



## Bioenergy 2019- Isolation of Bioethanol Producing Bacteria from Southern Part of Bangladesh (Sundarbans)

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Cellulase enzyme producing bacteria are available all over the region but besides this, mangrove forest contains highest amount of cellulose producing bacteria. From the southern part of Bangladesh seven types of soil sample was collected and five types of *Pseudomonas* strain, six types of *Bacillus* strain and five types of *Enterobacteriaceae* strain was found. After the extraction of enzyme from this bacterial strain these enzymes are taken for alcohol production. Cellulase enzyme are highly capable of converting carbon into sugar and after fermentation these sugars turn into alcohol or ethanol. *Pseudomonas* E1-P strain, *Bacillus* C1-Bt strain and *E. coli* T2-D2 strain shows highest O.D at 540nm in the spectrophotometry test among all the strain during the enzyme activity assay. After fermentation of over night their alcohol producing capability was tested by alcohol identification test. Thus, it can be deduced that mangrove soil contains the highest cellulolytic bacteria and it can be beneficial for bio-ethanol producing industry.

**Keywords:** Cellulase enzyme, fermentation, ethanol, Sundarbans.

### Introduction:

The engenderment of pristine ethanol ostensibly commenced in the 12-14th century along with amendments in the art of distillation sanctioning the condensation of vapors of lower boiling liquids. During the middle ages, alcohol was not only utilized for the engenderment or as a constituent of medical drugs, but withal for the manufacture of painting pigments and other chemical industries. It was only in the 19th century that this trade became an industry with cyclopean engenderment, due to economic ameliorations of the distilling process. Nowadays, ethanol is a paramount industrial chemical with emerging potential as a biofuel to supersede evanescent fossil fuels. The utilization of fossil fuel engenders sundry toxic gases which cause environmental quandaries. Consequently, the issues of ecumenical warming and energy crisis, which are engendered by utilizing fossil fuels are two major challenges in the expeditious growing world. Biofuels are one of the main renewable sources, which can contribute to surmount these quandaries. It has been dubbed a future fuel. It can be coalesced with gasoline to minimize the emission of CO<sub>2</sub>, NO<sub>2</sub> and hydrocarbons after combustion relative to that of gasoline alone.

In spite the sundry advantages of biofuel as a fuel the economic competitiveness with gasoline still remains an issue. To surmount this matter the utilization of frugal raw materials for the engenderment of biofuel is one of the most promising methods. In this regard, numerous endeavors to engender biofuel from sundry frugal and renewable re- sources such as from rice hulls, sugar cane leaves, bagasses, microalgae, sorghum, straw, industrial waste, in- dustrial, urban and agriculture residues and corn etc have been reported. These paramount sources contain adequate magnitude of carbohydrates, which can be utilized for biofuel engenderment. Two biological processes are needed for biofuel engenderment. One is saccharification that requires sundry enzymes, is the key step for sugar engenderment and the other one is through fermentation. Many microorganisms including yeast and bacteria convert the sugar and glucose to biofuel. However, there are some circumscriptions in utilizing sundry microorganisms for this resource because of the fact that the strains utilized for fermentation are mesophilic microorganisms which need extra operation cost and have some risk of contamination during bioethanol engenderment.

In this study, we have endeavored to identify some thermo tolerant microorganisms from the natural fermented sources of Bangladesh for bioethanol engenderment. There are several potential benefits of thermo tolerant microorganisms specially yeast for utilizing in the engenderment of industrial bioethanol which are as follows: thermotolerant yeast exhibits expeditious metabolic activity and a high fermentation rate with high product output. The viscosity of the fermentation broth decreases with incrementing temperature. Consequently, the energy required to maintain opportune agitation of the magnification media is truncated. The metabolic activity of microbes and frictional effects of agitation accommodates to engender astronomically immense magnitudes of heat. Thus, supplemental energy to maintain the vessels at the desired temperature as well as the cooling requisites after sterilization is minimized. Moreover, the chances of contaminations were withal minimized.

Here, we have accumulated 18-isolates from the sundry locations of Bangladesh in summer 2010 where temperatures raised from 35°C to 40°C or above. Accumulated samples were characterized morphologically, physiologically, biochem-

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ically and genetically. Among them, 2-strains isolated from the Tari and Pantavat encoded *Pichia galeiformis* and *Pichia guilliermondii*, respectively, engendered high amplitude of bioethanol under the conditions we employed here.

### Materials and Methods:

Microbial samples were amassed from the sundry sources and locations from Bangladesh in summer where temperatures fluctuate between 35°C - 40°C. Our targeted sample accumulation sites were Tari juice at around 35°C - 40°C), Pantavat (Pvt) (an overnight natural fermented rice in tap dihydrogen monoxide at around 35°C - 38°C), Sugarcane juice from the street and decomposed victuals materials (Dfm) from markets. Other sources can additionally be considered where obligatory.

Tari is a local name of sap of palm tree (*Phoenix robusta*), which was accumulated in a pot from the xylem tissue. This pot can carry about 15 - 20 liters of palm juice amassed in 12 h. Three samples (upper, middle and bottom layers) were amassed discretely from the pot by utilizing sterilized glass pipettes and the Tari commixed sample was amassed after commixed upper, middle and bottom layer sample by mixer and then accumulated sample from the middle position of the pot.

Sample amassment was carried out from April to June, 2010 in summer where temperatures fluctuate from 33 - 40°C by an enrichment technique in media containing sugar cane juice (8% total sugars), 0.05% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and 4% (v/v) ethanol at pH 4.5. After inoculation, cultures were incubated for 3 days in a rotary shaker at a predetermined temperature with shaking haste of 160 rpm. Enriched cultures were then streaked on agar plates containing the same medium and inoculated at sundry temperatures. Purified yeast cultures were kept on YPD agar plates and slants (1% yeast extract, 2% glucose, 2% peptone and 2% agar) and then stored at 4°C until use. Thermo tolerant yeast strains were culled predicated on their magnification performances at sundry temperatures like 37°C, 40°C and 42°C and other stresses. Cultures were then accumulated and screened further for their ethanol engenderment efficiency on above temperatures.

Accumulated microorganisms were utilized for isolation/screening purposes. In order to ascertain their optimum magnification environment, experiments were carried out in both solid and liquid medium under different physiological conditions. To understand the thermo tolerant nature of the isolates they were grown on ranges of temperature (35°C - 45°C). Microscopic studies were habituated to identify strain morphologically, after exposed to sundry physiological conditions during their magnification and multiplication. Furthermore, sequencing of 26S rDNA (Yeast D1/D2 region) was carried out further for identification/screening microorganisms from the sizably voluminous populations.

Cull microorganisms for high ethanol engenderment were conducted independently under sundry medium temperatures, pHs, and carbon sources as described in the results section.

Inoculums were rare by transferring one loop full of 24 h culture grown on a plate of YPD agar to an Erlenmeyer flask containing 50 ml of a sugar cane juice medium as described above. The inoculums were transferred at the rate of 1% to the screening medium, followed by incubation on a rotary shaker at sundry temperatures ranging from 30°C to 42°C in 250 ml Erlenmeyer flasks containing 100 ml of a basal sugar cane juice medium composed of sugar cane juice supplemented with glucose up to 18% total sugars and 0.05% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. Glucose concentrations were varied from 16% to 22% (v/v), differing by 5% (v/v) from one flask to the other.

Ethanol engendered in the fermentation medium was estimated by titration (potassium dichromate oxidation) method. Fresh potassium dichromate (33.882 g/l), ferrous ammonium sulphate (135.5 g/l) and diphenylamine solutions were utilized as reagent for estimation of ethanol concentration. The fermented sample was diluted ten times with distilled dihydrogen monoxide. Ten millilitre of the diluted sample was distilled against K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> (10 ml) containing concentrated H<sub>2</sub>SO<sub>4</sub> (5 - 6 ml). Then distilled product was titrated against freshly prepared ferrous ammonium sulphate solution with diphenylamine as a designator. Appearance of green color betokened the terminus point of the titration. Burette reading (amount ferrous ammonium sulphate) will be recorded to calculate the amount (in percentage) of bioethanol present in the sample.

The optimum temperature, which gave the highest cell dry weight, was at 37°C and temperature higher than 40°C, the magnification of maximum yeasts was decremented. Bioethanol fermentation was investigated at temperature higher than optimum temperature as shown in top panel. Because, yeast currently utilized for industrial fermentation is expeditiously inactivated at 33°C - 35°C. Paramount cooling costs would be eliminated, especially during the summer or in tropical countries, with fermenting temperatures of 40°C and above. Only a few screening surveys have been carried out for the competency of yeasts to grow in a flask at or above 40°C. Cells were grown to the YPD medium for 3-days in different mentioned temperatures. Cells were amassed and bioethanol engenderment rate was quantified as described in the Materials and Methods. The minimum 3.5 to maximum 7.5% v/v bioethanol were estimated from sundry tested temperatures. Among the different strains were examined here, Tari-6 gave the highest bioethanol at sundry tested temperatures. The highest 7.5% (v/v) and the lowest 6.0% (v/v) bioethanol were estimated from the strain Tari-6 at 37°C and 42°C, respectively. This result concludes again that the optimum cell magnification correlated with the maximum engenderment of bioethanol at 37°C as suggested anteriorly.

Medium pH is a paramount determinant for bioethanol engenderment. Yeast cells can increment fermentation in acidic condition.

Ergo, we have checked bioethanol activity after cultured cells in medium containing sundry pHs, which was adjusted by in-

tegrating either 1N HCl or 1N NaOH according to the protocol developed. Cell magnification rate was incremented along with the pH level increase from 4.0 to above (data not shown). No more paramount different was optically canvassed on the magnification rate at pH 4.5 to 7.0, but beyond that the magnification rates were decremented (data not shown). However, medium pHs had a paramount impact on bioethanol engenderment. Maximum and minimum bioethanol engenderment was optically canvassed when medium pHs was 4.5 and 7.0, respectively, as shown in bottom panel. Different samples have different rates of bioethanol engenderment. Samples isolated from the Tari-6 and Sugarcane juice engender maximum up to 7.0% and minimum up to 3.0%, bioethanol, respectively,

when the medium pH was 4.5.

**Conclusion:**

In this study, our main target was to identify some thermotolerant microorganisms from the natural sources of Bangladesh for bioethanol engenderment. After screening 18 samples morphologically, physiologically, biochemically and genetically (DNA sequencing), here we have concluded that the isolates from the Tari (*Pichia galeiformis*) and Pantavat (*Pichia guilliermondii*) are potential thermotolerant microorganisms engendered higher magnitude of bioethanol under the condition we employed here. Further research is required to establish temperature conditions and sundry parameters for optimum magnification and engenderment of bioethanol in laboratory scale.

Note: This work is partly presented at 5th World Bioenergy Congress during April 15-16, 2019 held at Tokyo, Japan