BIOPROCESS DEVELOPMENT FOR ECO-FRIENDLY MICROBIAL PRODUCTS AND ITS IMPACTS ON BIO-INDUSTRY ESTABLISHMENT IN BANGLADESH

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ABSTRACT

Eemphasis should be given to promote the industrial growth in Bangladesh for sustainable economic development while dependency on agricultural economy alone is at a stake due to urbanization, natural calamities (erosion, flood, salinity and droughts) and increase in people land ratio. The biotech industries based on microbial catalysis has got priority globally due to its unique potentialities over chemical process particularly in the bioprocess based on cheap agricultural raw materials, low energy involvement and rare environmental pollution. Considering the annual import of the bio-products, local market demand, availability of critical skilled man power and resources in Bangladesh, the biotech industries establishment should be believed favorable. In this connection, the regulating bodies should assess the advancement and potentialities of local existing Research & Development sectors and take essential steps for achieving the goals. In view of the above, our research development and findings on bioprocess for industrial enzymes for leather processing and mass production of *Bt* biopesticde for eco-friendly pest management are described. The results obtained are ready to be promoted to commercial level which is definitely a stimulatory and pioneering interface for the contemporary and near future biotech industries in Bangladesh.

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BACKGROUND

Biotechnology industry can spark industrial development in Bangladesh not only due to her dependency on decreasing land- people ratio and complexities on increasing productivities but prior and judicial commercial exploitation of microbial processes on local cheap raw materials could deliver agro-industrial products such as biofertilizer, biopesticides, biofuel including industrial enzymes (on use in textiles and garments, leather processing and poultry feed formulations) bio-pharmaceuticals and vaccines etc profitably, with impact on saving foreign currency, cleaner environments, food security, reduction of GHG and development of critical man power.

The potentialities of microorganisms have been successfully exploited by the scientists in the developed world and turned into a base for the development of giant new industries. The development of chemistry and physics in the mid 20^{th} century turned developed nations from agricultural to industrial arena. Although remarkable economic development was attained, there were problems of depletion of fossil fuel reserves, built up of huge pollution from chemicals which caused ecological imbalances. Fortunately, the development of microbial biotechnology and genetic engineering has come into play to face the new challenges' of 21^{st} century.

MICROORGANISM AS POTENTIAL TOOL IN INDUSTRIAL PRODUCTION PROCESS

The tiny microbes, invisible in naked eyes, can fit thousands altogether onto the head of a pin which are amazingly versatile and can be found almost everywhere. Some microbes can live in boiling water or frozen in ice, others can be fed on wood, plastic solid rock and even on toxic minerals. Some (only 5%) known as pathogens, a threat to environment, health and life that cause disease and contaminate products, may even be used as biological weapon. On the other hand, rest of the microbes (about 95%) are beneficial that help in producing useful products, maintaining clean environment and degrading pollutants. They also produce antidotes, fiber, food additives and fertilizers (5 F`s) useful for human welfare.

Among other disciplines, however, industrial microbiology and biotechnology hold a great promise to this end. This is because microbes, which are available from soil and its enzymes are industrially potential catalytic tools and can be considered as one of the useful resources for the advantages such as, (a) Microbes work at low temperature (30^{o}- 60^{o}C), thus offers an energy saving process unlike chemical process which occurs at high temperature and pressure, (b) the mild conditions produce high quality products with little or no by product,

(c) microbes are amenable to genetic manipulation to produce specific/target oriented or rare products which are not produced by non-biological system, (d) since growth rates of microbes are very fast (doubling time: 20- 60 min) thus less time is required for production, (e) offers eco-friendly processes and (f) agro-industrial cheap raw materials are very much available and thus supportive for the sustainability of the bio-industrial processes.

WHERE DOES BANGLADESH STAND?

Bangladesh, with her large population, is still dependant on agricultural productivity which is greatly affected by natural calamities such as erosion, cyclone, flood, drought and salinity etc. In addition, the indiscriminate use of chemical pesticides and fertilizers is causing pollutions to the soil and aquatic environment affecting public health and aquatic lives as well as emergence of resistance among pests causing loss to the agricultural productivity. In these circumstances, adequate priority should be given towards the biotech industrial development without which sustainable economic growth and greener environment can never be achieved. The development of industrial bio-products most relevant to the problems, maintenance of productivity and quality of existing bio-products and utilization of microorganisms as the resource for bio-products development are the essential domains in this area.

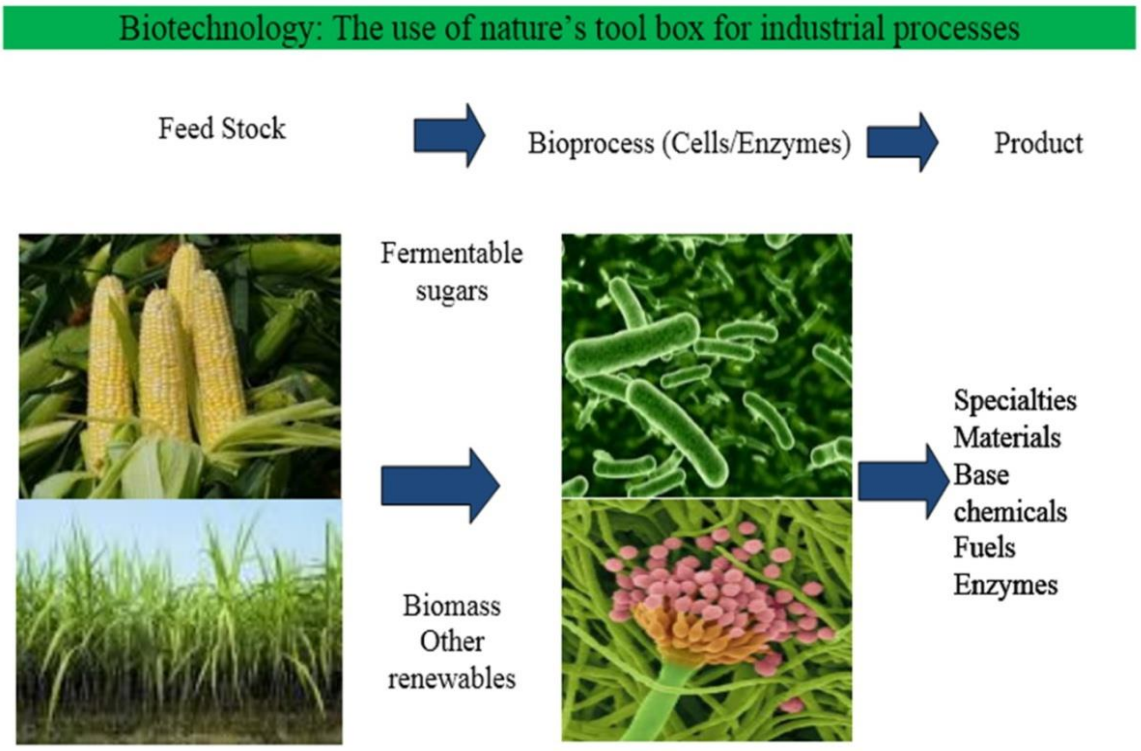

Figure 1: Production of various products using renewable raw materials

The development of microbial biotechnology (Fig 1) and its applications in increasing the production of foods, feeds, biofertilizers, biopesticides, plant growth factors, hormones, antibiotics, insulin, antibodies, interferon, diagnostic reagents, mono and polyclonal antibodies, are still in its infancy in Bangladesh. Many microbial products, except industrial grade ethanol from some distilleries, are being imported at the expense of hard earned foreign currencies. The fact is that the nation did not witness any appreciable development in this area. Although inadequate scientific policy, national commitment, scientific infrastructure, research budget and skilled human resources cannot be ruled out, the effective interaction/linkage between the scientists with applied sectors could contribute to such extent that significant development in the related area could be visible.

RESEARCH DEVELOPMENTS

Based on the above facts, bioprocess development of the bio-products[1-5] which are highly essential at present to meet up the immediate needs of the country, have been worked on in our laboratory (Table 1).

Table 1: Bio-products under development in our laboratory

Product Name	**Microbial source**	**Applications**	**Current Status**
Bating enzyme	*Bacillus* spp.	Leather Processing	Pilot plant level (PPL) completed
Soaking enzyme	*Bacillus* spp.	Leather Processing	PPL completed
Dehairing enzyme	*Bacillus* spp.	Leather Processing	Lab scale completed
Alkaline protease	*Bacillus* spp.	Cleansing aid in detergent	PPL completed
Xylanases	*Thermomyces lanuginosus*	Low quality jute fiber softening and Poultry feed ingredient	PPL completed
Biopolishing enzyme	*Trichoderma* spp.	Textile industry	R&D
Biopesticides	*B. thuringiensis*	Eco-friendly pest management in Agriculture	PPL on going
Anticancer protein & peptide antibiotics	*B. thuringiensis*	Pharmaceuticals	R&D

The present study covers the advances of bioprocess development of the enzymes i.e. alkaline proteases for its particular application in bating of hides and skins in leather manufacturing (Tanneries) and *Bacillus thuringiensis* (*Bt*) biopesticides for controlling the vegetable pest in Bangladesh.

BIOPROCESS DEVELOPMENT OF BATING ENZYMES FOR LEATHER INDUSTRIES (TANNERIES)

Leather is one of the exportable items of Bangladesh. There are about 220 tanneries and more than 75000 people are working as the direct employee[6]. Hide processing into the final leather is a multi steps process including soaking, dehairing and bating of raw hide during which proteolytic action is necessary and important to produce clean and supple pelts, and the good quality leather[7]. Most of the tanneries in Bangladesh exploit harsh chemicals for the processing of leather due to the high import costs of bating enzymes. In addition those who are importing the bating enzymes are also using the chemicals as blend. Importing of enzymes also takes long time during which the activity of the enzymes is decreased causing further expenses and this has been discouraging the use of bating enzymes in leather processing. On the other hand, local production of the bating enzyme will be cheaper not only due to its production on cheap raw materials but also enzyme preparation and uses in liquid state unlike dried enzyme being imported at high cost.

Based on the above facts, potential bacterial strain has been isolated, characterized and improved through genetic manipulation for the production of highly specific bating enzyme[8]. The genetic manipulation particularly mutation of the *Bacillus* sp. has yielded a high level of alkaline protease production in both lab scale and pilot plant level. The production conditions at pilot plant level were optimized on locally available very cheap raw materials such as soybean meal and molasses. The bioprocess conditions thus obtained supported a very high titer of protease (19000 U/hr/mL) production in fermentation culture (Fig 2).

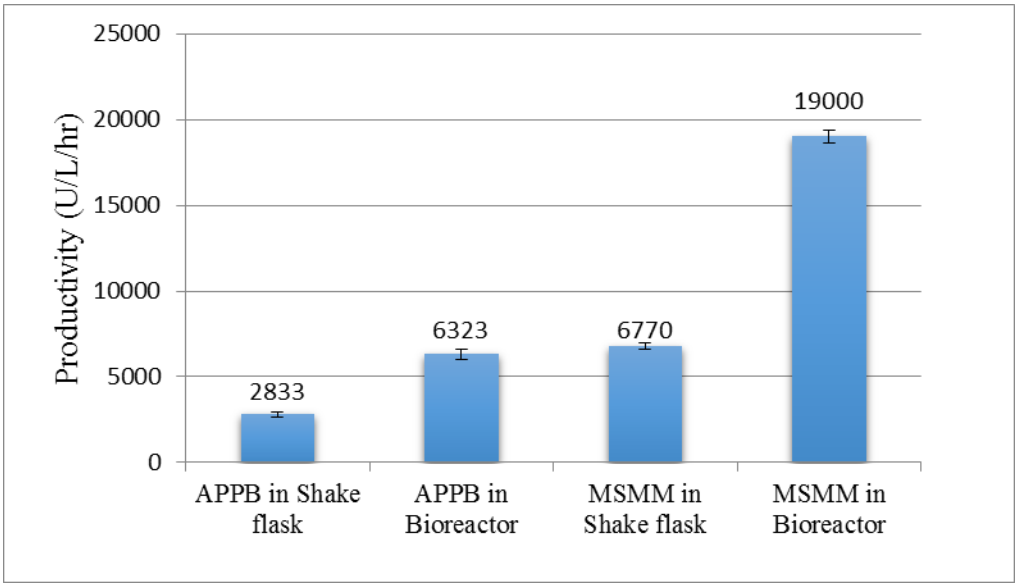

Figure 2: Productivity of bating enzyme on conventional APPB (Glucose-yeast extract) medium and designed MSMM (Molasses-soybean meal) medium by *Bacillus* sp.

APPLICATION OF THE ENZYME IN BATING OF SKIN AND HIDES:

The enzyme was recovered from the culture by a single step process of filtration (microfiltration) or centrifugation and this liquid enzyme was used directly for bating of the skin and hides both in a prototype pilot plant (Institute of Leather Engineering and Technology, Hazaribagh, University of Dhaka) and in Samina Tanneries Ltd, Hazaribagh, Dhaka.

For determining the bating activity of *Bacillus* protease, crude enzyme (2% of hide weight; 100mL equivalent to 60,100 U =34,841 LVU for 5 kg hide) was applied to the animal hide (1000kg) in presence of water. Cattle hide (after deliming) emerged in the enzyme preparations were rolled in a drum for about 60 minutes. Then the quality of the bated leather by both the *Bacillus* protease and commercial enzyme (Oropon K, TFL, UK) was tested in ISO laboratory, Institute of Leather Engineering and Technology, Hazaribagh, University of Dhaka.

The results of different qualitative tests of the enzyme treated leather (crust leather) such as tensile strength, percent of elongation, stitch tear strength, water vapor permeability, grain crack strength (Lastometer), and tongue tear strength tests (Table 2) indicated that *Bacillus* sp. bate was equally efficient to the commercial bate Oropon K. Also the bubble, thumb, cross section and pH tests (quantitative tests) of the treated leather (pelt leather) meet the

requirement of quality bating performance and comparable to the commercial enzyme. These results reflect the unique property of the *Bacillus* protease to selectively remove the non-structural proteins (eg. albumin, globulin, elastin etc) from the hides and skins. The wild type strain of *Bacillus* sp. was unable to hydrolyze the non-structural proteins and thereby was not suitable for bating purpose.

Table 2: Qualitative tests report of crust leather bated by *Bacillus* protease and commercial bating enzyme

Tests	0.5% commercial bating enzyme	2% protease enzyme from *Bacillus sp.*	Standard Value
Tensile Strength Test	202.49 Kg/cm^2	254.12 Kg/cm^2	200Kg/cm^2 (minimum)
% of Elongation Test	44.66%	55%	80% (maximum)
Stitch Tear Strength Test	121.20 Kg/cm	173.07 Kg/cm	80 Kg/cm (minimum)
Water Vapor Permeability Test	10.96 mg/cm^2	11.32 mg/cm^2	10 mg/cm^2 (minimum)
Grain Crack Strength (Lastometer) Test	28 Kg	20 Kg	20 Kg (minimum)
Tongue Tear Strength Test	63.06 Kg/cm	55.55 Kg/cm	45 Kg/cm (minimum)

OBSERVATIONS:

- A high level protease production was obtained on cheap raw materials for bating of leather by a genetically modified bacterium (Table 2).
- A single step recovery system of the bating enzyme from fermentation culture was obtained and the enzyme at liquid state can directly be used for batting - thus making the process cost effective.
- The bating enzyme has got prolonged stability at room temperature (data not shown).

The above results will thus facilitate the production of bating enzyme commercially in Bangladesh.

PRODUCTION OF BACILLUS THURINGIENSIS (BT) PESTICIDES BY BIOTECHNOLOGICAL APPROACH FOR THE CONTROL OF VEGETABLE PESTS IN BANGLADESH

The indiscriminate use of agricultural pesticides is causing serious health problems and environmental pollutions in many developing countries including Bangladesh. These pesticides are extremely hazardous and recalcitrant in nature which upon mobilization through irrigation, farming and flooding results in bioaccumulation and biomagnifications and thus exist in the food chain. They not only affect soil health and microbial flora, water bodies and aquatic lives i.e. fauna and fishes but also affect animal and human health[9,10]. Emergence of resistance in the pests is another major problem associated with chemical pesticides. This necessitates "Integrated pest management (IPM)" which accommodates all possible eco-friendly pest control methods by making greater use of economical, sustainable and environmentally safe alternatives such as the use of biopesticides. Research on biopesticides development necessitates the isolation of naturally occurring potential *Bacillus thuringiensis* strains and the determination of their specific target pests. No comprehensive study has been done in Bangladesh aiming at its large scale production and application for controlling the pests particularly with vegetables.

The study was, therefore, performed to isolate and identify *Bt* strains from different areas of Bangladesh effective against different pests affecting vegetables and fruits like cabbage, cauliflower, tomato, melon, potato, maize, peas etc[11]. This was followed by the characterization of toxin genes and proteins, selection of the most effective *Bt* isolates by bioassay, their large scale production and application in the field.

About 320 *Bacillus thuringiensis* was isolated and identified by biochemical and molecular techniques. These *Bt* strains were characterized for the content of potential *cry* genes active against specific pests affecting the agricultural crops and vegetables of Bangladesh (Fig 3). *cry1, cry2, cry4, cry8, cry10* and *cry11* genes[12] were searched in the assigned biotypes and *cry1* was most abundant in Bangladesh as 36% of tested isolates harbors this gene. Lepidoptera specific *cry1A* gene was searched in the isolates harboring *cry1* gene and 37% of tested isolates were positive for this gene. Searching for *cry2, cry3* and *cry9* genes are in progress.

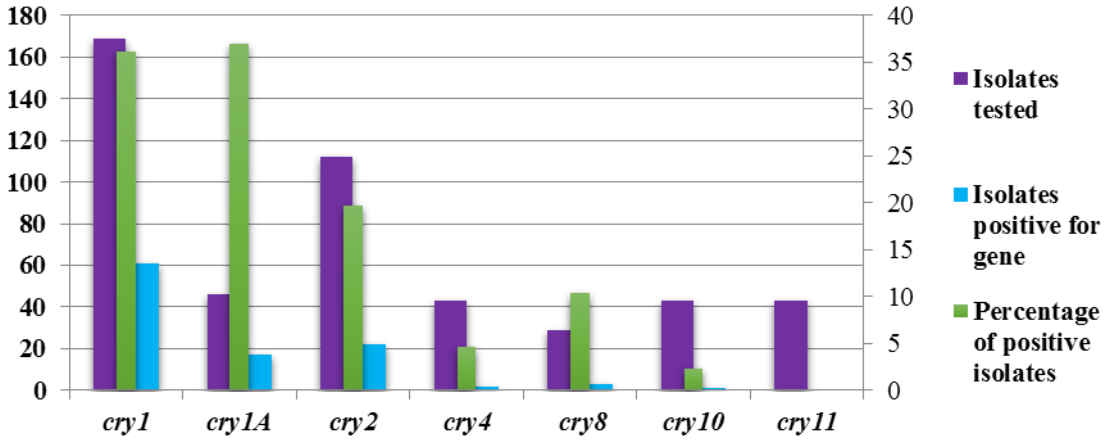

Figure 3: Abundance of *cry* genes in indigenous Bt strains

In vivo toxicity study was also performed to determine the efficacy of the potential *Bt* strains against certain prevailing vegetables pests in collaboration with the Entomological Laboratories of Atomic Energy Commission, Savar and Bangladesh Agricultural Research Institute, Gazipur. Certain indigenous *Bt* strains were thus found to be highly efficient against caterpillars affecting leafy vegetables such as cabbage and cauliflower (Figure 4). Strain *Bt* JSc1 protected pest infestation both in cabbage and cauliflower plants which is comparable to the protection provided by chemical pesticides in the same experiment (field at Narshingdi, Bangladesh).

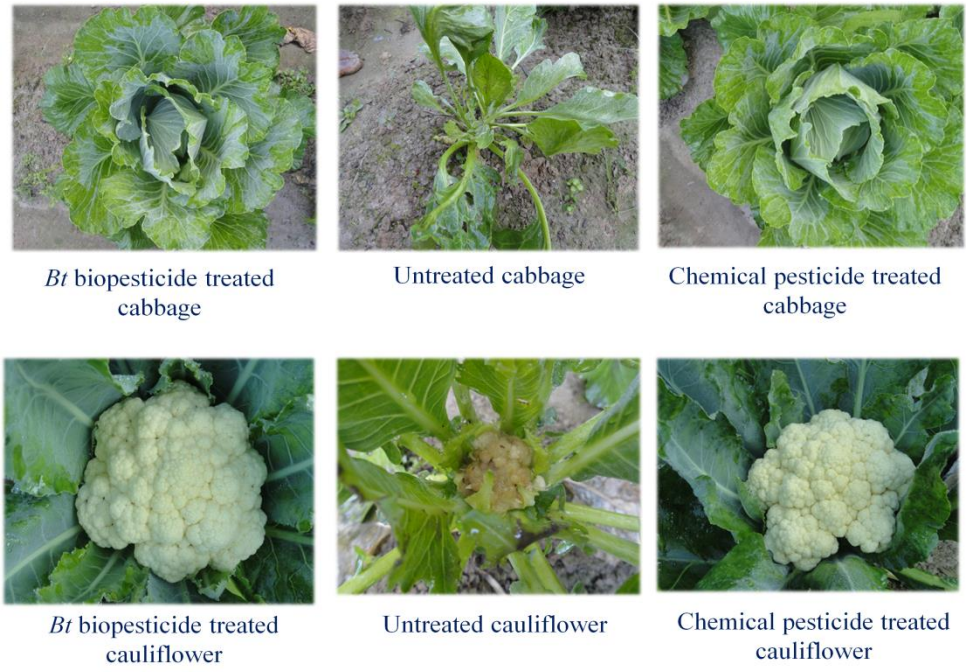

Figure 4: Field application of *Bt* JSc1 biopesticide on cabbage and cauliflower farming.

Bt JSc1 strain was then selected for large scale production of cry proteins in pilot plant bioreactor. Different agro-industrial cheap raw materials such as mustard seed meal[13], soybean meal, molasses and sea water (as the substitute for expensive mineral salts)[14] were used for optimization of production conditions in large scale bioreactor cultivation.

Table 3: Development of a cost effective medium for enhanced production of *Bacillus thuringiensis* δ-endotoxin after 48 hrs of cultivation

Media type and fermentation conditions	**Spore count (cfu/ml)**	**δ- endotoxin (mg/ml) Alkali soluble protein**
A	4.1×10^{5}	0.093
B	3.0×10^{7}	0.392
C	4.5×10^{7}	0.126
D	2.1×10^{10}	0.262
E	1.2×10^{10}	0.201
F*[1]	1.0×10^{9}	**2.741**
G	2.0×10^{9}	**1.661**
H	1.9×10^{8}	1.013
I	1.1×10^{8}	1.071
J	1.5×10^{8}	1.155
K	1.0×10^{8}	0.895
L	1.7×10^{8}	1.545
M*[2]	**2.5×10^{9}**	**2.497**
N	3.9×10^{7}	**1.509**

*[1]Semi-solid medium with growth promoter; *[2]Media containing of sea water and molasses

Sea water and molasses supported very high level of δ-endotoxin (cry protein) production under the optimized conditions (Table 3). The liquid preparation which contain both the toxin and sporulated *Bt* was found highly effective, stable and suitable for application in the field tested.

CONCLUDING REMARKS

A national study on *Bacillus thuringiensis* is nearly completed which generated a large collection of *Bt* strains (about 300) which are preserved for future use. Its cost-effective production technology is available & supply to the famers is possible as well. Further bioassay against agriculturally important pests is under progress jointly with BARI and home based *Bt* biopesticide production technology is under development which will help in promoting *Bt* biopesticide in Bangladesh.

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