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Research Article

Mutagenesis of *Bacillus* spp. using Ethyl Methane-sulfonate (EMS) for Improvement of Amylase Activity

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Abstract

An investigation has been carried out for the improvement of activity of amylase produced by *Bacillus* spp. using chemical mutagen i.e., ethyl methane-sulfonate. Fifteen isolates have been taken and are characterized as *Bacillus* spp. on the basis of biochemical characterization. Amylase activities of both mutated and non-mutated isolates of *Bacillus* spp. were determined at different pH and temperatures by using DNS method. Among non mutated isolate NBS-13 possessed highest amylase potential at temperature 60°C and pH 7.0. Lowest and highest Km value was observed in NBS-15 (0.046 mg/ml) and in NBS-8 (1.060 mg/ml), whereas maximum and minimum Vmax values was found in NBS-9 (6.024 µmole/min/mg and in NBS-2(0.270 µmole/min/mg) respectively. On the other hand, among mutated strains, MBS-11 and MBS-12 which depicted highest amylase values at pH 7.0 and pH 5.8 respectively. MBS-5 shows intense enzyme activity at 55°C. Lowest Km value was observed in MBS-12 (0.036 mg/ml) and maximum in MBS-14 (2.978 mg/ml), whereas maximum and minimum Vmax values was observed in MBS-12 (125 µmole/min/mg) and in MBS-6 (1.459 µmole/min/mg) respectively. Therefore, amongst these, two isolates i.e., NBS-9 (non mutated) and MBS-5 (mutated) shows intense enzyme activity at pH (7.0) and temperature (55°C) with specific activity 2.851 (µmole/min/mg) and 1.293 (µmole/min/mg) respectively and NBS-15 and MBS-12 are considered to have best binding capacities with substrate starch in non mutated and EMS-mutated strains. On the basis of overall observation, it is concluded that all the isolates are *Bacillus* species and having significant amylase production abilities and due to EMS-mutation there is improvement of amylase production abilities in general, especially those who are originally (in non mutated isolates form) poor in amylase activity.

Keywords: *Bacillus* spp., Amylase, EMS, DNS, pH, Vmax, Km.

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Introduction

In a group of industrial enzymes, 30% of the market alone is covered by amylases. Due to which the demands of microbial enzymes in industries increases day by day because of its economical production and various advantages in respect to biotechnological activities i.e., pharmaceuticals, renewable energy, liquefaction of starch, detergent, warp sizing of textiles, fibres, paper, foodstuffs, baking, clarification of haze formed in beer or fruit juices, etc [1]. From number of microorganisms, *Bacillus* spp. is the choice of microorganism because of maximum production of extracellular enzymes such as amylase, arabinase, cellulase, lipase, protease and xylanase [2]. Alpha amylase is an extracellular enzyme that hydrolyses α-D-(1,4) glycosidic linkage to produce different starch carbohydrates. It was the first enzyme produced from fungal source in 1894 which was used in many pharmaceutical benefits like for the treatment of digestive chaos. The mutant variants of *Bacillus* spp. has better ability to produce alpha amylase, which can be done by using various mutagenesis [3]. Different methods have been used to improve the enzyme production i.e., chemical method such as Ethyl Methane-sulfonate (EMS), N-methyl-N´-nitro-N-nitrosoguanidine (NTG), Ethidium bromide (Et Br), nitrous acid and ultra violet irradiations method were found to be suitable mutagens for the improvement of enzyme production [4]. In our study we use chemical method i.e., EMS for the betterment of enzyme activity.

Materials and Methods

Microorganism and culture conditions

Soil samples were collected from dumping sites of cereal brans of different mills of Jammu region and incubated at 80°C for the extraction of amylase. Isolation was done by serial dilutions and pour plate method using the peculiar media i.e. MYP (Phenol Red Egg Yolk Polymixin Agar Base with pH 7.2 for 24 h at 28±2°C. The obtained pure

cultures were stored in refrigerator at 4°C for further use. The isolates screened for biochemical characterization was done as per the procedures of Cappuccino and Sherman (1992) which includes Starch hydrolysis, Urease test, Gelatin Hydrolysis test, Simmons citrate test, Casein hydrolysis and Hydrogen Sulfide(H_2S) gas production test [5].

Improvement of culture through mutant Ethyl Methane-sulfonate for enzyme activity

From the maintained pure cultures, inoculate a loop of bacteria in 5 ml of non-tendler medium accompanied by consistent shaking at 120 rpm for 24 hours. Then, after 24 hours, chemical mutagen EMS 200 µl (50 µl/ml) were mixed into it and placed it in a shaker at 120 rpm for 1-2 hours. The bacterial suspension so obtained was transferred to a sterilized centrifuge tubes aseptically and centrifuged at 5000 rpm for 20 minutes**.** The chemical mutagen (EMS) was removed by discarding the supernatant from the bacterial cells. After washing, 10 ml of sterilized phosphate buffer (pH 7.2) was added in a cell pellet to made bacterial suspension. Then, bacterial suspension were added to the petriplates containing MYP agar medium by spread plate method and incubated at 28 ± 2°C for 24 hrs. After 24 hours, the bacterial colonies were picked up and transferred to nutrient agar slants.

Amylase Activity

The mutant variants were grown up in LB (Luria Broth) medium at 55°C with shaking at 120 rpm for 24 hrs. The supernatant i.e. cell free filtrate was used for enzyme assay after removal of cells by centrifugation at 10,000 rpm for 10 mins. Amylase activity was determined according to method given by (Miller 1959) [6]. 50 µl of Enzyme extract was added in a test tube containing 1ml of 1% (w/v) soluble starch in 0.1M phosphate buffer (pH 7.0) and then the mixture was incubated at 55°C for 10 min. 3 ml of DNS reagent were added to it for terminating the reaction and kept for 10 mins at constant heating in boiling water bath. After developing yellow to orange colour, absorbance was read at 540 nm. By using Bradford method protein content was also estimated [7].

Effect of different Temperatures and pH's on Enzyme Activity

Enzyme activity of the *Bacillus* isolates (non mutated as well as mutated) at different temperatures ranging from 30°C to 60°C and at different pH ranging from 5.8 to 7.4 were determined [8].

Effect of substrate on amylase activity

Effect of substrate was assessed by measuring amylase activity at different substrate concentrations. The reaction mixtures were made separately taking substrate concentrations *viz*. 0.1, 0.2, 0.3,0.4 of 1% (w/v) soluble starch in 10 ml of 0.1M phosphate buffer (pH 7) respectively and 50 µl of the enzyme was added to each of them and then incubated at 55°C for 10 min. The reaction was stopped by adding 3 ml DNS reagent. Enzyme unit and specific activity at different substrate concentrations were calculated following above mentioned way.

Results and Discussion

Biochemical Characterization of Bacillus spp. isolated from soil samples

Fifteen isolates isolated from soil samples on the basis of their growth on *Bacillus* specific solid medium i.e. MYP Agar Base. Single colonies of *Bacillus* spp. were picked and streaked on the Agar plates. The pure cultures thus obtained were stored at 4°C for further analysis. A total of fifteen isolates were selected and examined for colony and morphological characteristics. The colour of the colonies was slimish cream. The isolates formed irregular, smooth and created type of colonies. Gram staining reaction showed positive results and microscopic observations revealed that the isolates were rod shaped bacterium. As reported by other researchers, isolates of *Bacillus* spp. are rod-shaped, gram positive aerobic bacteria and are famous for their potential to produce extracellular amylases having great industrial importance [9].

Fifteen isolates were characterized as *Bacillus* species on the basis of their physical characteristics (colony, morphology and gram reaction). All the fifteen isolates showed positive results for biochemical tests *viz.* starch hydrolysis, casein hydrolysis, Simmon's citrate, Gelatin hydrolysis and H_2S-gas production tests, but were found to be negative for urease reaction with varying intensities. These above observations advocated the bacterial isolates as *Bacillus* species. The characterization was done by using the methodologies incorporated in literature [10, 11]. The result of the biochemical characterization of the *Bacillus* isolates was presented in **Table 1**.

Table 1 Biochemical characterization of isolated *Bacillus* species

Isolate	Starch hydrolysis	Casein hydrolysis	Simmons's Citrate test	Urease test	Gelatin hydrolysis test	H_2S production
BS-1	+++	++	+ve	-ve	+ve	+ve
BS-2	+++	+++	+ve	-ve	+ve	+ve
BS-3	+++	+++	+ve	-ve	+ve	+ve
BS-4	+++	++	+ve	-ve	+ve	+ve
BS-5	++	++	+ve	-ve	+ve	+ve
BS-6	+	+++	+ve	-ve	+ve	+ve
BS-7	++	+	+ve	-ve	+ve	+ve
BS-8	+	+++	+ve	-ve	+ve	+ve
BS-9	+++	++	+ve	-ve	+ve	+ve
BS-10	+	++	+ve	-ve	+ve	+ve
BS-11	++	++	+ve	-ve	+ve	+ve
BS-12	+++	++	+ve	-ve	+ve	+ve
BS-13	+	++	+ve	-ve	+ve	+ve
BS-14	+++	++	+ve	-ve	+ve	+ve
BS-15	+++	++	+ve	-ve	+ve	+ve

+ = low positive reaction, ++ = moderate positive reaction, +++ = highest positive reaction, -ve = negative reaction, +ve = positive reaction.

Amylase production ability of non mutated and EMS-mutated* Bacillus *isolates at ambient temperature and pH (at 30^0Cand pH7.0)

Non-mutated isolates of *Bacillus* spp. and their EMS_{200} mutants were assessed for amylase activity at ambient temperature and pH (at 30°C and pH 7.0). The amylase activities of all fifteen non mutated isolates of *Bacillus* spp. were assayed for starch degrading abilities and were found statistically varied among the isolates. The specific activities of the isolates were ranged from 0.180 to 2.851 µmole maltose/min/mg protein. Among these isolates, NBS-9 isolate showed highest amylase activity i.e., 2.851 µmole maltose/min/mg protein followed by NBS-13, NBS-14, NBS-15 i.e., 1.490, 1.600, 1.492 µmole/min/mg protein respectively and lowest amylase activity was found in NBS-10 i.e., 0.180 µmole maltose/min/mg protein followed by NBS-2, NBS-8, NBS-1 i.e., 0.242, 0.306, 0.342 µmole maltose/min/mg protein respectively at pH 7 and room temperature (30°C). Amylase activity of isolates of *Bacillus* spp. was found in the range 2.33 and 2.00 IU [12].

In order to improve the amylase production efficiency, the *Bacillus* isolates were mutated by using chemical mutagen i.e., Ethyl Methane-sulfonate (EMS-200) treatment. The amylase activities of all mutated isolates of *Bacillus* spp. were determined. The specific activities of the mutated isolates significantly changed from the values of their corresponding non mutated isolates. Amylase produced by the mutated isolates ranged from 0.587 to 1.293 µmole maltose/min/mg protein with maximum amylase production in MBS-5 with specific activity 1.293 µmole/min/mg protein and lowest amylase production in MBS-4 i.e.0.587 µmole/min/mg protein.

A comparative study of variation in amylase activities among non mutated and EMS-mutated isolates was depicted in **Figure 1**. However, EMS mutation caused a mixed effect on the isolates showing amylase activity range in mutants from 0.587 to 1.293 µmole/min/mg protein. MBS-5 showed highest amylase activity of 1.293 µmole/min/mg protein followed by MBS-3, MBS-12, MBS-11, MBS-14 having 0.925, 0.886, 0.870, 0.865 µmole/min/mg protein respectively which indicates that non mutated isolates having high amylase activities previously have been decreased due to mutation whereas the isolates that have comparatively low amylase activity earlier got improved in amylase production upon mutation. EMS-mutation was reported to enhance the amylase activity at pH 7 [13]. Therefore, NBS-9 among non mutated isolates and MBS-5 among EMS-mutated isolates are found to be best isolates that have more amylase production potential.

Effect of different temperatures on amylase activity of non-mutated* Bacillus *isolates at pH 7.0

Amylase production abilities of *Bacillus* isolates were studied under different temperatures (30, 40, 50 and 60°C) to know exact temperature for the optimal production of amylase by an individual isolate and also to understand the effect of high temperature on amylase production in the different isolates. The results showed that amylase activities were significantly varied with change in temperature (i.e. temperature 30, 40 and 50°C) and also among isolates. The non mutated *Bacillus* isolates NBS-1, NBS-4, NBS-6, NBS-7, NBS-8, NBS-10 and NBS-11exhibited maximum amylase activity at temp 30°C (0.208, 0.390, 0.456, 0.225, 0.131, 0.193, 0.467 µmole/min/mg protein

respectively)whereas the isolates *viz*.NBS-2 and NBS-14 showed maximum amylase activity at 40°C (0.685 and 0.666 µmole/min/mg protein). The isolates NBS-3, NBS-5, NBS-9, NBS-12, NBS-15 showed maximum amylase activity at 50°C(0.685,0.597, 0.654, 0.100, 0.309 µmole/min/mg protein) respectively. However, only one isolate i.e., NBS-13 recorded highest amylase specific activity (0.556 µmole maltose/min/mg protein) at 60°C. A comparative study of variation in amylase activities with rise of temperature among non mutated isolates were depicted in **Figure 2**. Studies were conducted on activity of amylase produced by *Bacillus megaterium* and found that the amylase activity increases due to rise in temperature from 30°C to 40°C but followed by a sharp decline at 50°C [14]. Another study reported the maximum amylase production at 60°C and minimum at 30°C by *Bacillus* spp. [15].

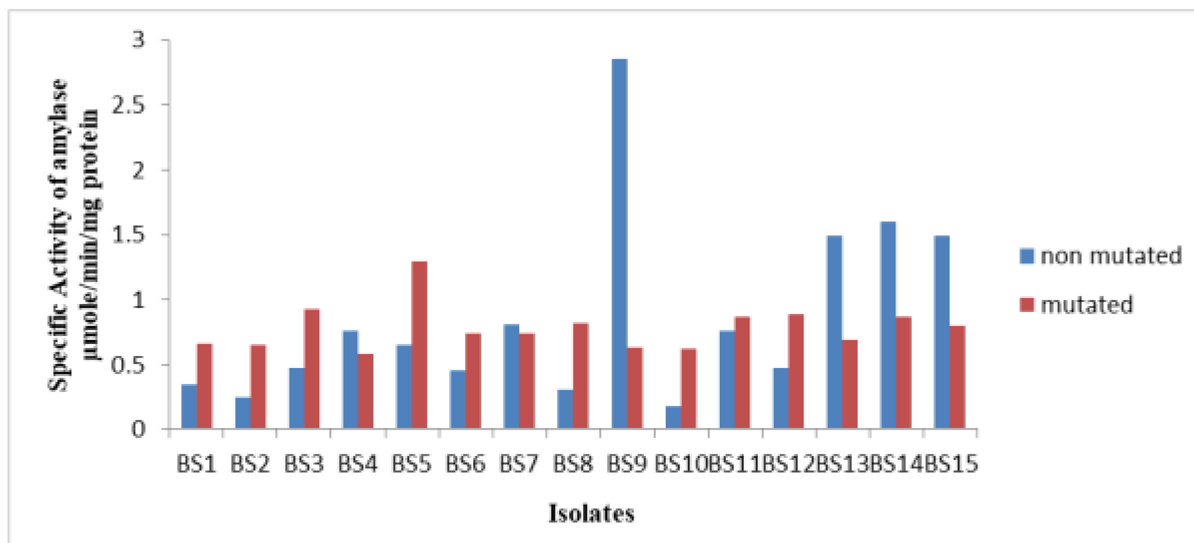

Figure 1 A comparative study of variation in amylase activities among non mutated and EMS mutated isolates

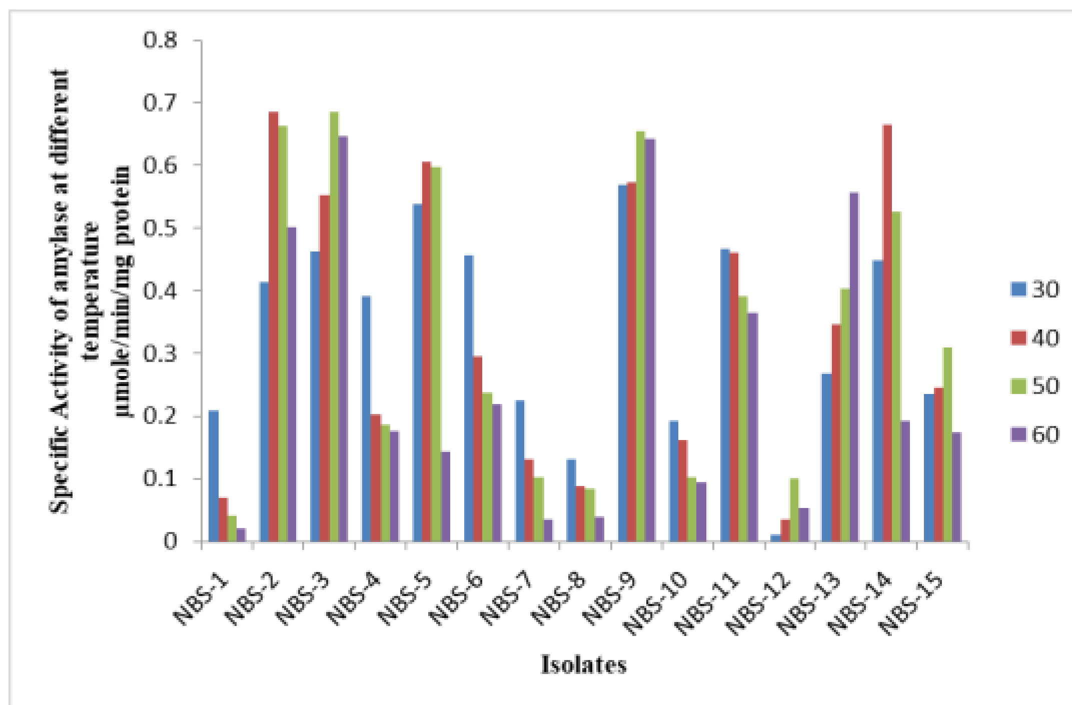

Figure 2 A comparative study of variation in amylase activities with rise of temperature among non mutated isolates

Effect of different temperatures on amylase activity on EMS-mutated* Bacillus *isolates at pH 7.0

The amylase production efficiency of EMS-mutated isolates was studied to assess the impact of EMS-mutation on amylase production ability with change of temperature. The results showed that amylase activities were significantly varied with treatments and also among mutated isolates. All EMS-mutated isolates possessed optimal amylase activity at 30°C. However amylase production abilities in all isolates were sustained up to 60°C. On mutation, the MBS-5, MBS-7, MBS-8, MBS-14 and MBS-15 isolates recorded better amylase activities at 60°C. MBS-7 having amylase

activity 1.165 µmole/min/mg protein was best among the mutated isolates which possessed maximum amylase activity at 60°C. The mutated isolates possessed maximum amylase activity at 30°C but these all isolates were sustained upto 60°C. A comparative study of variation in amylase activities with rise of temperature among EMS-mutated isolated isolates was depicted in **Figure 3**. Maximum α-amylase production was noticed by the EMS- mutant strain of *B. licheniformis* when the incubation temperature was adjusted at 37°C and the enzyme activity markedly declined when the temperature was increased up to 43°C [16]. It might be due to the fact that 37°C is the optimal temperature of growth of bacterial culture and subsequently for enzyme production. In addition, high temperature might have reduced the moisture contents of the fermentation medium and growth of the organism resulting in the decreased enzyme production [17].

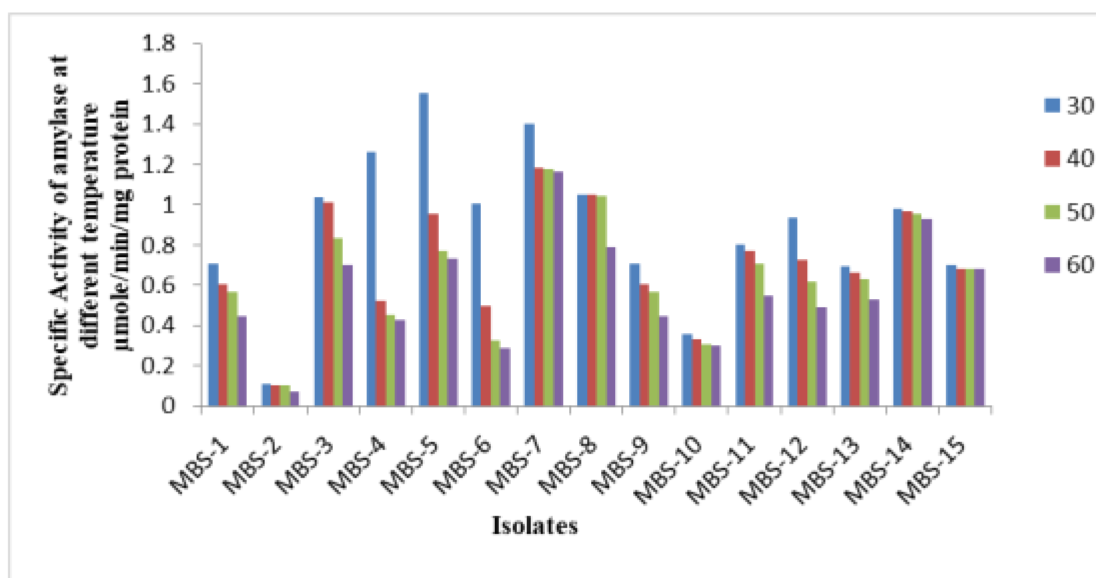

Figure 3 A comparative study of variation in amylase activities with rise of temperature among EMS-mutated *Bacillus* isolates

Effect of different pH on amylase activity of non mutated **Bacillus** *isolates at fixed temperature (30°C)*

Amylase production abilities of *Bacillus* isolates were studied under different pH (5.8, 6.2, 6.6,7.0, 7.4) to know the exact pH for the optimal production of amylase by an individual isolate. The amylase activity varied significantly with change in pH and also among isolates. Among the non mutated isolates, the maximum and minimum amylase activities exhibited by NBS-1(0.422 µmole/min/mg protein) and NBS-10(0.143 µmole/min/mg protein) at pH 6.6 followed by NBS-3(0.421 µmole/min/mg protein) and NBS-8(0.380 µmole/min/mg protein) at pH 5.8 while rest of isolates showed maximum activity at pH 7 ranging from 0.232 to 1.437 µmole/min/mg protein. A comparative study of variation in amylase activities among non mutated *Bacillus* isolates with change in pH was depicted in **Figure 4**. However, pH 5.8 to 7.4 also supported the production of amylase. Similar results were earlier reported by other researchers. Maximum amylase production was observed at pH range of 6 to 8 (maximum amylase yield 1.67 mg/ml/min) in different isolates of *Bacillus* spp. but in most of cases, amylase activity was found at pH 7 [14, 18]. Another study revealed that optimal pH for amylase production in *Bacillus* species was recorded at pH 8.0 [19].

Effect of different pH on amylase activity of EMS- mutated isolates

Besides non mutated *Bacillus* isolates, the amylase production efficiency of Ethyl Methane-sulfonate (EMS)-mutated isolates were studied to assess the effect of EMS-mutation on amylase production ability with change in pH. The results showed that amylase activities were significantly varied with treatments (i.e., with changing pH) and also among mutated isolates. The mutated isolates *viz*. MBS-1, MBS-6, MBS-11, MBS-13 and MBS-15 recorded specific activities of 0.786, 0.680, 2.203, 0.473, 0.756 µmole/min/mg protein respectively possessed highest amylase activity at pH 7 followed by MBS-2, MBS-3, MBS-5, MBS-7 having 0.680, 1.433, 0.527, 0.086 µmole/min/mg protein respectively exhibited maximum amylase activity at pH 6.6.Whereas, MBS-4, MBS-8, MBS-9, MBS-10 and MBS-12 with activities 0.159, 0.553, 0.408, 0.899, 2.270 µmole/min/mg protein showed maximum amylase activity at pH 5.8. The isolate MBS-14 (specific amylase activity 0.593 µmole maltose /min/mg protein) recorded maximum amylase activity at pH 6.2. Results showed that EMS-mutation had mixed type effect on pH for amylase production abilities. A comparative study of variation in amylase activities among EMS-mutated *Bacillus* isolates with change in pH was

depicted in **Figure 5**. However, maximum isolates showed better effect towards acidic medium. The differential effect of initial pH (6.0, 6.5, 7.0 and 7.5) on alpha amylase production by the mutant isolate of *Bacillus amyloliquefaciens* EMS-6 [16]. The enzyme activity was found maximum at pH 6.5 and minimum at pH 6.0.

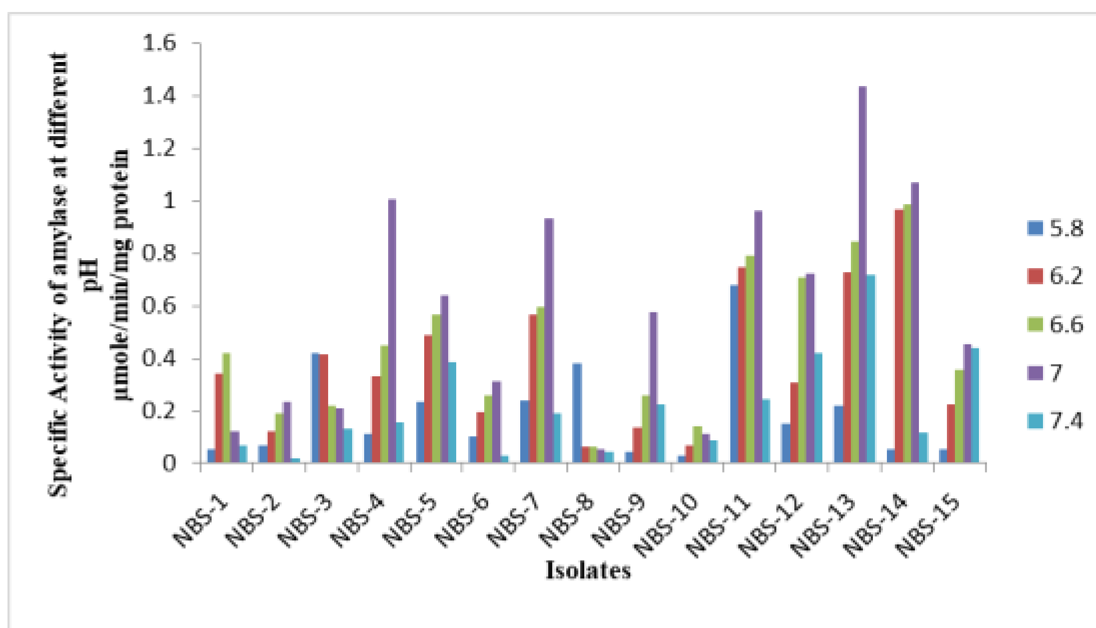

Figure 4 A comparative study of variation in amylase activities with change of pH among non -mutated isolated *Bacillus* isolates

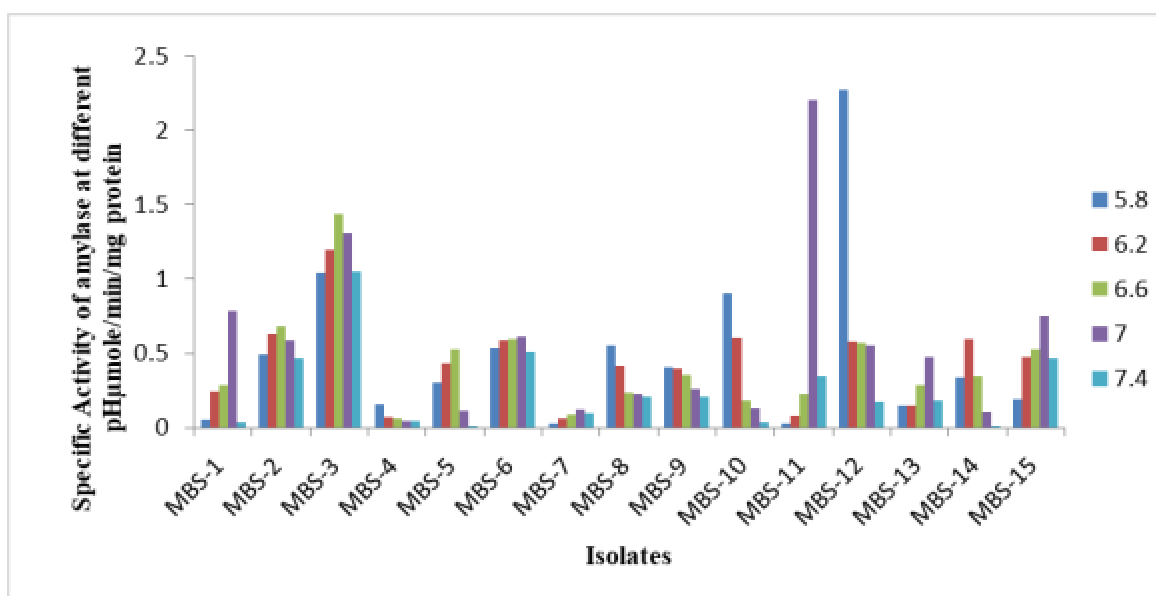

Figure 5 A comparative study of variation in amylase activities with change of pH among EMS mutated *Bacillus* isolates.

Enzyme kinetics of non mutated and EMS-mutated **Bacillus** *strains at pH 7.0 and temperature 30^{0}C*

The kinetic constants (Vmax, km,) for amylase were determined by incubating fixed amount of enzyme with varied concentrations of soluble starch as a substrate (0.1 to 1.2%). The enzyme followed the Michalis Menten kinetics of catalysis. The Km and Vmax values of various enzymes are difficult to match as they depend upon the substrate used and also the reaction conditions. In present research work, the Vmax and Km of amylase were derived from the Line weaver Burke plot. Low values of Km indicate high affinity of the enzyme for the substrate. Mutation caused improvement in enzyme quality in some strains showing rather lower Km value as compared to non mutated strain whereas, in some strains Km values increased and hence declined enzyme quality. The value of Km depends on the enzyme and also the substrate, in addition as conditions like temperature and pH. Among non mutated strains, minimum K_m value was observed in NBS-15 (0.046 mg/ml) and maximum in NBS-8 i.e., (1.060 mg/ml) and

maximum Vmax was observed in NBS-9 (6.024 µmole/min/mg and minimum were observed in NBS-2 (0.270 µmole/min/mg) whereas in mutated strains minimum Km value observed in MBS-12 (0.036 mg/ml) and maximum km value were observed in MBS-14 (2.978 mg/ml) and maximum Vmax was observed in MBS-12 (125 µmole/min/mg) and minimum was observed in MBS-6 (1.459 µmole/min/mg). Enzyme kinetics parameters of amylase activities of non mutated and EMS- mutated strains of *Bacillus* spp. at pH 7 and temperature 30°C strains was depicted in **Figures 6** and **7**. So, EMS-mutation caused improvement in enzyme quality in some strains showing rather lower Km value as compared to non mutated strain, whereas in other enzyme quality declined as increase in Km occurred on mutation. An improvement in enzyme- substrate binding reaction rate also recorded due to EMS-mutation of *Bacillus* isolates [20, 21]. These observations support our study. Thus, the results obtained confirmed that chemical mutagenesis technique is a crucial tool in improvement of *Bacillus* spp. for increasing amylase production potential.

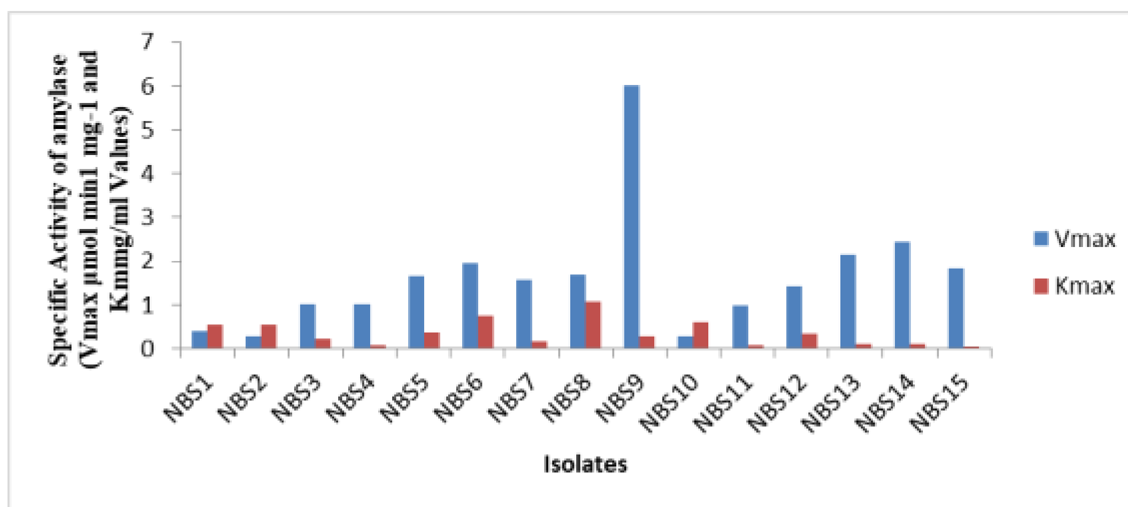

Figure 6 Enzyme kinetic parameter (V_{max} and K_m Value) of non-mutated Strains

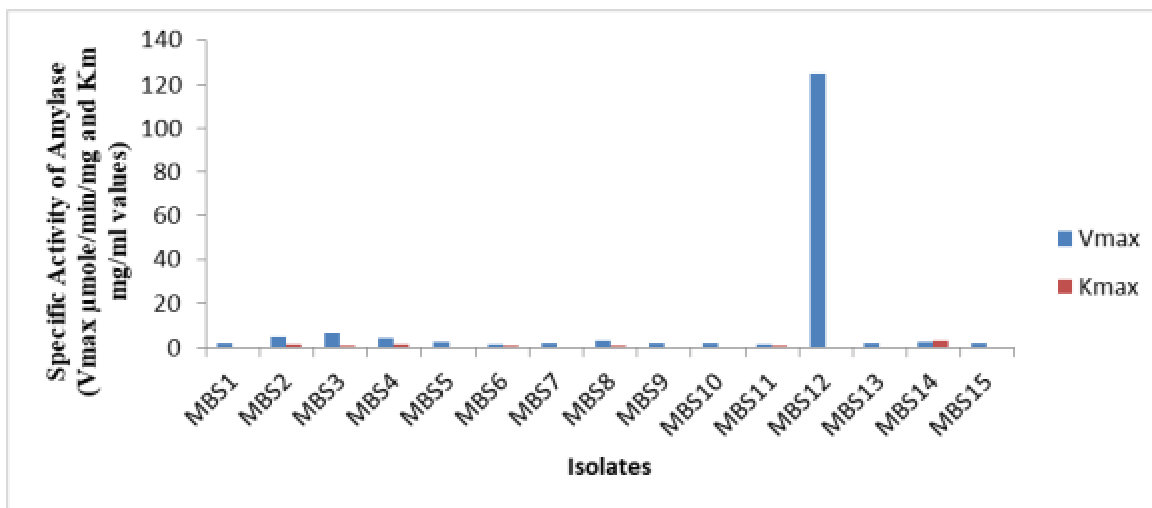

Figure 7 Enzyme kinetic parameter (V_{max} and K_m Value) of EMS-mutated

Conclusion

On the basis of overall observations, it is concluded that all the isolates of *Bacillus* spp. isolated from soil samples collected from dumping sites of cereal brans of different mills have significant amylase production abilities. EMS-mutation causes improvement of amylase production abilities in general, especially those having poor amylase activity.

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References

[1] Sun, H., Zhao, P., Ge, X., Xia, Y., Hao, Z., Liu, J., Peng, M. 2010. Recent advances in microbial raw starch degrading enzymes. Applied Biochemistry and Biotechnology, 160:988-1003.

[2] Joo, M. H., Hur, S. H., Han, Y. S., Kim, J. Y. 2007.Isolation, identification, and characterization of Bacillus strains from the traditional Korean soybean-fermented food, Chungkookjang. Journal of Applied Biological Chemistry, 50:202-210.

[3] Shiau, R., Hung, H., Jeang, C. 2003.Improving the thermostability of raw starch digesting amylase from a Cytophaga sp. by site-directed mutagenesis. Applied and Environmental Microbiology, 69:2383-2385.

[4] Haq, Z., Ashraf, H., Rani, S.2001. Biosynthesis of alpha-amylase by chemically treated mutant of Bacillus subtilis GCBU- 20. Pakistan Journal of Biological Sciences, 2:73-75.

[5] Cappucinu, J. G., Sherman, N.1992.Microbiology, a laboratory manual.The Benjamin publishing company inc. California.

[6] Miller, G. L. 1959.Dinitrosalicyclic acid reagent for determination of reducing sugar. Journal of Analytical Chemistry, 31:426-428.

[7] Bradford, M. M. 1976 A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72:248-254.

[8] Bessler, C. F., Schmitt, J., Maurer, K., Schmid, D. R. 2003. Directed evolution of a bacterial amylase: toward enhanced pH-performance and higher specific activity. Protein Science, 12:2141-2149.

[9] Swain, M. R., Kar, S., Padmaja, G. 2006. Partial characterization and optimization of production of extracellular α-amylase from Bacillus subtilis isolated from culturable cowdung microflora. *Polish Journal of Microbiology*, 55:289-296.

[10] Ahmed, Alkando, A., Ibrahim, H. M. 2011.A potential new isolate for the production of a thermostable extracellular α- amylase. African Journal of Bacteriology Research, 3:129-137.

[11] Kaur, A., Kaur, M., Samyal, M. L., Ahmed, Z. 2012.Isolation, characterization and identification of bacterial strain producing amylase. Journal of Microbiology and Biotechnology Research, 2:573-579.

[12] Kunal, M., Verma, S., Tanta, R. 2011.Isolation of amylase producing Bacillus species, from soil sample of different regions in Dehradun and to check the effect of pH and temperature on their amylase activity. Journal of Pharmaceutical and Biomedical Sciences, 12:1-8.

[13] Khodayari, F., Cebeci, Z., Ozcan, D. B. 2014.Improvement of enzyme activity of a novel alkaline and thermophile Bacillus spp.CU-48, producing α-amylase and CMcase by mutagenesis. International Journal of *Chemical* and *Natural Science*, 2:97-103.

[14] Oyeleke, S. B., Oduwole, A. A. 2009. Production of amylase by bacteria isolated from a cassava waste dumpsite in Minna, Niger state, Nigeria. African Journal of Microbiology Research, 3:143-146.

[15] Senthil, Kumar, P. K., Uma, C., Saranraj, P. 2012.Amylase production by Bacillus spp. using cassava as substrate.International Journal of Pharmaceutical and Biological Archive, 3:300-306.

[16] Haq, I.U., Ali, S., Saleem, A., Javed, M. M. 2009. Mutagenesis of Bacillus licheniformis sthrough ethyl methane-sulfonate for alpha amylase Production. Pakistan *Journal of Botany*, 41:1489-1498.

[17] Markkanen, P. H., Suihko, M. L.1974. The use of UV-radiation in the improvement of enzyme production by Bacillus subtilis. Chemistry Letters, 4:89-92.

[18] Daniel, R. M., Peterson, M. E., Danson, M. J. 2010. The molecular basis of the effect of temperature on enzyme activity. Journal of Biochemistry, 425:353-360.

[19] Olajuyigbe, F. M., Ajele, T. O. 2005. Production dynamics of extracellular protease from Bacillus species. African Journal of Biotechnology, l4:776-779.

[20] Samanta, S., Das, A., Halder, S. K., Jana, A., Kar, S., Mohapatra, P. K. D., Pati, B. R., Mondal, K. C. 2014. Thermodynamic and kinetic characteristics of an α-amylase from Bacillus licheniformis SKB4. Acta Biologica Szegediensis, 58:147-156.

[21] Hossain, S. M. Z., Haki, G. D., Rakshit, S. K. 2006. Optimum production and characterization of thermostable amylolytic enzymes from B. stearothermophilus GRE1. Journal of Chemical Engineering, 84:368-374.

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