Studies on Phloem Sap of *Caryota urens* and Screening of Yeast Strains for Potential Industrial Applications

By

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Abstract

Biochemical and microbial changes that occur during fermentation of Caryota urens phloem sap were studied with a view to upgrading the local fermentation industry and to developing new biotechnological process by recognizing yeasts suitable to local conditions.

First sample of sap which was taken after keeping the sterilized pot for 10 hours, was apparently unfermented. Paper chromatographic studies done with unfermented sap revealed that the main type of sugar was sucrose, while glucose and fructose were in trace amounts. The content of reducing sugar of unfermented sap, measured by Lane and Eyon method, was very low (0.14 %, w/v). However this started to increase after 24 hours and reached a maximum value (4.1 %, w/v) after 24 hours. The alcohol concentration, measured by ebulliometer, increased only after appreciable quantities of reducing sugar was formed. Maximum alcohol concentration of 7 %, v/v was obtained on the fifth day. The unfermented sap had a pH around 7. This decreased substantially and reached a constant value around 4 after the fourth day of fermentation. Decrease in pH coincided well with increase of acidity.

Microbial analysis of fermenting sap revealed that the type of organisms present in the sap changed with time. At the beginning of fermentation the sap contained mainly bacteria with a colony count in the order of 10^7 cells/ml. The yeast cell number increased after 24 hours and reached a maximum of 8×10^7 cells/ml after third day. During the later stages of fermentation, there was a drop in the viable yeast cell count, while the bacteria count increased again to 10^6 cells/ml.

A total of eleven yeast strains were isolated from fermenting phloem sap, some of these were identified as species of Candida , and species of Saccharomyces. A strain which fermented inulin , isolated from fermenting sap and it's identification was not possible. This may be a new strain that has not been reported earlier.

These yeast were examined to determine their suitability for high temperature ethanol fermentation and for single cell protein production.

When the yeast isolates were screened for their ability to ferment sucrose to ethanol at 40 °C only five strains gave substantial yields, (ie more than 50 % of the theoretical maximum). Out of these, three strains were selected for further studies. For the purpose of selecting the best strain for alcohol fermentation at 40 °C , certain

parameters of these strains were checked. These strains had different growth rates at 40 °C when checked using a complete synthetic medium. Strain S 2-5 had the highest growth (2.2×10^6 cells/ml/h) while S11 F-3 had the lowest growth rate (1×10^6 cells/ml/h) . Lower growth rate would favour high ethanol production and hence, S11 F-3 seems to be better in this aspect. When fermentation tests done with these three strains (S 2-5, S11 F-3, S5 MB-29) at different temperatures, 30, 35 and 40 °C in complete synthetic medium with 20 % sucrose, the highest alcohol concentration of 14.2 %, v/v was obtained with strain S11 F-3 at 40 °C. At 35 °C, all three strains gave more than 9 %, v/v alcohol. At 40 °C strain S11 F-3 performed better, giving an alcohol yield of around 9.2 %, v/v. With increase of temperature from 30 °C to 40 °C, the ethanol yield of strain S11 F-3 decreased by about 35 % while the strain S5 MB-29 and S 2-5 decreased about 36.5 % and 40 % respectively. When the temperature was increased from 35 °C to 40 °C the decreased in ethanol yield was only about 10 % in the case of strain S11 F-3. With the molasses medium, at 40 °C , S11 F-3 gave highest alcohol yield of 8.4 %. This value is very much higher than the value obtained with Baker's yeast when used under the same conditions (4.7 %) Maximum CO_2 productivity (g/h), final ethanol concentration (%,v/v), yield efficiency [(g ethanol/g glucose)/ 0.511] and maximum cell growth rate (No.of cells/ml/h) were used

as selection criteria of thermotolerant yeast. The strain S11 F-3 which gave highest alcohol yield gave higher values with all the above parameters except growth rate.

Four yeast strains which were unable to give high alcohol yields in fermentation experiments were tested for their nitrogen contents and higher growth rates were selected for further studies (S5 M-15 and S8 M-15). Their optimum growth temperature was around 35 °C. The moisture contents were in the range of 6.5-7.3 %, while the ash contents were around 7 % on dry weight basis. Two strains showed considerably good growth in molasses medium and produced about 4 g cell dry weight/100 ml of molasses. Amino acid composition of two strains were determined using HPLC method (Pico-Tag) and strain S8 M-15 consisted of 82 % of essential amino acids, while strain S5 M-15 consisted of 70 % essential amino acids from the total amino acid content of each of the strains. Thiamine and riboflavin contents were analyzed by HPLC method (Hagg) and two strains contained about 0.2 mg riboflavin and 0.1 mg thiamine per gram of dried cells.

The results suggest that changes during natural fermentation of Caryota urens phloem sap is very much similar to coconut sap fermentation and among the isolated strains, strain S11 F-3 could be regarded as the best strain for production of alcohol at higher temperature while strains S5 M-15 and S8 M-15 which could be grown more

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