Standardization of Natural Indigo Fermentation Dyeing: Identification of Bacteria in the Fermentation Vat

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1. INTRODUCTION

Indigo-blue is one of the oldest natural dyes for blue color and is traditionally produced from indican by innate enzyme in indigo plant. Indigo is insoluble and, therefore, for commercial dyeing, it is converted to the colorless and soluble leuco-form by chemical reduction using alkaline sodium dithionite, in a process called vatting[1]. This conventional procedure generates environment-polluting, highly alkaline effluents, in addition to by-products containing sulphur[2]. Thus, we expect that the processes using microorganism participated in indigo production and/or reduction may solve these problems in a environmentally friendly manner[3], also providing another possibility of improvement for low reproducibility and non-uniformity. In these context, we here identify the bacterial population or community resident in a reactor traditionly used. 16S rRNA sequences-based metagenomic approach pave a way for understanding and interpreting the bacterial community related to natural indigo fermentation, and will be a basis for the process mimic formulated by artificial one.

The objective of this study is to standardize bacterial fermentation dyeing of natural indigo. Bacteria involved in fermentation are identified by biosystematically for exploring more efficient, green, and reproducible indigo dyeing process.

2. EXPERIMENTAL

Natural indigo fermentation

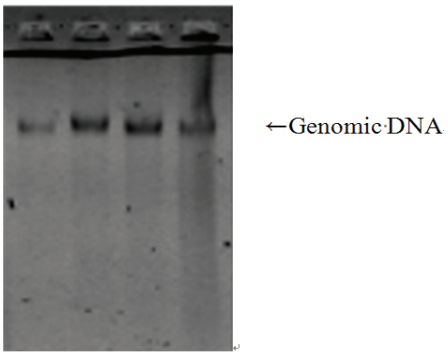
Natural indigo and lye were mixed at a ratio of 1:10 and kept in a ceramic vat at 27-30°C for up to 120 days. Fermenting liquor(30cc) was sampled in a regular basis of time.

Analysis of bacterial community

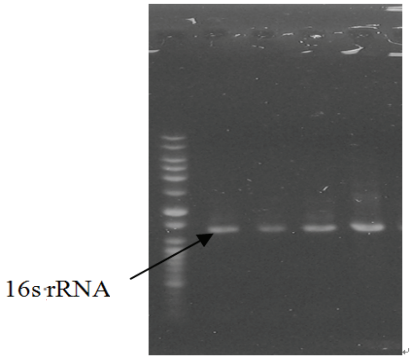
1) Sampling an aliquot of fermentation broth in a certain time interval.
2) Total genomic DNA extraction from whole community of strains.
3) PCR amplication of 16s rDNA to amplipy a single or few copies of a particular DNA sequence and to amplipy 16s rDNA from genomic DNA using universal primer.
4) Cloning of 16s rDNA and prepare library of 16s rDNA from community strains.
5) Mapping of 16s rDNA using several restriction enzymes that cut DNA at specific sequences of DNA
6) DNA sequence analysis of selected clone from library.

3. RESULTS AND DISCUSSION

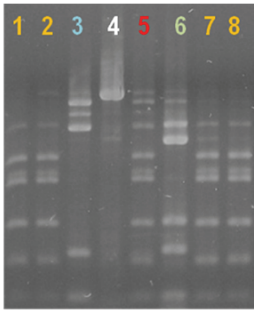
Metagenomic DNA from well fermented broth



Amplified 16s rDNA

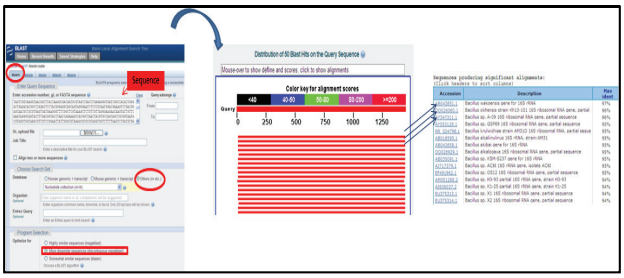


16s rDNA analysis using restriction enzyme digestion



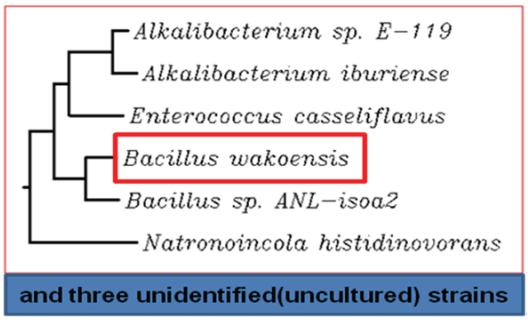
DNA sequence analysis

The whole sequence analysis of selected clone from library was carried out.



Phylogenetic tree

Evolutionary relationships between genera of identified 6 strains were confirmed.



4. CONCLUSIONS

1) As fermentation made progress, the pH of vat was decreased.
2) The reduction state of indigo was not maintained below pH 10, resulting that no dye uptake was obtained.
3) Six identified and three unidentified bacterial strains were isolated from indigo fermentation vat.
4) The major strain was confirmed to be the strain *Bacillus sp*. ANL-isoa2 (about 95 %) among the identified organisms .
5) This result indicated a possibility that Bacillus sp. ANL-isoa2 is able to reduce indigo.
6) These results provided a basis for understanding the participation of bacterial strains in the biological process of well-fermented natural indigo case.

5. ACKNOWLEDGEMENTS

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6. REFERENCES

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