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IMPROVEMENT OF AMYLASE ENZYME ACTIVITY OF A THERMOPHILLIC *BACILLUS SP.* BY MUTAGENESIS AND ITS APPLICATION IN DESIZING OF FABRIC INTEXTILE INDUSTRY

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ABSTRACT:

Textile industry particularly the chemical processing sector has always been a major share in the global pollution. The advantage of this amylase is that they work well for desizing fabrics because they can remove the coating of starch without damaging the yarn.The aim of the study is to keep up with the increasing demands of amylase enzymes in the textile industry, improvements are beingdone to enhance the enzyme production by mutagenesis. It was observed that due to EMS-mutation there is an improvement in amylase production abilities of mutant strains, especially those who were originally poor in amylase activity. Genetic modificationof the organism is a great method to improve the amylase enzyme activity of the wild type strain. Further specific study needs to bedone in order to utilize these strains on a commercial scale.

Key Words: Textile industry, Desizing, Fabric, EMS (Ethyl methane sulphonate), Wild type, Mutant

1. INTRODUCTION:

Textile industry particularly the chemical processing sector has always been a major share in the global pollution. Due to constantlyincreasing level of pollutants, governments of many countries have imposed strict limitations on the release of pollutants into the environment. Processes which contribute in reducing pollution are gaining ground all over the world. Amylase enzymes act on α- 1, 4 glycosidic bonds and hydrolyze them into dextrin and maltose. Desizing is a process of removing starch from fabric. The advantage of this amylase is that they work well for desizing fabrics because they can remove the coating of starch without damagingthe yarn.

Thermo stability is a feature of most of the enzymes sold for bulk industrial usage and thermophilic organisms are therefore of special interest as a source of novel thermostable amylase. Today enzymes have become an integral part of the textile processing. The uses of harsh chemicals in the textile industry have been replaced by enzymes. Along with this, enzymes are used in the textileindustry because they accelerate reactions, act only on specific substrates, operate under mild conditions, are safe and easy to controland can biologically degradable i.e. biodegradable.

The aim of the study is to keep up with the increasing demands of amylase enzymes in the textile industry, improvements are beingdone to enhance the enzyme production by mutagenesis. Different mutagens such as nitrous acids or ethyl methane sulphonate (EMS) can be used for the mutation of bacteria. It was observed that due to EMS-mutation there is an improvement in amylase production abilities of mutant strains of *Bacillus sp.* in general, especially those who were originally (in non mutated isolates form)poor in amylase activity.(3)

2. MATERIALS AND METHODS:

2.1. Enrichment and isolation of amylase producing bacteria from soil:

2.1.1. 1ml of soil suspension was inoculated into Luria Bertani broth and incubated at 55°c for 1 week.

2.1.2. After 1 week 1ml of enriched sample was transferred in starch broth and kept for incubation at 55°c for 24 hours.

2.1.3. One loopful of suspension was streaked on starch agar plate and incubated at 55°c to obtained amylase producing purestrain.

2.2. Determination of starch hydrolyzing activity of the isolate:

2.2.1. The plates were flooded with Grams iodine to determine amylase enzyme activity.

2.2.2. Grams staining and endospore staining of the isolates were done.

2.3. Genetic modifications of strain A and B which had shown poor amylase activity using EMS to increase the yield of theenzyme:

2.3.1. Cells were actively grown in tendler's non synthetic medium for 24 hrs and then treated with EMS (200µl/ml) to obtainmutant strains.

2.4. Determination of amylase enzyme activity given by wild type strain and mutant strain was evaluated by desizing offabric:

2.4.1. Partial purification of enzyme was carried out. (2)

2.4.2. Weight of the fabric was taken before desizing.

2.4.3. Desizing: The purified enzyme + fabric containing starch + one drop of Tween 80 (used as a surfactant) incubation at 55°cfor 24 hours.

2.4.4. Fabric was washed in water, dried and weight of the fabric was taken.

2.4.5. Fabric was immersed in tegewa's iodine and colour of the fabric was compared with Tegewa scale.

3. RESULTS:

3.1. Enrichment and isolation of amylase producer:

Agricultural field soil sample was enriched in Luria Bertani broth and then transferred to starch broth.

3.2. Determination of starch hydrolyzing activity of the isolate:

From the starch broth two amylase enzyme producing strains were obtained. The wild type strain A and B were found to be poor inamylase activity as shown in fig 2 and fig 3. These strains were found to be Gram positive non endospore forming rods as shown in table 1.1.

Table 1.1: Microscopic characteristics of the isolates

Culture Type	Gram Nature	Endospore Staining
Wild type strain A	Gram positive rods	Non endospore forming
Wild type strain B	Gram positive rods	Non endospore forming

Fig 2: Amylase enzyme activity given by wild type strain A

Fig 3: Amylase enzyme activity given by wild type strain B

3.3. Treatment of isolates with EMS mutagen:

Later these two strains were treated with chemical mutagen that is ethyl methane sulfonate. It was observed that there was a significant improvement in the activity of amylase enzyme as shown in fig 4 and fig 5.

Fig 4: Amylase enzyme activity given by Mutant strain A

Fig 5: Amylase enzyme activity given by mutant strain B

3.4. Determination of amylase enzyme activity given by wild type strain and mutant strain was evaluated by desizing offabric:

The amylase enzyme obtained from wild type strain A and B & mutant strain A and B were purified using ammonium sulfate followed by dialysis. Desizing of the fabric was performed and starch degrading activity of the enzyme was determined. The colourof the fabric which was desized by enzyme obtained from wild type strain A and B correspond to the number 1 and 2 on Tegewa scale. On the other hand the colour of the fabric which was desized by enzyme obtained from mutant strain A and B correspond tothe number 7 on Tegewa scale as shown in fig 6.

Fig 6: Desizing of fabric

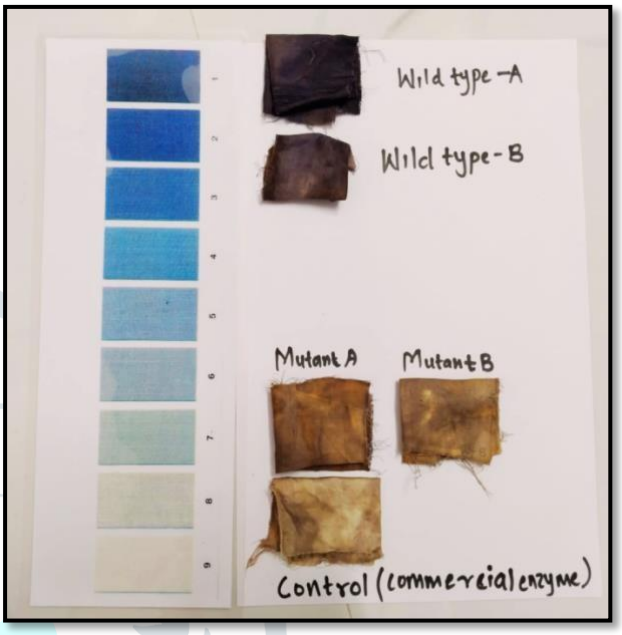

Fig 7: After desizing comparison of fabric colour with Tegewa scale

Table 1.2: Evaluation of desizing of fabric

Sr. No.	Culture Type	Method	Incubation time(hrs) and temperature (55°C) for desizing	Weight before desizing(gm)	Weight after desizing(gm)	Difference in the weight of fabric(gm)	Tegewa scale reading
1	Wild type strain A	Purified enzyme + fabric containing starch+ one drop Tween 80	24	1.05	0.96	**0.09**	1
2	Wild strain B	Purified enzyme + fabric containing starch+ one drop Tween 80	24	0.96	0.83	**0.13**	2
3	Mutant strain A	Purified enzyme + fabric containing starch+ one drop Tween 80	24	0.96	0.87	**0.09**	6
4	Mutant strain B	Purified enzyme + fabric containing	24	0.95	0.86	**0.09**	7

		starch+ one drop Tween 80					
5	Control (commercial enzyme)	Purified enzyme + fabric containing starch+ one drop Tween 80	24	1.00	0.89	**0.11**	8

After desizing there was a significant decrease in the weight of the fabric was observed. When the fabric was immersed in Tegewa's iodine solution and colour was compared with Tegewa scale as shown in table 1.2. Desizing done by enzyme produced by wild type strain is not effective whereas it was observed that enzyme produced by EMS treated organisms has effectively desized the fabric.

4. DISCUSSION:

Amylase enzyme produced by strain A and B was obtained from soil sample. The starch degrading activity given by both the strainswas poor. Therefore improvement of strains was done by chemical mutagenesis. Ethyl methane sulphonate was used as a chemicalmutagen. EMS causes point mutation in the DNA of the organism. Precautionary measures were taken while working with EMS.

There was a significant difference in the amylase enzyme activity given by wild type strain and mutant strain on the starch agar plate. Partial purification of enzyme was done using ammonium sulfate followed by dialysis. Evaluation of amylase enzyme activityof wild type and mutant strain was done by desizing of fabric. After desizing there was a significant reduction in weight of the fabric. The fabric was immersed in Tegewa iodine and colour was compared with Tegewa scale. The colour of the fabric which was desized by enzyme obtained from wild type strain A and B correspond to the number 1 and 2 on Tegewa scale. On the other hand the colour of the fabric which was desized by enzyme obtained from mutant strain A and B correspond to the number 7 on Tegewa scale.

Genetic modification of the organism is a great method to improve the amylase enzyme activity of the wild type strain. This is a minor step to reduce water pollution caused by used of chemicals. Further specific study needs to be done in order to utilize these strains on a commercial scale.

5. AKNOWLEDGEMENT:

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