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Production of L-glutaminase by *Streptomyces rochei* Detected from the Lime Stone Quarries of Deccan Trap

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The present study focuses on exploration of an actinomycete from limestone quarries of deccan trap for the production of L-glutaminase. *Streptomyces rochei*, a promising producer of L-glutaminase was confirmed based on 16S rDNA sequence (DMQ-14: JQ889270) analysis. At optimum level of process parameters (pH-8.0; temperature - 40^{0}C ; inoculum size-1x10^8 spores/ml and agitation-200 rev/min.) a linear increase (9.86±0.025 IU; 12.28±0.010 IU; 13.46±0.015 IU; 15.78±0.02 IU respectively) in the production of L-glutaminase by *Streptomyces rochei* was achieved. Among the nutritional sources, Starch and L-glutamine at 1.0 % w/v proven to be the best carbon and nitrogen sources for the enhanced production (25.42±0.040 IU and 30.24±0.01 IU respectively) of L-glutaminase. $MgSO_4$, $7H_2O$ (0.05% w/v) proved to be the most suitable metal ion for further increase (31.55±0.020 IU) in the production of L-glutaminase. Thus, with all the optimized conditions, the maximum production of L-glutaminase was 31.55±0.020 IU.

Key words: L-glutaminase, Actinomycetes, Limestone quarry, Submerged system, Optimization, 16S rDNA.

L-glutaminase (EC 3.5.1.2) is an amidohydrolase enzyme which generates L-glutamic acid and ammonia from L-glutamine (Archibald 1944). This cellular enzyme deaminates L-glutamine and acts as a proteolytic endopeptidase, which hydrolyses the peptide bonds present in the interior of protein molecules. It is ubiquitous from the presence point of view in plants, animals and microbes both in prokaryotes and eukaryotes. Among some well studied genera in microbes worth mentioning from study perspective are *E. coli* (Prusiner and Stadtman, 1976), *Pseudomonas* sp. (Jyoti *et al*., 2011), *Brevibacterium* sp. (Imada *et al*., 1973), *Vibrio costicola* (Jeyaprakash *et al*., 2010), *Streptomyces rimosus* (Sivakumar *et al*., 2006), *Streptomyces avermitilis* and *Streoptomyces labedae* (Abdallah *et al*., 2012), *Streptomyces gresius* (Muthuvelayudham *et al*., 2013), *Hypocrea jecorrnea* (Bulbul *et al*., 2013) , *Zygosaccharomyces* sp. (Iyer and Singhal, 2010), *Bacillus* sp. (Tadikamalla *et al*., 2011) and *Micrococcus luteus* k-3 (Masuo *et al*., 2005) etc.

Efforts to increase the glutamate content of soya sauce using salt and thermo tolerant glutaminase have drawn much attention (Nandakumar *et al*., 2003). The action of glutaminase plays a major role as therapeutic agent in cancer and HIV. (Kumar and Chandrasekaran, 2003). It also plays an important role in biosensor as a monitoring agent for glutamine level measurement (Kashyap *et al*., 2002). A speciality chemical called theanine also used to be produced by this enzyme following c-glutamyl transfer reaction. Use of this enzyme as a flavour enhancer has become a successful alternative against the use of commercial flavour enhancer in Chinese preparations and an allergen by action for individuals (Renu *et al*., 2003).

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Production of enzyme was influenced by variety of physical and nutritional parameters and factors affecting the production in recent years had received attention as of its great demand in clinical application and also in food industries. Optimization of parameters can in turn influence enzyme synthesis and cell yield (Pandey *et al*., 2003). It is reported to be produced in both submerged and solid state systems by a number of myriad sources. From the compatibility perspective in mass production and as well as beneficial application aspect extracellular enzyme producer as choice of source is always attractive. Several reports (Dastager *et al*., 2007a, 2007b, 2008) are there from our research laboratory pertaining to the isolation of novel and potential actinomycetes. Different bioactive molecules have been screened from actinomycetes (Vishalakshi *et al*., 2009 and Ameena *et al*., 2010). An attempt has been made in the present investigation to isolate and screen actinomycetes from lime stone quarries of deccan trap, for the extracellular L-glutaminase.

METHODS

Collection and processing of soil samples

Soil samples from the regional lime stone quarries, agricultural fields and crab mount soil samples from the sites of mangrove, near Salim Ali Bird Sanctuary, were collected for the isolation of actinomycetes. The samples collected were cleaned, dried and subjected for phenolic, heat and calcium carbonate treatment (Kuster *et al*., 1963).

Isolation and basic identification of actinomycetes

Actinomycetes were isolated from the treated soil samples by serial dilution technique on Starch Casein Agar – SCA **(**Starch 10, K_2HPO_4 2.0, KNO_3 2.0**,** NaCl 2.0**,** Casein 0.3**,** $MgSO_4$, $7H_2O$ 0.05, $FeSO_4$,$7H_2O$ 0.01 and Agar 20-gL^{-1}**)**. Growth of actinomycetes after the incubation of three days at 35^0C, were identified based on the standard colony characters. The important microscopic features namely Gram staining, mycelial branching and sporulation pattern of the selected colonies were recorded. The identified colonies of actinomycetes were subjected for the biochemical characters, utilization of sugars and amino acids (Shirling and Gottlieb, 1966; Buchanan *et al*., 1974). The confirmed isolates of actinomycetes were sub cultured on SCA and preserved at 4^0C.

Screening of actinomycetes for L-glutaminase

The identified isolates of actinomycetes were screened qualitatively by rapid plate assay (Gulati *et al*., 1997) for the synthesis of L-glutaminase on Starch Glutamine Mineral (SGM) Medium - Starch 10, K_2HPO_4 2.0, NaCl 2.0, $MgSO_4$, $7H_2O$ 0.05, $FeSO_4$, $7H_2O$ 0.01 and Agar 20 gL^{-1} along with L-glutamine and 1%, phenol red (2.5% alcoholic stock solution) at pH 6.8. Selected isolates of actinomycetes were subjected for quantitative screening by broth culture assay (Imada *et al*., 1973) using SGM Medium. 1 ml of 0.01% Tween 80 spore suspension of 5 days old test isolate ($1x10^8$ spores/ml) were inoculated into 100 ml medium (pH 7.0) and incubated for six days at 35^0C. 5 ml of incubated broth was drawn at every 24 hrs and assayed for L-glutaminase activity.

Production of L-glutaminase

A batch wise bioprocess (Krishnakumar *et al*., 2011) was carried out using a selected potential isolate of *Streptomyces* sp. DMQ-14 in a 250 ml Erlenmeyer flask containing 100 ml of SGM Medium (pH 7.0). After sterilization of the medium at 121^oC for 15 min. 5 ml suspension of five days culture with spore count $1x10^8$ spores/ml was inoculated separately and incubated at 35^oC for a week. The fermentation was carried out at both static (at 35^0C) and shake (180 rpm, 35^0C) conditions. The enzyme activity of the fermented broth was determined at every 24 hrs.

Assay of L-glutaminase

5 ml of the culture broth was withdrawn and centrifuged at 6,000-8,000 rpm for 10 minutes. The enzyme assay was carried out with the supernatant obtained as per Imada *et al*. (1973). One IU of L-glutaminase is the amount of enzyme which liberates 1 mmol of ammonia per ml per minute (mmol/ml/min). Ammonium sulphate (6mM) was used as standard (Sivakumar *et al*., 2006).

Influence of process variables

Important physicochemical process variables such as pH, temperature, agitation, inoculum size and nutritional parameters such as carbon sources (Glucose, Fructose, Maltose, Mannitol and Starch - 0.5 to 2.5 % w/v), nitrogen sources (Beef extract, Malt extract, Calcium nitate, L-glutamine (0.25 to 1.25 % w/v) and metal ions ($MgSO_4$, $7H_2O$, $MnSO_4$, $7H_2O$, $CaSO_4$, $7H_2O$, $FeSO_4$, $7H_2O$, $CoSO_4$, $7H_2O$ - 0.05 to 0.25 % w/v) were examined at different range/concentrations in

batch wise bioprocess under submerged system as mentioned earlier. One factor at a time approach (Iyer and Singhal, 2010) was employed to understand the influence of process variables on the production of extracellular L-glutaminase. All the values are measured in triplicate and their standard deviation and standard error of mean were calculated using a statistical software Graph Instat pad 3.1 version.

16S rDNA analysis

The genomic DNA of the *Streptomyces* isolate DMQ-14 was extracted (Rintala and Merja Kontro, 2001) and purified by DNA wizard column - Promega Wizard. The nucleotide sequence was obtained from Department of Biotechnology, University of Helsinki, Finland and submitted to NCBI. BLAST search comparison was made against the Genbank databases and the related strains were selected for alignment by CLUSTAL X program (Thompson *et al*., 1997). The evolutionary history was inferred using the Neighbor-Joining method and the evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al*., 2004). Phylogenetic analyses were carried out employing MEGA4.

RESULTS

Isolation and identification of actinomycetes

Colonies obtained on Starch casein agar were identified as actinomycetes based on aerial / substrate mycelium and sporulation pattern. In all six colonies obtained from mangrove soil, two from limestone quarry soil and one from agricultural field soil were further confirmed as genus of *Streptomyces* based on biochemical properties, showing positive for hydrolysis of gelatin and starch; reduction of hydrogen peroxide and nitrate; negative for hydrogen sulphide production. The physiological properties further confirms the genus and to some extent the species, based on utilization of specific sugars and amino acids. All these morphological, biochemical and physiological characters of the test isolates are as presented in Table 1.

Screening of actinomycetes for L-glutaminase production

The confirmed isolates of actinomycetes belonging to the genus *Streptomyces* were screened and graded based on the intensity (+ / ++ / +++) of pink colour developed from yellow, indicating maximum synthesis (Table 2) of L-glutaminase. Four isolates namely DMQ-13, DMQ-14, DMM-8 and DMM-10 chosen being highly positive for the synthesis of L-glutaminase were subjected to quantitative screening for the production of L-glutaminase (Figure 1).The isolate *Streptomyces* DMQ-14 has shown maximum production (15.1±0.03 IU) of L-glutaminase, followed by DMQ-13 (12.4±0.04 IU) and the least production was shown by DMM-8 (8.92±0.03 IU), followed by DMM-10 (7.61±0.03 IU).

Effect of process variables on the production of L-glutamianse

The effect of important physicochemical and nutritional process variables on the production of L-glutamianse by the potential isolate of *Streptomyces* DMQ-14 in a batch wise bioprocess were evaluated. The pH 8.0 (Table 3: 9.9±0.025 IU), Temperature 40°C (Table 4: 12.3±0.01 IU), Inoculum size of 1 X 10^8 spores /ml (Table 5: 13.5±0.02 IU) and agitation 200 rev/min (Table 6: 15.78±0.02 IU) were recorded to be optimum for the maximum production of L-glutaminase. Among the nutritional process variables, 1.00% of Starch (Figure 2: 25.42±0.04 IU), 1.00 % of L-glutamine (Figure 3: 30.24±0.01 IU) were proved to be effective for the maximum production of L-glutaminase at the end of 120 h of fermentation. The effect of salt (sodium chloride) at various concentrations on the production of L-glutaminase was also examined. The maximum production of L-glutaminase was recorded (Figure 4) at 2.0 % concentration (23.0±0.05 IU) on 120 h of fermentation. The influence of 0.05% of $MgSO_4$, $7H_2O$ (Figure 5: 31.35±0.02 IU) were proved to be effective for the maximum production of L-glutaminase at the end of 120 h of fermentation.

Molecular characterization of the potential isolate

Molecular characterization of the potential isolate of *Streptomyces* DMQ-14 was carried out by 16S rDNA analysis. The optimal tree with the sum of branch length = 0.2277795 was plotted. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were shown next to branches. The tree was drawn to scale; with branch lengths same unites as those of the evolutionary distances used to infer the

Table 1. Morphological, biochemical and physiological properties of actinomycetes

	Isolates								
Properties	DMM-2	DMM-8	DMM-10	DMM-11	DMM-12	DMM-13	DMQ-13	DMQ-14	DMS-3
Aerialmycelium	Gray	Dark gray	Dark gray	Gray	Gray	Dark gray	Dark gray	Gray	Whitish gray
Substrate mycelium	Pale yellow	Pale yellow	Pale yellow	Colorless to yellow	Colorless to yellow	Pale yellow	Yellow brown	Red orange	Light brown
Sporulation features	Spiral Gray Smooth	Straight chain Gray Smooth	Straight chain Gray Smooth	Open spiral Gray Smooth	Straight chain Gray Smooth	Straight chain Gray Smooth	Open loop Gray Warty	Straight chain Gray Smooth	Open spiral White
				Hydrolysis of					
Casein	+	+	+	+	+	+	+	+	+
Gelatin	+	+	+	+	+	+	+	+	+
Starch	+	+	+	+	+	+	+	+	+
				Reduction of					
H_2O_2	+	+	+	+	+	+	+	+	+
Nitrate	+	+	+	+	+	+	+	+	+
H_2S Production	-	-	-	-	-	-	-	-	-
				Utilization of sugars					
Arabinose	+/-	+	+	+	+/-	+	+	+	+
Fructose	+	-	+	+	+	-	+	+	+
Galactose	+	-	+	+	+	-	+	+	+
Raffinose	-	+	+/-	-	-	+	+	-	-
Rhamnose	+	+	+	+	+	+	+	+/-	+
Xylose	+	+	+	+	+	+	+	-	+
				Utilization of amino acids					
Cysteine	+	+	+	+	-	-	+	+	+
Glutamine	+	+	+	+	+	+	+	+	+
Hydroxyproline	+	+	+	+	-	-	+	-	+
Phenylalanine	+	+	+	+	-	-	+	+	+
Tryptophan	+	+	+	+	-	-	+	+	+
Valine	+	+	+	+	-	-	+	+	+

Table 2. Qualitative screening of *Streptomyces* for the synthesis of L-glutaminase

Isolates	Coloration at different incubation period (h)					
	24	48	72	96	120	144
DMM - 2	-	-	+	+	+	+
DMM - 8	-	+	++	++	+++	+++
DMM - 10	-	-	+	++	+++	+++
DMM - 11	-	+	+	+	+	+
DMM - 12	-	-	+	+	+	+
DMM - 13	-	+	+	+	+	+
DMQ - 13	-	+	+++	+++	+++	+++
DMQ - 14	-	+	++	+++	+++	+++
DMS - 3	-	+	+	+	++	++

+++: High; ++: Moderate; +: Low; -: No colour

Table 3. Effect of pH on the production of L-glutaminase by *Streptomyces*

pH	Enzyme activity (IU) at different fermentation Period (h)						
	24	48	72	96	120	144	168
7.0	2.6 ± 0.02	3.2 ± 0.59	5.16 ± 0.03	5.85 ± 0.04	6.64 ± 0.04	6.16 ± 0.03	5.75 ± 0.04
7.5	3.5 ± 0.025	4.3 ± 0.025	5.46 ± 0.03	5.76 ± 0.025	6.57 ± 0.02	6.33 ± 0.035	5.46 ± 0.02
8.0	3.8 ± 0.025	5.5 ± 0.025	6.3 ± 0.02	7.8 ± 0.02	9.9 ± 0.025	8.7 ± 0.02	8.2 ± 0.025
8.5	3.7 ± 0.025	5.7 ± 0.025	6.6 ± 0.025	7.3 ± 0.025	8.5 ± 0.025	8.2 ± 0.025	7.7 ± 0.025
9.0	3.5 ± 0.011	5.5 ± 0.015	6.2 ± 0.017	7.2 ± 0.01	8.2 ± 0.02	8.1 ± 0.025	7.5 ± 0.02

Table 4. Effect of temperature on the production of L-*glutaminase* by Streptomyces

Temp.	Enzyme activity (IU) at different fermentation Period (h)						
(°C)	24	48	72	96	120	144	168
30	3.7 ± 0.200	4.3 ± 0.020	4.7 ± 0.035	6.3 ± 0.025	8.3 ± 0.030	7.7 ± 0.035	6.3 ± 0.025
35	4.3 ± 0.025	4.6 ± 0.025	6.7 ± 0.025	7.9 ± 0.020	9.5 ± 0.020	8.8 ± 0.023	7.9 ± 0.020
40	5.3 ± 0.010	6.5 ± 0.011	7.8 ± 0.025	9.2 ± 0.015	12.3 ± 0.010	10.5 ± 0.015	9.2 ± 0.015
45	3.5 ± 0.015	5.9 ± 0.025	7.2 ± 0.015	8.6 ± 0.025	11.9 ± 0.003	10.6 ± 0.025	8.6 ± 0.025
50	4.5 ± 0.020	3.9 ±0.025	5.3 ± 0.020	6.7 ± 0.02	10.4 ± 0.015	8.2 ± 0.020	6.7 ± 0.020

Table 5. Effect of inoculum size on the production of L-glutaminase by *Streptomyces*

Inoculum	Enzyme activity (IU) at different fermentation Period (h)						
size	24	48	72	96	120	144	168
1×10^5	3.3± 0.15	3.9± 0.02	3.7± 0.02	4.3± 0.015	6.9± 0.02	5.7± 0.02	5.2± 0.02
1×10^6	4.9± 0.02	5.3± 0.016	5.9± 0.025	6.3± 0.02	7.9± 0.02	7.2± 0.02	6.5± 0.02
1×10^7	5.5± 0.03	6.3± 0.015	7.2± 0.02	8.5± 0.015	12.2± 0.005	11.8± 0.015	10.5± 0.015
1×10^8	6.2± 0.015	8.5± 0.015	11.2± 0.010	11.7± 0.015	13.5± 0.015	12.8± 0.015	12.5± 0.015
1×10^9	5.5± 0.015	6.2± 0.025	6.8± 0.015	6.5± 0.015	8.7± 0.01	8.3± 0.01	7.5± 0.015

Table 6. Effect of agitation on the production of L-glutaminase by *Streptomyces*

Agitation	Enzyme activity (IU) at different fermentation Period (h)						
(rpm)	24	48	72	96	120	144	168
140	3.6± 0.2	4.1± 0.41	4.2± 0.203	5.0± 0.59	7.1± 0.148	6.0± 0.036	5.4± 0.598
160	3.9± 0.015	4.7± 0.005	4.5± 0.01	5.9±0.15	6.9± 0.02	6.3± 0.598	5.4± 0.01
180	4.3± 0.015	4.9± 0.02	5.6± 0.02	6.3± 0.02	7.6± 0.02	6.5± 0.015	6.1± 0.015
200	5.8± 0.02	6.3± 0.02	8.9± 0.02	9.9± 0.02	15.8± 0.02	12.7± 0.02	7.3± 0.02
220	4.7± 0.03	5.1± 0.02	8.5± 0.02	9.7± 0.02	14.5±0.015	15.3± 0.015	12.5± 0.025

phylogenetic tree. The phylogenetic tree (Fig. 6) reveals that, the test isolate *Streptomyces* DMQ – 14 has got 99.00% similarity with the type strain *Streptomyces rochei* NR041091 indicating the confirmation of the potential strain as *Streptomyces rochei*. GenBank accession number for the nucleotide sequence of the potential isolate is JQ889270.

DISCUSSION

Isolation and screening

The literature available is limited to the isolation and screening of bacteria and fungi only (Jeyaprakash *et al*., 2010; Iyer and Singhal, 2010) for the synthesis of L-glutaminase. Very few cultures of actinomycetes have been isolated from

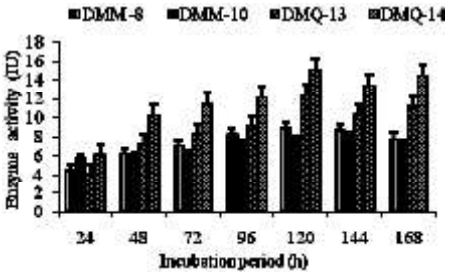

Fig. 1. Screening of *Streptomyces* for the production of L-glutaminase

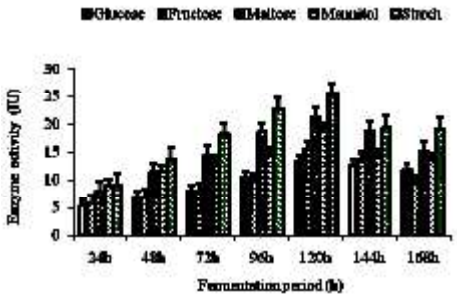

Fig. 2. Effect of carbon sources on the production of L-glutaminase by *Streptomyces*

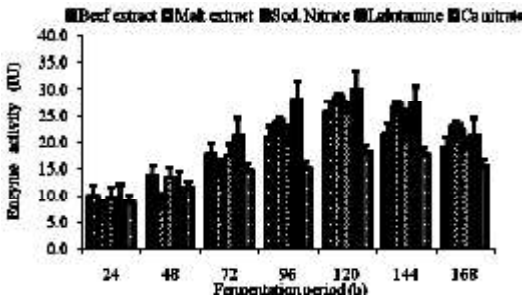

Fig. 3. Effect of nitrogen sources on the production of L-glutaminase by *Streptomyces*

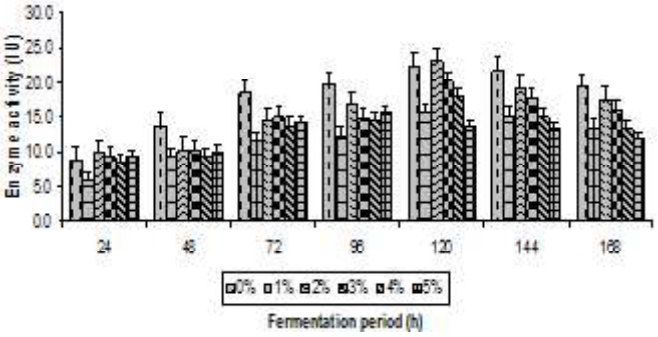

Fig. 4. Effect of sodium chloride on the production of L-glutaminase by *Streptomyces*

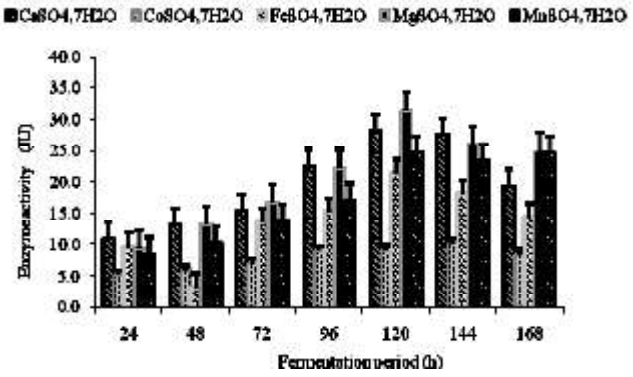

Fig. 5. Effect of metal ions on the production of L-glutaminase by *Streptomyces*

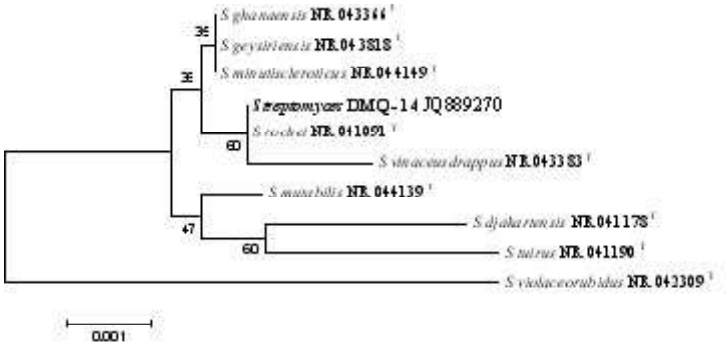

Fig. 6. Phylogenetic tree of the potential isolate of *Streptomyces* DMQ - 14

different sources by several researchers aiming at the production of L-glutaminase. Actinomycetes isolated from marine water (Sivakumar *et al*., 2006; Krishnakumar *et al*., 2011) and mangrove samples (Balagurunathan *et al*., 2010) for the production of L-glutaminase were reported from India. Detection and biotechnological exploration of several actinomycetes were reported (Dastager *et al*., 2007; Vishalakshi *et al*., 2009; Ameena *et al*., 2010; Syed and Dayanand, 2012a, 2012b) from our research laboratory. In the present study, actinomycetes were isolated and screened for the synthesis and production of L-glutaminase. An isolate of *Streptomyces* DMQ-14 obtained from the soil of limestone quarry was proved to be efficient, (15.1±0.03 IU) for the synthesis of L-glutaminase. The harsh environment and typical physiological conditions of limestone quarries proved to be a

good ecological niche for the prominent and potential isolate of actinomycetes.

Process optimization

A batch wise bioprocess was optimized with important physicochemical and nutritional variables for the production of L-glutaminase under submerged system. Several reports are available on the process standardization by submerged system (Iyer and Singhal, 2010; Jeyaprakash *et al*., 2010) and solid state system (Prabhu *et al*., 1997; Pandey *et al*., 2003) for the production of L-glutaminase by employing either bacterial or fungal cultures. Relatively, not much information is available regarding the production of L-glutaminase by actinomycetes. The pH 8.0, temperature 40ºC, agitation 200 rev/min and $1x10^8$ spores/ml inoculums size were proved to be optimum for the maximum production of L-glutaminase under submerged system by *Streptomyces* DMQ-14. Agitation and aeration were considered (Banik *et al*., 2011) as most critical parameters used for process scale–up and determination of productivity of enzyme. Actinomycetes being filamentous, sporulating and highly aerobic naturally performs better in these conditions. Incorporation of several carbon sources had shown enhanced activity from 23.0±0.05 IU to 25.42±0.04 IU. Among them, starch promoted the maximum activity (25.42±0.04 IU) compared to other sources. This result similarizes very rare in recent surveyed literatures indicating a natural adaptation of the organism to the carbon source already used in the said medium all over the process. The same source is also used in another literature tested with *Pseudomonas* sp. in submerged fermentation where it showed better activity (Jyoti *et al*., 2011). Maltose (21.29±0.05 IU) is the second best source for the production of L-glutaminase and almost equivalent to mannitol (19.03±0.05 IU). This result is quite befitting with the report of Sivakumar *et al*. (2006). But, to our surprise, the activity of glucose (13.29±0.041 IU) contradicts with the literature pertaining to the influence of carbon sources (A patent: Yuasa *et al*., 1999), dealing with the production of L-glutaminase by an yeast. It appears that, glucose acts as a repressor or very slowly assimilated in the medium than the other carbon sources. Nitrogen can be an important limiting factor for the microbial production of enzymes (Chandrasekaran *et al*., 2000). L-glutamine act as the optimum nitrogen source (30.24±0.01 IU) among all sources tested indicating quite similar results with the previous researchers (Prabhu *et al*., 1997; Krishnakumar *et al*., 2011) and very recently by Thadikamala *et al*. (2011). It depicts that, indeed the amide nitrogen of glutamine was source of amino groups in a wide range of biosynthetic processes and it also frequently involved in protein active or binding sites (Jeyaprakash *et al*., 2010). Next suitable source was malt extract (28.3±0.02 IU) which similarizes with the value of 16.6 IU by Krishnakumar *et al*. (2011) and 15.61 IU by Sivakumar *et al*. (2006). Sodium nitrate (25.5±0.02 IU) found to be the optimum source among inorganic nitrogen sources for L-glutaminase production which is quite similar with the results of *Vibrio sp*. reported by Jeyaprakash *et al*. (2010) and *Aspergillus* sp. by Prasanth *et al*. (2009). Malt and beef extract are not much significantly higher in values from each other, that is quite equivalent as organic nitrogen source. It is also supported with the literature of Sivakumar *et al*. (2006) being tested with actinomycetes. While, calcium nitrate has shown least value (18.3±0.03 IU) for optimum production of L-glutaminase, whereas it is promoting a good yield in case of production of L-glutaminase by actinomycetes (Sivakumar *et al*., 2006). Among metal ions tested, magnesium sulphate proved to be the optimum one in comparison to other metal ions tested and it also similarizes with the observations of Jeyaprakash *et al*. (2010) for the production of L-glutaminase by *Vibrio* sp. Whereas, $CoSO_4$ (10.03±0.03 IU) had the least effect on the production of L-glutaminase indicating its role as suppressor for the yield of L-glutaminase in submerged fermentation as well as a poor growth inducing cofactor.

Characterization

Characterization and confirmation of any potential isolate is very important and essential before it is being submitted to the culture deposit centers. Although, the potential isolate of an actinomycete DMQ-14, was confirmed as the genus *Streptomyces* based on morphological, biochemical and physiological properties, molecular characterization was essential to confirm its species level. The molecular characterization by 16S rDNA gene sequence reveals that, 1417 nucleotide base pairs consisting of Adenine -22.4%, Guanine –

33.9%, Cytosine – 25.7% and Thymine -18.0%; with AT:GC ratio of 40.4:59.6. Blast analysis denoted 99.00% similarity to *Streptomyces rochei* family. Thus, confirming the potential isolate DMQ-14 as *Streptomyces rochei*.

CONCLUSIONS

The present study reveals that, limestone quarries would be the better ecological niche for the occurrence of potential actinomycetes. *Streptomyces rochei* DMQ-14 proved to be relatively a potential strain for the production of L-glutaminase under submerged system in comparison with any of the reported isolates of actinomycetes. The modified basic starch casein medium as starch glutamine mineral medium proved to be the best for the production of L-glutaminase. Halo tolerant nature of the enzyme would be advantageous as a potent commercially and industrially applicable L-glutaminase, which requires to be explored further.

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