Physiological Characterization and Protein Profiling of four Yeast Strains used in Traditional Brewing by the Nyshi and Apatani Tribes in Arunachal Pradesh, India

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Received 12 February; accepted 14 March; published online 01 April; printed 16 April 2013

ABSTRACT

Traditional alcoholic brewing among the Nyshi and Apatani tribes of Arunachal Pradesh have different indigenous protocols and therefore the perpetuation of yeast strains also differ. The results obtained suggest that the species showed differences in terms of their physiological and biochemical activities. Isolation of pure cultures showed that originally a common starter culture was used by the tribes which eventually underwent a variation and selection in parallel with their segregation pattern due to different protocols for fermentation thus yielding variants that got selected either due to cultural selection or extensive selection pressure. Protein profiling of the strains using SDS-PAGE have revealed that the strains are indeed variants at the genetic level. The strains might therefore be mutagenic variants, which arose during segregation through time immemorial. The isolated strains have shown a preference of growth at neutral pH; a character not usually observed in most yeast species, which generally prefer an acidic medium for growth. Growth of one of the isolates (B) was found to be higher compared to others under optimum conditions while another strain (D) showed an appreciable thermo-tolerance and maximum growth at 35°C. Notwithstanding the experiments performed and results obtained, it can be predicted that careful identification and selection can yield industrially important and beneficial yeast strains.

Keywords: Nyshi, Apatani, Arunachal Pradesh, brewing, Saccharomyces spp., protein profiling, thermo-tolerance

Abbreviations: SDS-PAGE - Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis; YPD – Yeast Peptone Dextrose

1. INTRODUCTION

The various ethnic tribes of the North-eastern states of India are known to produce local wine and beverages. Wine or beer is a part and parcel of their tradition and culture of this part of the country. It is used as a recreational drink and as a stimulant mark of honour when offered to respectable guest. It is prepared in all households. It is drunk in all social, political, cultural and religious gatherings or occasions. Libation of wine or beer is made to the spirit to obtain blessings. It also contributes much towards lamenting the solidarity. It is also said to have medicinal efficacies and is a nourishing as well as stimulating drink. Wine or beer is considered a wholesome drink, age and sex being no bar (Sengupta, 1994). According to Singh (1995), the Nyshi and Apatani are two of the most important tribes of Arunachal Pradesh. Each of the tribes has different traditional protocols and methods for fermentation and alcohol production. The types of substrates used by them for fermentation differ subject to availability, nutritional requirements and environmental conditions. Despite the observed differences, a cursory look suggest that originally a common starter culture was used for fermentation which eventually resulted into the perpetuation of different yeast strains due to years of segregation and selection pressure (Das, 1986). However, no detailed work on isolation of pure yeast strains for traditional brewing practices by the Nyshi and Apatani tribes of Arunachal Pradesh has been reported so far. The present work aims to characterize the different yeast strains and subsequent protein profiling using SDS-PAGE to bring about the importance of isolating pure strains which can ultimately be used in effective and large scale fermentation processes. Moreover, identifying locally perpetuated yeast strains and exploiting the beneficial ones from the consortia will provide an insight to the age old social, cultural and economic values of the local tribal population by effecting their indigenous production of beer and wine. Characterization of the different strains has been based on thermo-tolerance experiments as well as tolerability of the strains to various pH ranges of 5.0, 5.5, 6.0, 6.5 and 7.0.

2. SCOPE OF THE STUDY

The present study aims to accomplish the following objectives:
1) Sub- culture of pure strains of yeasts used in traditional brewing;
2) Growth behavior study
3) Characterization of pure cultured yeast strains based on the parameters namely,
   (a) Tolerance under different temperature ranges of 26°C, 35°C and 40°C as well as different pH levels of 5.0, 5.5, 6.0, 6.5 and 7.0

SDS-PAGE

SDS-PAGE is a powerful method of protein analysis developed in the mid 1960s. It can be used to separate all types of proteins, including those that are insoluble in water. Membrane proteins, protein components of the cytoskeleton and proteins that are part of large macromolecular aggregates can be resolved into polypeptides by size. It also provides information about the molecular weight and the subunit composition of any protein complex (Alberts, et al., 2002).

Thangjam Gopeshwor Singh,
Physiological Characterization and Protein Profiling of four Yeast Strains used in Traditional Brewing by the Nyshi and Apatani Tribes in Arunachal Pradesh, India, Indian Journal of Science, 2013, 3(6), 35-38,
http://www.discovery.org.in/ijis.htm

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(b) Protein profiling of the whole cell protein extracts of the strains using SDS-PAGE method.

2.1. Materials
Start culture as yeast cakes were procured separately from Nyshi and Apatani Tribes.

2.1.1. Chemicals
YPD solid and liquid media for sub-culturing as well as characterization study of yeast strains were prepared as described by Atlas & Parks, 1993. Preparation of stock solution, working solution, resolving gel solution, stacking gel solution, electrophoretic gel solution, gel fixing solution, gel staining solution and gel de-staining solution for SDS-PAGE for protein profiling were performed according to procedures described by Laemmli (1970) and Ausubel et al. (1997a and 1997b).

2.2. Methodologies

2.2.1. Sub-culturing of the yeast strains
Sub-culturing of pure yeast strains was done by streaking the isolates onto YPD solid media in 50mm Petri plates. The work was done very carefully in a laminar flow cabinet to prevent any type of contamination. All the equipments and media were first autoclaved at 15 lb pressure. The strains were then incubated in a BOD incubator at 28°C. Streaking was done repeatedly onto new plates until pure growths were achieved (Atlas & Parks, 1993). Regular observations were made after 2nd, 4th, 5th and 6th days of incubation. After each 6th day of incubation, the strains were again streaked onto next set of Petri plates. After four repeated streaking, pure cultures of four yeast strains could be obtained.

2.2.2. Study of growth behaviour
Growth behavior study of the pure cultures was done following the methodology of Adams et al., (1997). The pure cultures obtained during successive streaking were inoculated into YPD liquid media (pH 7.0) incubated at 28°C and growths were calculated based on extracellular protein secreted by the isolates in culture. For the same, 1ml of the incubated cultures were diluted 10 times and the protein content was determined by observing the extinction coefficient at 660nm in a UV-VIS spectrophotometer (Systronics, India) following the methodology as described by Lowry et al. (1951) using Folin-Ciocalteau reagent. The protein equivalent was calculated from a standard protein assay curve of Bovine serum albumin (BSA) and plotted against time of incubation.

2.2.3. Temperature tolerance of the strains
For thermo-tolerance study of the yeast isolates, the strains were inoculated into normal YPD liquid media (pH 7.0) which were then subjected to temperatures of 28°C, 35°C and 40°C for a time span of three days each. After the third day, growth was determined following the methodologies of Lowry et al. (1951) and Adams et al., (1997) described above.

2.2.4. Sensitivity of the yeast isolates at different pH
Growth behaviors of the isolates subject to different pH ranges of 5.0, 5.5, 6.0, 6.5 and 7.0 incubated at 28°C were observed to investigate their pH tolerance. For the same, methodologies of Lowry et al. (1951) and Adams et al., (1997) described above were followed.

2.2.5. Protein profiling

2.2.5.1. Determination of Whole Cell Protein Content
The total whole cell protein content of the four isolates was determined following the methodology as described by Lowry et al. (1951) using Folin-Ciocalteau reagent and using purified Bovine Serum Albumin (BSA) as a standard. The protein equivalent was calculated from a standard protein assay curve of Bovine serum albumin (BSA).

2.2.5.2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)
Protein profiling of the yeast strains was done by performing SDS-PAGE following the procedure of Laemmli (1970) and described by Ausubel et al. (1997a, 1997b). The gel electrophoresis was carried out in a vertical slab gel electrophoretic apparatus (Vertical Dual Mini Gel System, Bangalore Genei, India) with 12% resolving gels of 0.75mm thickness and stacking gels of 5% acrylamide mix. The gels made were 12cms in height and 14cms in width. The electrophoretic buffer used was 1X Tris-glycine.

One litre of 1X electrophoretic buffer was poured into the lower and the upper electrophoretic tanks, sufficient to create a current. The gels with loaded sample preparations were run at 10mA until the tracking dye reached the resolving gel and then the current was increased to 15mA. The gels were generally run for 5-6 hours at a constant power supply and at room temperature. The temperature of the gel was maintained at approximately 10-15°C by providing a water bath with running tap water. The electrophoretic run was stopped as soon as the dye front reached approximately 2cms above the bottom line of the gel. After electrophoresis, the gel was fixed in fixative for 4-5 hours, washed twice with deionized water and then stained with Comassie Brilliant Blue R-250 for 5-6 hours. The gel was transferred to destaining solution and destained for 8-12 hours as per requirements for better resolution of bands. The constituents required for preparation of fixative, staining and destaining solutions were prepared as described by Ausubel et al., (1997a).

3. RESULTS AND DISCUSSION

The investigations carried out with the different starter cultures yeasts of the Nyshi and Apatani tribes of Arunachal Pradesh yielded appreciable results.

3.1. Isolation and culture of pure strains
The four strains isolated and cultured showed that they were hugely different in terms of their morphology and growth pattern. They were named as A, B, C and D for convenience. All the strains showed profuse growth in consortia during the first plating from the starter culture. However, after subsequent culturing, they showed differences in colour, morphology and growth pattern. Strain D was bit yellowish while the remaining three strains were greyish white and opaque in colour. Their growth behavior in solid YPD media showed interesting results where isolates A and B had highest rate as well as longest exponential growth, isolate C and D showed slowest growth rates (Figure 1).

Thermo-tolerance study revealed that isolates A, B and D had maximum growth activity at 35°C whereas isolate C depicted slowest growth at all the experimented temperature ranges. Ranges 28°C and 40°C were seen with good growth activities among the three isolates, namely A, B and D (Figure 2). Results of the pH sensitivity study showed remarkably fine sensitiveness of the four isolates to the different pH ranges subject to exposure during the experiment. It was seen that the strains depicted poor tolerability at more acidic condition than alkaline. During the first 24 hours of incubation, marked tolerability from all the four isolates were seen in all the pH ranges followed by increase in growth during the next 48 hours of incubation. Similar observations were found in each pH ranges among all the isolates (Figure 3 and Figure 4).
3.2. Whole cell protein analysis

Total whole cellular protein content in all the isolates varied considerably. It was observed that the isolate B showed the maximum amount of whole cell protein content followed by isolates A and D. Isolate C depicted a marked decrease in whole cell protein content which directly corresponds to growth of the isolates in YPD media (Table 1). However the studies nullify the presence of adenine deficient yeast strains as was reported earlier by Sarma (1999) on strains isolated from cakes used by tribes of Assam.

3.3. Protein profiling

Protein profiling of all the four yeast isolates were obtained using SDS-PAGE which depicted a subsequent amount of information of the isolates studied (Table 2). A band of 199.6 kDa was present in three of the isolates viz. B, C, and D but absent in A. One particular band of 173.2 kDa was observed in isolate C and not in others. Another band of 168 kDa was observed isolates C & D and not in A & B. Isolate C depicted the characteristic presence of another band of 116.7 kDa.

Table 1 Cellular protein content of the yeast isolates

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Yeast strain</th>
<th>Protein content in µg/ml</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>95.4</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>116.4</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>55.9</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>73.2</td>
</tr>
</tbody>
</table>

Table 2 Band patterns of yeast isolates in SDS-PAGE

<table>
<thead>
<tr>
<th>S. No</th>
<th>Isolate A</th>
<th>Isolate B</th>
<th>Isolate C</th>
<th>Isolate D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>199.6kDa</td>
<td>199.6kDa</td>
<td>199.6kDa</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>173.2kDa</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>168kDa</td>
<td>168kDa</td>
<td>-</td>
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<tr>
<td>4</td>
<td>-</td>
<td>116.7kDa</td>
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<td>5</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<td>6</td>
<td>88.2kDa</td>
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<td>-</td>
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<td>7</td>
<td>85.4kDa</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>8</td>
<td>59.5kDa</td>
<td>59.5kDa</td>
<td>-</td>
<td>59.5kDa</td>
</tr>
</tbody>
</table>
lower molecular weight bands of 111.4 and 88.2 kDa were observed in isolate B but was absent in others. A very low molecular weight band of 59.5 kDa was present in three isolates except isolate C. The band pattern studied provided a clear indication that all the isolates were different at genetic level to some extent. Isolate C might represent a different clad of origin provided the genetic origin is evaluated using advanced molecular techniques.

4. CONCLUSION
Protein profiling of the strains in the present study has revealed that the strains are indeed variants at the genetic level as was evident from expression of whole cell proteins. The strains might therefore be mutagenic variants which arose during segregation through time immemorial. A clear and vivid picture can be obtained by subjecting the strains to various advanced molecular analyses. Interestingly, all the isolated strains have shown a preference of growth at neutral pH; a character not usually observed in most yeast species which generally prefer an acidic medium for growth. Growth of one of the isolates (B) was found to be higher compared to others under optimum conditions while another strain (D) showed an appreciable thermo-tolerance and maximum growth at 35°C which was clearly astonishing. Notwithstanding the experiments performed and results obtained, it can be predicted that careful identification and selection can yield industrially important and beneficial yeast strains.

SUMMARY OF RESEARCH
1. The present study within the limit of available resources and techniques has yielded appreciable amount of information on the differences of yeast strains used for traditional brewing among the two tribes of Arunachal Pradesh.
2. The protein profiling study has revealed that the yeast strains used were of common origin which eventually were segregated and evolved into genetically different species of yeast under different cultural and environmental selection pressures.

FUTURE ISSUES
The results have clearly indicated the importance of scientific characterization of the yeast strains so that they can be used for large scale production of traditional brews. The characterization, however, need further study using advanced techniques so that the strains can be identified at the species level.

DISCLOSURE STATEMENT
There is no financial support for this work from any funding agencies.

ACKNOWLEDGEMENTS
My deep sense of gratitude goes to Late Prof. Sharda Khandelwal, Head, Indira Gandhi Academy of Environmental Education Research and Ecoplanning (IGAEERE), Jiwaji University, Gwalior, for providing me the support and encouragement to conduct the present work. I am also highly grateful to Dr. Subhas Chandra Tiwari, Senior Lecturer, Department of Applied Sciences (Forestry), North Eastern Regional Institute of Science and Technology (NERIST), Arunachal Pradesh and Dr. Hridip Kumar Sarma, Research Associate, Department of Applied Sciences (Forestry), North Eastern Regional Institute of Science and Technology (NERIST), Arunachal Pradesh for the technical support and laboratory to conduct the work flawlessly.

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