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Research Article

NOVEL *GEOBACILLUS THERMOLEOVORANS* KNG 112 THERMOPHILIC BACTERIA FROM BANDARU HOT SPRING: A POTENTIAL PRODUCER OF THERMOSTABLE ENZYMES

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ABSTRACT

Objectives: Herein, industrial important thermostable bacteria and their thermozymes have been studied for their relative resistance to the various harsh environments. The façade nature of amylase was studied by partial optimization from a novel *Geobacillus* strain indicates that the enzymes may be potential candidates for industrial biocatalyst.

Methods: Routine microbiological methods have employed for the isolation of novel *Geobacillus* strain from hot springs. The strain was further characterized by various phenotypic and genotypic methods, such as 16s rRNA gene sequencing methods. Furthermore, the strain was partially optimized for the production of thermostable amylase enzymes such as various temperatures, pH, carbon sources, substrate concentration, and agitation speed.

Results: A Gram-positive, aerobic, and non-motile Geobacillus sp. were isolated and characterized from a hot spring of Karnataka. Various biochemical tests for the strain IC5 revealed that the bacteria are capable of growing at the temperature 55°C and pH 7–8. These tests also revealed that the strain IC5 also tolerated 8% (w/v) NaCl and has amylolytic and lipolytic enzyme activities. Furthermore, the 16S rRNA gene sequence of the strain indicated that the strain IC5 belongs to the genus Geobacillus was in close resemblance with the gene of Geobacillus thermoleovorans EC-5 with 99% of similarities. The partial optimization and characteristics of G. thermoleovorans KNG 112 amylase were shown the optimum activity at 50°C–60°C and pH 8. The strain used starch as a carbon source with an agitation speed of 120rpm for maximal amylase production.

Conclusion: The present study on G. *thermoleovorans* KNG 112 revealed that the bacteria could withstand harsh environmental conditions and capable of producing critical industrial thermozymes such as amylase and lipase.

Keywords: Amylase, Lipase, Thermophilic, Geobacillus *thermoleovorans*, Phylogenetic.

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INTRODUCTION

Microbiological exploration of hot springs has bought a paradigm shift in both science and society in general. Globally, it is regarded as one of the most rewarding studies that established a good microbial arsenal of human knowledge [1,2]. It has enriched our scientific knowledge of understanding toward thermophilic lifestyles and also provided a number of biological resources having potential industrial applications that require processing at harsh conditions, including high temperature and pH [3,4].

Hot springs were arisen by the emergence of geothermally heated groundwater in volcanically active regions [5]. Hot springs were found throughout the world but they are more concentrated only in certain regions of the world. Hot springs vary widely in their temperature, chemical composition, and pH [6]. Worldwide, geothermal areas which are favorable habitats for thermophilic organisms were limited to a restricted number of sites in New Zealand [8], Jordan [9], United States [10], Italy [11], and Turkey [12]. In India, nearly 400 thermal springs under seven geothermal provinces were distributed [13].

Thermophilic bacilli were grown at a temperature from 45°C to 70ºC and these were possible isolates from different environmental conditions such as hot springs, cold habitats, petroleum reservoirs, deep ocean-basin cores, and deep sea hydrothermal vents [14]. Thermophilic bacteria were first characterized by Miquel which was found to form aerobic spore and able to grow at 70°C [15] and also many thermophilic bacteria have been characterized [16]. The most important characteristic of thermophilic organisms is their ability to produce thermostable enzymes with higher operational stability and longer shelf life [17]. Among bacterial sources, several strains of *Bacillus* and *Geobacillus* were exploited for thermostable α-amylase production in industries [18,19].

α-Amylases (EC 3.2.1.1, 1,4-α-D-glucanglucanohydrolyase) hydrolyze starch to a range of products such as glucose and maltose or specific maltooligosaccharide or mixed maltooligosaccharides [20,21]. Amylase can be obtained from several bacteria, yeast, fungi, and actinomycetes; however, enzyme from bacterial and fungal sources has dominated applications in industries [22]. They were employed in industries for various purposes such as glucose and maltose-forming α-amylases in alcohol fermentation and sugar syrup formulation and maltooligosaccharide forming α-amylases in food processing [23,24]. Amylases also play a significant role in starch, detergent, beverage, and textile industries and their commercial production from microorganisms represent 25%–33% of the world enzyme market [25]. Industrial production of enzymes can be made economical by utilizing low cost substrates such as agricultural byproducts in the production medium [26].

Microbial lipases have also been immensely used for biotechnological applications in dairy, detergents, and textile industries as well as surfactant and oil-processing industries. In fact, they were also been widely used in pharmaceutical industries in the production of enantiomerically pure chemicals, since they have a number of unique characteristics, coupled with in distinct substrate specificity [27], stable, and active in organic

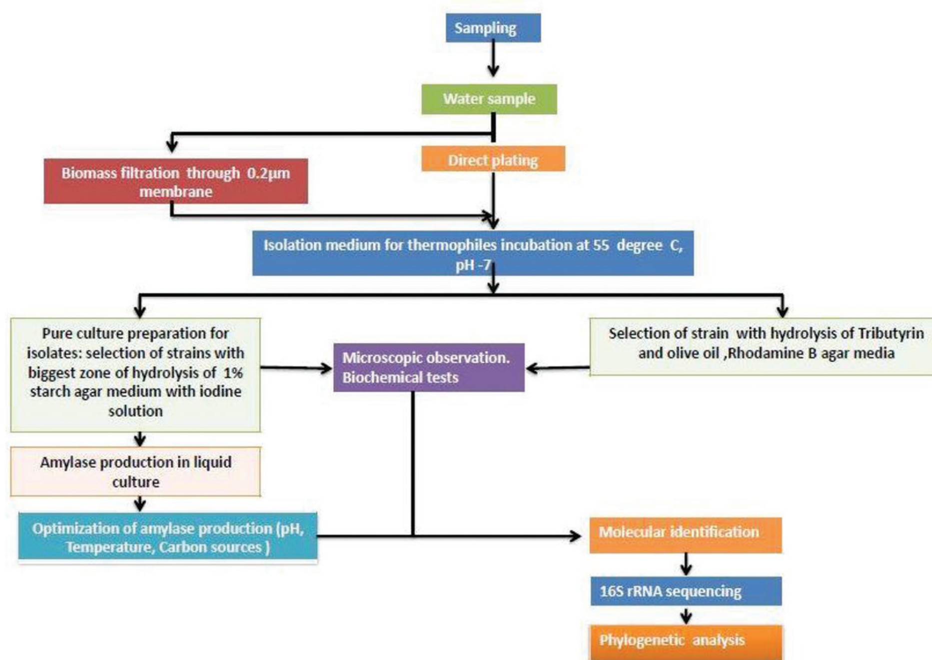

Fig. 1: Detailed flow chart of bacterial isolation, identification, and screening for amylase production and its optimization

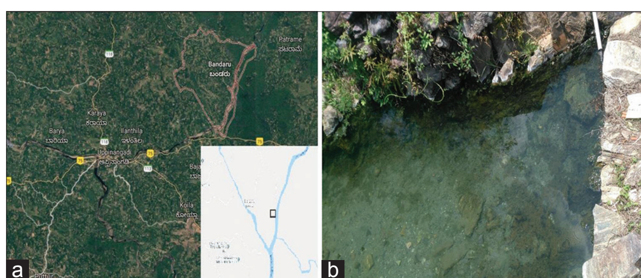

Fig. 2: (a) Map showing the location of hot water spring in Karnataka, India, (b) sampling site

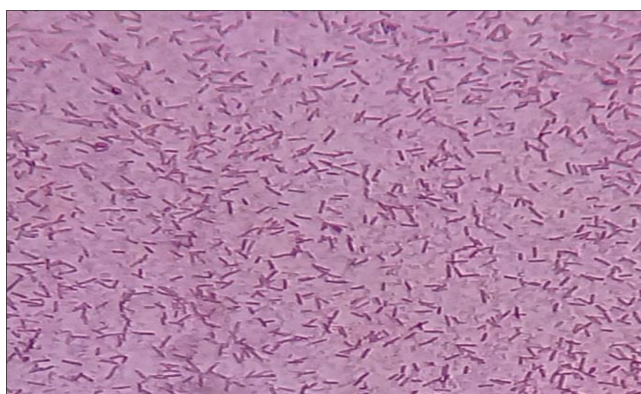

Fig. 3: Microscopic image of newly isolated *Geobacillus* species from Bandaru hot spring

solvents [28]. They do not require cofactors [29], exhibit a high degree of regioselectivity, and possess a wide range of substrate specificity for the conversion of various unnatural substrates [30].

Present work envisages the isolation and characterization of amylase and lipase producing bacteria, from natural source "Bandaru" hot Spring in Karnataka. This work describes the effects of culture conditions on amylase production in submerged fermentation under controlled conditions of carbon sources, temperature, pH, starch concentration, and agitation speed in a laboratory shaking incubator.

METHODS

Source of strain and media

Water sample was collected from hot water springs located on the bank of river Nethravathi (Belthangady taluk), Dakshina Kannada district, Karnataka state, India. The temperature and pH of the thermal water were recorded immediately after sampling. One milliliter of water sample was serially diluted in saline (0.9% NaCl) and plated onto solid PBTA media containing (peptone 1.5%, beef extract 0.2%, tryptone 0.5%, NaCl 0.1%, and agar 2%). Plates were sealed with parafilm and incubated at 60°C for 12 h to identify possible microorganism existing in the water samples. The various colonies were picked, isolated in pure culture and then stored at −20°C.

Chemicals

All chemicals were purchased from HiMedia Laboratories, Mumbai. Tryptone was procured from Rasayan laboratories.

Screening of amylase and lipase producing bacteria

Screening of amylolytic bacteria using starch

The amylase production of the isolates was determined by subculturing in SYPB media, which contains 2% starch, 1% yeast extract, 0.1% peptone, 0.1% beef extract, 0.05% $MgSO_4$, 0.04% $CaCl_2$, and 2% agar, pH-7 [31]. The plates were incubated at 60°C for 24 h. An appearance of clear zone around the colonies after flooding 1% iodine solution indicates the amylase activity of the isolates. Among those, the bacteria showing higher amylase activity were selected for further amylase enzyme production.

Screening of lipolytic bacteria using tributyrin

The lipase producing bacteria screening was performed in Petri dishes using medium constituted by 0.5% peptone, 0.3% yeast extract, 0.1% tributyrin, and 2% agar at pH 7.0 [32]. The isolated pure cultures were inoculated and plates were incubated at 60°C for 24 h. After incubation, a clear lipolytic halo appeared around the colonies. The isolated strains which displayed higher halos were selected as promising bacterial lipase producers using tributyrin as substrate.

Screening of lipolytic bacteria using Rhodamine B olive oil

The isolated bacteria were screened for lipolytic activity using tributyrin in Petri dishes using medium consists of nutrient broth, 0.8%; NaCl, 0.4% and agar-agar 2% at pH-7. The medium was autoclaved and cooled till 60°C. Then, 3% olive oil and 1 ml of Rhodamine B (1 mg/ml) were added [33]. The medium was thoroughly shake and spread onto Petri dishes, later bacterial strains were inoculated and incubated at 60°C for 48 h. The extracellular lipase enzyme production was identified as an orange fluorescent halo under ultraviolet (UV) light at 350 nm.

Biochemical and physiological characterization of isolate

Bacterial growth was determined by measuring the OD_{600} (Eppendorf Biospectrometer, Germany) in liquid media. Effect of temperature on bacterial growth was analyzed in PBT liquid broth media, between 40 and 90°C. The effect of salinity was determined; various amounts of NaCl were directly weighed to a 25 ml medium with the desired NaCl concentration (0%–1%) at 55°C. For effect of pH, the medium was adjusted with 5 M HCl or 10 M NaOH (in 0.5-unit steps) to obtain pH values ranging from 4 to 12 and recorded after 3 days at 55°C. The isolate was determined by conducting fermentation of D-cellobiose, D-xylose, D-galactose, glycerol, inositol, and D-lactose. The hydrolysis of starch, tributyrin, gelatin, casein and utilization of citrate, and acetate was also determined. Gram's reaction was determined using the HiMedia Gram Stain Kit according to the manufacturer's instructions.

Sequencing and phylogenetic analysis

The pure colonies of isolated bacteria were selected with a sterilized toothpick, and suspended in 0.5 ml of sterilized saline in 1.5 ml centrifuge tube and were centrifuged at 10,000 rpm for 10 min. After removal of supernatant, the pellet was suspended in 0.5 ml of InstaGene Matrix (Bio-Rad, USA) and incubated at 56°C for 30 min and then heated at 100°C for 10 min. After heating, the supernatant can be used for polymerase chain reaction (PCR). 1 µl of template DNA was added in 20 µl of PCR reaction solution. 27F/1492R universal primers (1492R 5'TACGGYTACCTTGTTACGACTT3' 27F 5' AGAGTTTGATCMTGGCTCAG 3') were used and 35 amplification cycles at 94°C for 45 s, 55°C for 60 s, and 72°C for 60 s were performed. DNA fragments amplified were about 1400 bp in the case of bacteria. Positive control (*Escherichia coli* genomic DNA) and a negative control in the PCR were included. Unincorporated PCR primers and dNTPs from PCR products were removed using the Montage PCR Clean up kit (Millipore). The purified PCR products were sequenced using BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, USA). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied Biosystems, USA). The sequence was aligned and submitted to NCBI database using Clustal X software. The phylogenetic tree was constructed using the neighbor-joining tree making an algorithm of MEGA 7 and deposited in GeneBank with the accession number KX824759.

Identification of molecular base sequence report using Sequencher version 5.2 software (Gene codes Corp, MD, USA). The base frequency report indicates the percentage of nucleotides such as adenine, thymine, cytosine, and guanine in individuals. The report also showed the nucleotide frequency, dinucleotide frequency, and trinucleotide frequency percentage with different nucleotide combinations.

Motility assay

Motility of the strain was tested using a modified method [34], semisolid motility test medium (1% agar) in 18 mm × 150 mm glass culture tubes. The bacterial culture was inoculated into the medium using a sterile straight needle to one-half the depth of the tube. Tubes were incubated at 55°C for 3 days and results were observed at 24, 48, and 72 h.

Antibiotic resistance profile

Drug resistance is the major difficulty of this era, which is leading toward morbidity and mortality [35]. Resistance to antibiotics was determined on PBTA media. The plates were amended at the following concentration in mg/ml: Amoxicillin (10), norfloxacin (15), erythromycin (10), cefixime (5), cephalexin (10), and cefpodoxime (5). Plates were streaked with *Geobacillus thermoleovorans* KNG 112 and were incubated at 55°C. The plates were observed daily for resistance up to 4 days.

Partial optimization of amylase production

Effect of temperature, pH, and carbon sources on amylase production

To study the effects of temperature and pH on enzyme production and growth of the organisms, the strain was grown in the basal media at different pH (5–10) and temperature (30°C–85°C). The optimum temperature and pH for maximum enzyme production were obtained after working out a series of experiments for the bacterial strain. The different carbon sources such as lactose, starch, sucrose, maltose, and glucose were used for the production of amylase.

Effect of agitation rate on amylase production

The effect of agitation rate was determined by incubating the culture at 55°C with different agitation speed 80, 100, 120, and 140 rpm. The bacterial growth was investigated spectrophotometrically at 600 nm.

Estimation of protein

The protein concentration of culture filtrate was estimated by the Bradford method [36], bovine serum albumin was used as standard. 250 µl of Bradford reagent was mixed with 50 µl of appropriate diluted enzyme sample and incubated for 10 min at room temperature. Optical density was measured at 595nm against a blank prepared with 50 µl buffer. The standard curve was constructed using bovine serum albumin was a standard known protein.

Amylase activity assay

The amylase activity was determined by measuring the amount of reducing sugars released during starch hydrolysis using the dinitrosalicylic method [37]. The reaction mixture containing 0.5 ml of 1% (w/v) soluble starch in 20 mM Tris–HCl, pH 7.4, and 0.5 ml of enzyme solution was incubated for 30 min at 60°C. The reaction was stopped by adding 1 ml of DNS solution and the mixture was boiled for 10 min. After cooling, the mixture was diluted 4 times with double distilled water and the absorbance at 540 nm was measured. One unit of enzyme activity was defined as the amount of enzyme that releases 1 µmol of reducing sugar equivalent to maltose per minute.

RESULTS AND DISCUSSION

Isolation of bacteria

Initially, six strains were isolated from hot water sample. Based on their morphological characteristics, only three strains have shown both amylolytic and lipolytic activity. The selected strain was named as IC5 for further characterization. Species belonging to genus *Geobacillus* have been isolated from many natural hot springs [38,39].

Biochemical identification of bacteria

The isolated bacteria from hot spring water were found to be a member of *Geobacillus* and identified as Gram-positive, aerobe, and non-motile rods. Cells formed cream circular colonies with a diameter of 2.0–2.5 mm and smooth margins on PBTA medium at 60°C. The temperature growth range was between 35 and 80°C with an optimum growth at 55°C. The pH growth range was from 5.5 to 9.5 with an optimum pH 7.5. The isolate was grown on PBTA medium having pH 7.5 at 60°C unless otherwise stated and subculturing was performed on the same medium. The isolate was maintained as a glycerol stock at −70°C for further studies.

The growth of isolate was observed up to 8% NaCl (w/v). The isolate utilized different carbon sources and shown positive results for D-cellobiose, glycerol, D-xylose and negative for D-galactose, inositol, and D-lactose. The isolates hydrolyzed starch and tributyrin and have not hydrolyzed casein and gelatin (Table 1).

Molecular identification

Approximately 1080 bp 16s rRNA gene product was sequenced for the isolated bacteria. This sequence, containing 1140 bp constituted, was by nucleotides with 1079 bp database query. 16S rRNA gene sequence of strain KNG 112 showed the highest homology (an identity of 99.9%) to 16S rRNA gene sequence of *G. kaustophilus* C4. A phylogenetic tree was constructed using 16S rRNA sequence of several microorganisms, which demonstrated that the closest neighbor of strain KNG 112 was *G. thermoleovorans* EC-5 (Fig. 4).

Analysis of nucleotide frequency

The partial sequences of 16s rRNA gene of *G. thermoleovorans* KNG 112 strain containing nucleotide, dinucleotide, and trinucleotide frequency percentage were determined using sequencer software version 5.2 (Table 2).

Table 1: Comparative physiological characteristics of thermophilic *Geobacillus thermoleovorans* KNG 112 isolated from Bandaru hot spring with reference strains

	G. KNG 112	*G. thermocatenulatus* (DSM 730^{T})	*G. thermodenitrificans* (DSM 466^{T})	*G. thermoleovorans* (DSM 5366^{T})
pH range	5.5–9.5	6.5–8.5	6.0–8.0	6.2–7.8
Optimum pH	7.5	6.8–7.0	6.8–7.0	6.8–7.0
Temperature range	35°C –80°C	42ºC–69°C	45°C–70°C	35°C–78°C
Optimum temperature	55°C	55ºC–60°C	55°C–60°C	55°C–65°C
Gram reaction	+	ND	ND	ND
Shape of the cell	Rod	ND	ND	ND
Motility	-	+	+	-
NaCl stability	0%–8%	0–5	0–5	0–4
Hydrolysis test				
Starch	+	-	-	+
Casein	-	+	-	-
Gelatin	-	-	-	-
Tributyrin	+	ND	ND	ND
Production of acids				
D-Cellobiose	+	+	+	+
D-Galactose	-	+	+	-
Glycerol	+	+	-	+
Inositol	-	ND	ND	ND
D-Lactose	-	-	+	+
D-Xylose	+	+	-	+

Detailed description of characteristic features of Taxa, 1. *G. thermoleovorans* KNG 112; 2. *G. thermocatenulatus* (DSM 730^{T}); 3. *G. thermodenitrificans* (DSM 466^{T}); 4. *G. thermoleovorans* (DSM 5366^{T}). *G. thermoleovorans: Geobacillus thermoleovorans*, *G. thermodenitrificans*: *Geobacillus thermodenitrificans*, ND:

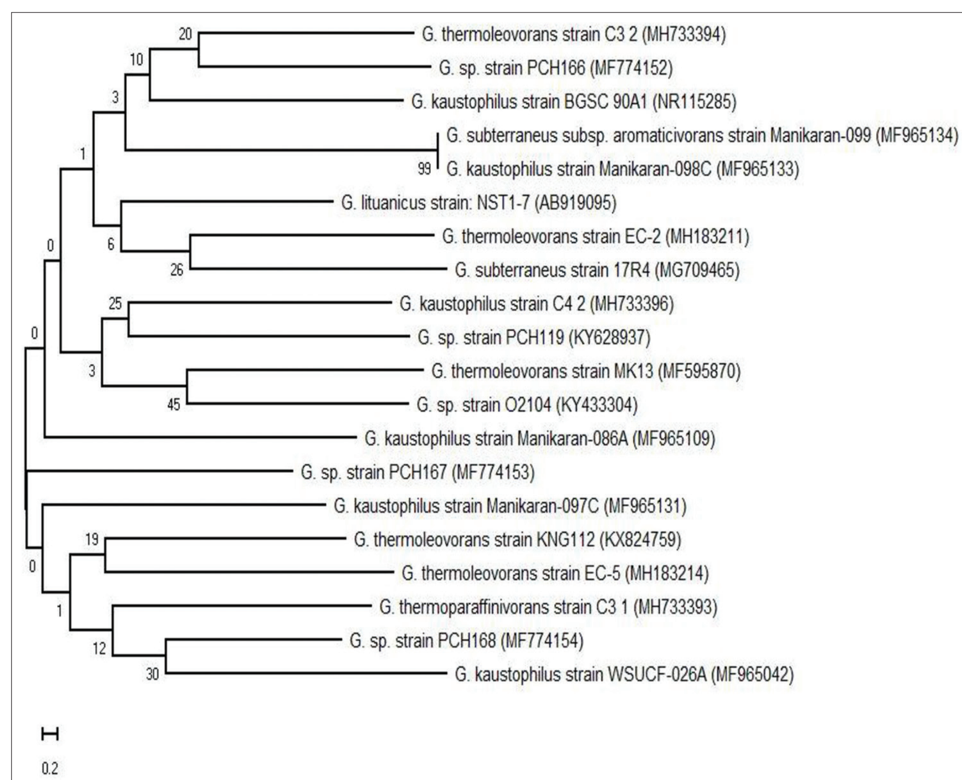

Fig. 4: Evolutionary relationships of the selected hot spring isolated strain 16s rRNA gene sequences with publically associated taxa. Phylogram was inferred using the neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap tests (500 replicates), is indicated at branching points

Table 2: Nucleotide frequency, dinucleotide frequency, trinucleotide frequency percentage with different nucleotide combinations of 16s rRNA sequence of *Geobacillus thermoleovorans* KNG 112

Base	Total	%
A	246	22.80
C	272	25.21
G	372	34.48
T	189	17.52
Ambiguity	0	0.00

Base	Total	%	Base	Total	%	Base	Total	%	Base	Total	%
AA	69	6.40	CA	55	5.10	GA	85	7.88	TA	37	3.43
AC	63	5.84	CC	71	6.59	GC	98	9.09	TC	40	3.71
AG	84	7.79	CG	94	8.72	GG	121	11.22	TG	72	6.68
AT	29	2.69	CT	52	4.82	GT	68	6.31	TT	40	3.71

Base	Total	%	Base	Total	%	Base	Total	%	Base	Total	%
AAA	14	1.30	CAA	15	1.39	GAA	24	2.23	TAA	16	1.49
AAC	20	1.86	CAC	16	1.49	GAC	19	1.76	TAC	8	0.74
AAG	25	2.32	CAG	17	1.58	GAG	30	2.79	TAG	12	1.11
AAT	10	0.93	CAT	7	0.65	GAT	11	1.02	TAT	1	0.09
ACA	12	1.11	CCA	12	1.11	GCA	21	1.95	TCA	10	0.93
ACC	17	1.58	CCC	18	1.67	GCC	24	2.23	TCC	12	1.11
ACG	23	2.14	CCG	23	2.14	GCG	38	3.53	TCG	10	0.93
ACT	11	1.02	CCT	18	1.67	GCT	15	1.39	TCT	8	0.74
AGA	16	1.49	CGA	17	1.58	GGA	29	2.69	TGA	23	2.14
AGC	28	2.60	CGC	25	2.32	GGC	32	2.97	TGC	13	1.21
AGG	27	2.51	CGG	33	3.06	GGG	37	3.44	TGG	24	2.23
AGT	13	1.21	CGT	19	1.76	GGT	23	2.14	TGT	12	1.11
ATA	6	0.56	CTA	8	0.74	GTA	14	1.30	TTA	9	0.84
ATC	2	0.19	CTC	14	1.30	GTC	15	1.39	TTC	9	0.84
ATG	11	1.02	CTG	18	1.67	GTG	26	2.41	TTG	17	1.58
ATT	10	0.93	CTT	12	1.11	GTT	13	1.21	TTT	5	0.46

Starch and lipid degradation

The genus *Geobacillus* produces a large variety of extracellular enzymes; amylases are particularly considered for industrial importance [40]. This study deals with the screening of amylolytic and lipolytic enzyme-producing bacteria and production condition optimization and partial characterization of crude extracellular amylase produced by novel *G. thermoleovorans* KNG 112. *G. thermoleovorans* KNG 112 amylase activity was monitored on starch agar media. After 24 h of incubation, clear zone was observed around the strain after flooding iodine solution (Fig. 5).

For lipid degradation, the strain produced clear zone around the colonies on the tributyrin agar medium. The agar plates containing olive oil and Rhodamine B have pinkish color and an impervious appearance. Lipase production was observed by irradiating plates with UV light at 350 nm. After 48 h of incubation, colonies have shown orange fluorescent halos around the colonies of lipase producing strains after longer incubation. IC5 showed a fluorescent halo (Fig. 6). It has been recognized that *Geobacillus* species can degrade various triacylglycerol.

Effect of temperature, pH, and carbon sources on amylase production

The extracellular amylase production of the bacterium was studied in SYBT broth media. In our study, *G. thermoleovorans* KNG112 has shown maximum activity at 55°C, where 30°C–80°C temperature ranges were also been observed. Most of the *Geobacillus* species were shown maximum activity at 55°C–70°C. The same optimum temperature was also observed in the optimization of amylase production from *Anoxybacillus flavithermus* [41]. In the series of pH ranges 5–10, the maximum amylase production was achieved at pH 7.5 (Table 3).

Further studies on enzyme production in shake-flask cultures were carried out using *G. thermoleovorans* KNG 112. The organism was used for extracellular amylase production in shake-flask culture using PBTA media (0.1% yeast extract, 0.25% peptone,0.1% beef extract, 0.005% $MgSO_4$.0.005% $CaCl_2$, and 2% soluble starch, pH-7.5) [30], for 36 h of incubation at 55°C and enzyme activity obtained was 0.76 U/ml. To enhance the production of the enzyme,

Fig. 5: The amylase production capability test of newly isolated *Geobacillus* was done by starch hydrolysis on the starch agar plate assay method. Bacterial isolate was streaked on the starch agar plate and plates were incubated at 55°C for 24 h.

various parameters associated with the production of amylase were studied in the medium used for the enzyme production. Optimization of culture conditions is very important for maximum microbial growth and enzyme production by microorganisms [42]. Among the physical and chemical parameters, optimum temperature, pH range, carbon and nitrogen sources are most important for enzyme production by microbes [43].

The time course in shake culture fermentation, the maximum rate of enzyme production was increased with the increase in the fermentation period and the maximum activity was reached after 36 h of incubation (Table 3). At that time, protein concentration obtained was 3.51 mg/ml.

From the time course study in shake culture, it was found that the rate of enzyme production was increased with the increase in the

Table 3: Effect of culture condition for extracellular amylase production from *Geobacillus thermoleovorans* KNG 112

Culture condition	Amylase activity (U/ml)	Relative activity (%)	Total soluble protein (mg/ml)
Initial pH			
5	0.32	26.67	0.75
5.5	0.53	44.64	0.98
6	0.77	64.84	1.13
6.5	0.83	72.78	1.21
7	1.12	93.29	1.94
7.5	1.20	100.00	2.17
8	1.14	95.60	2.20
8.5	0.83	69.54	2.11
9	0.73	61.06	1.89
9.5	0.56	46.95	1.75
10	0.39	33.07	1.69
Incubation temperature			
30	0.36	50.12	1.21
37	0.43	55.68	1.85
45	0.80	92.34	2.21
55	0.87	100	2.02
60	0.83	95.85	1.89
65	0.81	93.09	1.68
70	0.67	77.47	1.58
75	0.63	72.68	1.14
Incubation period			
12	0.63	71.09	2.56
24	0.73	91.34	3.51
36	0.76	100	3.42
50	0.66	86.24	3.22
60	0.57	71.09	3.03
90	0.47	69.92	2.94

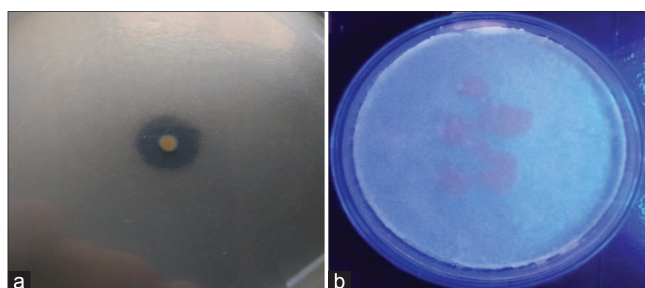

Fig. 6: Zone of clearance due to the hydrolysis of tributyrin on TA media by isolated *Geobacillus* species, the plate was incubated at 55°C for 24 h. The isolate has shown orange halos under ultraviolet light at 350 nm, which indicates the isolate was capable to produce lipase on olive oil Rhodamine B agar media.

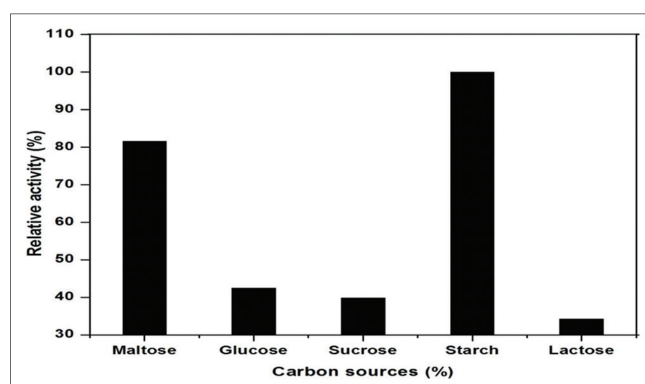

Fig. 7: The effect of different carbon sources on enzyme production was studied using loopfull of inoculum in 100 ml basal medium. The fermentation was carried out at 55°C at 120 rpm for 40 h. Absorbance was measured at 540 nm with spectrophotometer and enzyme activity was presented on the y axis and carbon sources was on X-axis

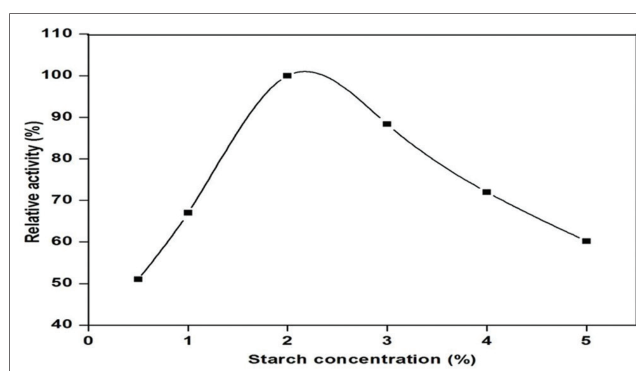

Fig. 8: The effect of starch concentration on the amylase production by *Geobacillus thermoleovorans* KNG 112 at 55°C and pH 7.5 in shake flask culture

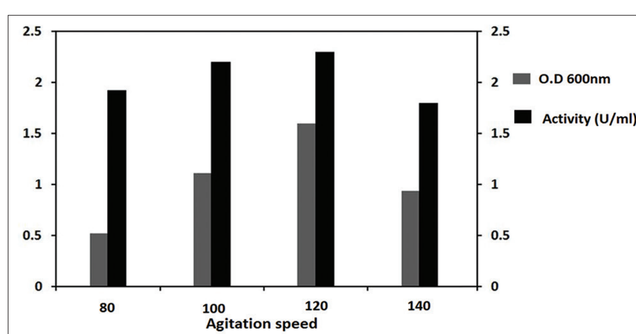

Fig. 9: Effect of agitation speed on amylase production

fermentation period and reached its maximum activity after 48 h incubation (Table 3).

Fig. 8 shows the effect of starch concentration, maximum amylase production was at 2%. The maximum extracellular amylase was

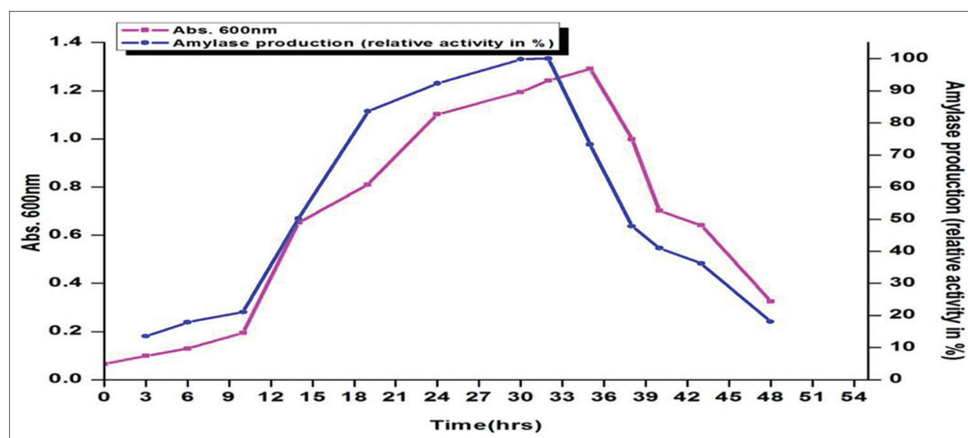

Fig. 10: Amylase was produced at various stages of bacterial growth in SYBT media and was incubated at 55°C and pH of 7.5. The activity was assayed in the supernatant of culture. The optical density of bacterial growth was measured at 600 nm using biospectrophotometer

produced with an increase in the starch concentration up to 2%. At higher concentration (more than 2%) of starch, the production of amylase was decreased, same results were obtained for the production of α-amylase by an extreme thermophile *Bacillus thermoleovorans* NP54 [44].

Fig. 9 shows that the maximum production of amylase was at 120 rpm speed. The data exposed that shaking is very essential for production of amylase; enzyme production was declined after shaking speed reaches 150 rpm due to the damage of bacterial cell at higher shaking speed [45].

Antibiotic susceptibility

The growth performance of *G. thermoleovorans* KNG 112 was studied in the presence of a variety of antibiotics. The strain was susceptible to cephalexin (10mg/ml), and resistant to norfloxacin (15), erythromycin (10), cefixime (5), and cefpodoxime (5).

CONCLUSION

The thermophilic novel bacteria *G. thermoleovorans* KNG 112 was isolated and primary enzymatic production potential was determined and characterized. This is the first investigation describing the isolation and characterization of novel *Geobacillus* strain from Bandaru hot spring Karnataka, India. The diversity in phenotypic and enzymatic analysis among *G. thermoleovorans* strains indicated the presence of subspecies. These capable results can be exploited further for production of industrially viable thermostable enzymes. The present study envisages the capability of thermophilic bacteria *G. thermoleovorans* KNG 112 for the production of biotechnologically important amylolytic and lipolytic thermo stable enzymes. The optimal condition of the amylase production was achieved at 55°C and pH-7.5, while 2% starch achieved maximum amylase production and 120 rpm agitation speed. The maximum growth and amylase production was recorded for 36hrs of incubation.

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AUTHOR'S CONTRIBUTIONS

The authors declare that this work was done by the authors named in this article.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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