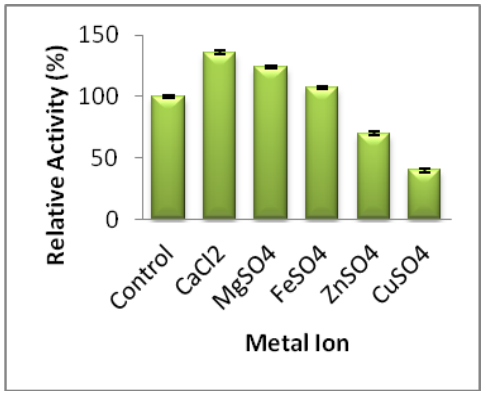

Figure 5. Effect of metal ions on amylase activity of *B. subtilis* (KX247637).

IV. Conclusion

With increasing growth of enzyme market and amylase demand, the present study has been taken up with a view to explore the hot spring as its possible source. Moreover, such study is a prerequisite for tapping the biotechnological potential of the microorganisms from varied, unique ecosystems. To the best of our knowledge, this study is first of its kind to explore the bacterial diversity of hot springs of Munger, Bihar, India. The findings improve our understanding of microbial diversity and community composition in acidic hot springs. The explored hot spring has emerged as potent sink of thermoactive amylase producing bacteria having optimum activity at temperature between 50-80 ℃. The *B. subtilis* RK6 is capable of producing amylase having thermal and alkali-tolerant nature and the dual extreme characteristics of the enzyme is suitable for application in starch liquefaction, detergent processing and other starch based industries.

Acknowledgement

The first author would like to thank Department of Science and Technology, Govt. of India, New Delhi, India for grant to carry out this research work under grant no. [IF-131075] and Microbial Biodiversity Laboratory, Department of Botany, Patna University, Patna, Bihar, India for providing laboratory facilities.

References

[1] A. Verma, M. Gupta, and P. Shirkot, "Isolation and characterization of thermophilic bacteria in natural hot water springs of Himachal Pradesh (India)," *The Bioscan*, vol. 9, no. 3, pp. 947-952, 2014, .

[2] Sujeeta, K. Malik, S. Mehta, and K. Sihag, "Isolation and screening of amylase producing fungi," *International Journal of Current Microbiology and Applied Sceiences*, vol. 6, no. 4, pp. 783-788, 2017.

[3] S. Das, S. Singh, V. Sharma, and M. L. Soni, "Biotechnological applications of industrially important amylase enzyme," *International Journal of Pharma and Bio Sciences*, vol. 2, pp. 486-496, 2011.

[4] P. Sharma, S. Gupta, A. Sourirajan, and K. Dev, "Characterization of extracellular thermophilic amylase from *Geobacillus* sp. isolated from Tattapani hot spring of Himachal Pradesh, India," *Current Biotechnology*, vol. 4, no. 2, pp. 202-209, 2015.

[5] A. Homaei, M. Ghanbarzadeh, and F. Monsef, "Biochemical features and kinetic properties of alpha amylases from marine organisms," *International Journal of Biological Macromolecules*, vol. 83, pp. 306-314, 2016.

[6] D. H. Tambekar, S. D.Tambekar, A. S. Jadhav, and P. B. Bhule, "Extraction and Partial characterization of thermostable, alkaline tolerant a-amylase from *Bacillus oryzaecorticis*," *Bioscience Discovery*, vol. 8, no. 2, pp. 206-210, 2017.

[7] K. D. Hiwase, "Isolation, production and characterization of enzyme amylase produced from bacteria isolated from bat guano of Lonar Lake," *International Journal of Applied Research*, vol. 3, no. 4, pp. 810-817, 2017.

[8] M. Kuddus and P. Ramteke, "Recent developments in production and biotechnological applications of cold-active microbial proteases," *Critical Reviews in Microbiology*, vol. 38, no. 4, pp. 330-338, 2012.

[9] M. K. Panda, M. K. Sahu, and K. Tayung, "Isolation and characterization of a thermophilic *Bacillus* sp. with protease activity isolated from hotspring of Tarabalo, Odisha, India," *Iranian Journal of Microbiology*, vol. 5, no. 2, pp. 159-165, 2013.

[10] A. Singh and E. Subudhi, "Structural insights of microbial community of Deulajhari (India) hot spring using 16s-rRNA based metagenomic sequencing," *Genomics Data*, vol. 7, pp.101-102, 2016.

[11] I. Hauli, B. Sarkar, T. Mukherjee, and S. K. Mukhopadhyay, "Isolation and identification of a novel thermo-alkaline, thermostable, SDS and chelator resistant amylase producing *Anoxybacillus* sp. IBA from hot spring of Bakreswar, West Bengal (India): First report," *Advances in Applied Sciences Research*, vol. 4, no. 5, pp. 202-212, 2013.

[12] K. Deep, A. Poddar, and S. K. Das, "*Anoxybacillus* suryakundensis sp. nov, a moderately thermophilic, alkalitolerant bacterium isolated from hot spring at Jharkhand, India," *PLOS ONE*, vol. 8, no. 12, e85493, 2013.

[13] A. Gehlani, R. Patel, A. Mangrola, and P. Dudhagara, "Cultivation-independent comprehensive survey of bacterial diversity in Tulsi Shyam hot springs, India," *Genomics Data*, vol. 4, pp. 54-56, 2015.

[14] P. Ranawat and S. Rawat, "Exploration of thermophilic bacteria from hot springs of Garhwal Himalayas and their screening for industrially important enzyme production," *Environment Conservation Journal*, vol. 18, no. (1&2), pp. 149-158, 2017.

[15] G. T. Mehetre, A. S. Paranjpe, S. G. Dastager, and M. S. Dharne, "Complete metagenome sequencing based bacterial diversity and functional insights from basaltic hot spring of Unkeshwar, Maharshtra, India," *Genomics Data*, vol. 7, pp. 140-143, 2016.

[16] S. Bisht and A. Panda, "Isolation and identification of new lipolytic thermophilic bacteria from an Indian hot spring," *International Jouranl of Pharma and Biosciences*, vol. 2, no. 2, pp. 229-235, 2011.
[17] A. Ghati, K. Sarkar, and G. Paul, "Isolation and characterization and molecular identification of esterolytic thermophilic bacteria from an Indian hot spring," *Current Research in Microbiology and Biotechnology*, vol. 1, no. 4, pp. 196-202, 2013.
[18] N. Sharma, G. Vyas, and S. Pathania, "Culturable diversity of microorganism found in hot springs of Northern Himalayas and explore their potential for production of industrially important enzymes," *Scholars Academic Journal of Biosciences*, vol. 1, no. 5, pp. 165-178, 2013.
[19] G. L. Miller, "Use of dinitrosalicyclic acid reagent for determination of reducing sugar," *Analytical Chemistry*, vol. 31, pp. 426-428, 1959.
[20] E. Rikhvanov, N. Varakina, D. Sozinov, and V. Voinikov, "Association of bacteria and yeasts in hot springs," *Applied and Environmental Microbiology*, vol. 65, no. 9, pp. 4292-4293, 2017.
[21] V. S. Prajapati, U. B. Trivedi, and K. C. Patel, "A statistical approach for the production of thermostable and alkaliphilic alpha-amylase from *Bacillus amyloquefaciens* KCP2 under solid-state fermentation," *3 Biotech.*, vol. 5, no. 2, pp. 211-220, 2015.
[22] W. D. Crab and C. Mitchinson, "Enzymes involved in the processing of starch to sugars," *Tibtech.*, vol. 15, pp. 349-352, 1997.
[23] M. M. Gromiha, M. Oobatake, and A. Sarai, "Important amino acid properties for enhanced thermostability from mesophilic to thermophilic proteins," *Biophysical Chemistry*, vol. 82, no. 1, pp. 51-67, 1999.
[24] S. Zhang, Y. He, H. Yu, and Z. Dong, "Seven N-terminal Residues of a thermophilic xylanase are sufficient to confer hyperthermostability on its mesophilic counterpart," *PLOS ONE*, vol. 9, no.1, e87632, 2014.
[25] Y. Suzuki, N. Ito, T. Yuuki, H. Yamagata, and S. Udaka, "Amino acid residues stabilizing a *Bacillus* alpha amylase against irreversible thermoinactivation," *The Journal of Biological Chemistry*, vol. 264, no. 32, pp.18933-18938, 1989.
[26] M. Asgher, A. M. Javaid, S. U. Rahman, and R. L. Legge, "A thermostable a-amylase from a moderately thermophilic *Bacillus subtilis* strain for starch processing." *Journal of Food Engineering*, vol. 79, no. 3, pp. 950-955, 2007.
[27] S. Negi and R. Banerjee, "Characterization of amylase and protease produced by *Aspergillus awamori* in a single bioreactor," *Food Research International*, vol. 42, no. 4, 443-448, 2009.
[28] E. C. M. J. Bernhardsdotter, J. D. Ng, O. K. Garriott, and M. Pusey, "Enzymatic properties of an alkaline chelator-resistant α-amylase from an alakaliphilic *Bacillus* sp. Isolate L1711," *Process Biochemistry*, vol. 40, no. 7, pp. 2401-2408, 2005.
[29] M. Lu, S. Wang, Y. Fang, H. Li, S. Liu, and H. Liu, "Cloning, expression, purification, and characterization of cold-adapted α-Amylase from *Pseudoalteromonas arctica* GS230," *The Protein journal*, vol. 29, no. 8, pp. 591-597, 2010.
[30] A. Pandey, P. Nigam, C. R. Soccol, V. T. Soccol, D. Singh, and R. Mohan, "Advances in microbial amylases," *Biotechnology and Applied Biochemistry*, vol. 2, pp. 135-152, 2000.
[31] L. L. Lin, C. C. Chyau, and W. H. Hsu, "Production and properties of a raw-starch-degrading amylase from the thermophilic and alkaliphilic *Bacillus* sp. TS-23," *Biotechnology and Applied Biochemistry*, vol. 28, no. 1, pp. 61-68, 1998.
[32] S. Chakraborty, *et al.*, "Characterization and stability studies on surfactant, detergent and oxidant stable α-amylase from marine haloalkaliphilic *Saccharopolyspora* sp. A9," *Journal of Molecular Catalysis B: Enzyme*, vol. 68, no. 1, pp. 52-58, 2011.
[33] C. Vieille and G. J. Zeikus, "Hyperthermophilic enzymes: sources, uses and molecular mechanisms for thermostability," *Microbiology and Molecular Biology Review*, vol. 65, no. 1, pp. 1-43, 2001.
[34] R. Gupta, P. Gigras, H. Mohapatra, V. K. Goswami, and B. Chauhan, "Microbial α-amylases: A biotechnological prospective," *Process Biochemistry*, vol. 38, pp. 1599-1616, 2003.

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