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CHARACTERIZATION OF YEAST FOR ETHANOL AND WINE PRODUCTION USING FERMENTATION TECHNOLOGY

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ABSTRACT
Commercial wine yeast strains of the species *Saccharomyces cervisiae* have been selected to satisfy many different, and sometimes highly specific, oenological requirements. As a consequence, more than 200 different strains with significantly diverging phenotypic traits are produced globally. This genetic resource has been rather neglected by the scientific community because industrial strains are less easily manipulated than the limited number of laboratory strains that have been successfully employed to investigate fundamental aspects of cellular biology. However, laboratory strains are unsuitable for the study of many phenotypes that are of significant, scientific and industrial interest. Here we investigate whether a comparative transcriptomics and phonemics approach, based on the analysis of five phenotypically diverging industrial wine yeast strains, can provide insights into the molecular networks that are responsible for the expression of such phenotypes. For this purpose, some oenological relevant phenotypes, including resistance to various stresses, cell wall properties and metabolite production of these strains were evaluated, and aligned with transcriptomic data collected during alcoholic fermentation.

KEYWORDS: fermentation, phenotypes, strains, wine

INTRODUCTION

The production of pure ethanol apparently begins in the 12-14th century along with improvements in the art of distillation permitting the condensation of vapors of lowers boiling liquids. Now, ethanol is an important industrial chemical with emerging potential as a biofuels to replace vanishing fossil fuels. Ethanol may be produced commercially by chemical synthesis or biosynthesis. Chemical synthesis is by hydration of ethylene (C_2H_4). For the biosynthesis, in the fermentation process, yeast uses monosaccharide as a carbon source and then converts to ethanol via glycosis under anaerobic conditions. The overall reaction can be summarized as follow:

$$\underset{\text{Glucose}}{C_6H_{12}O_6} + 2\ ADP + 2\ Pi \xrightarrow{\text{Yeast}} \underset{\text{Ethanol}}{2\ C_2H_5OH} + 2\ CO_2 + 2\ ATP + 2\ H_2O$$

At the beginning of the 20th century, several kinds of raw materials were exploited for ethanol production, such as molasses or agricultural production, and the possibility of hydrolyzing lignocellulosic materials was investigated. Carbohydrate-rich raw materials suitable for ethanol production can be classified into three groups of agricultural products: which all sugar, starch and lignocelluloses. The first raw material group, sugar refers to sugar-beet as well as sugarcane and molasses. The second group, starch from such crops as cassava, cereals and potatoes. The last group, lignocelluloses, covers waste materials from the harvesting of agricultural crops such as rice straw, corn cob and sugarcane waste. In general, industrial yeast strains are able to grow and efficiently ferment ethanol at pH values of 3.5-6.0 and temperatures of 28-30° C, with efficiency dropping off rapidly at higher temperature. According to there are several potential benefits of thermo tolerant yeast for using in the production of industrial alcohol.

MATERIALS AND METHODS

A--ISOLATION AND SCREENING OF WINE YEASTS

Wine yeasts were isolated from silage samples, at Suranaree University of Technology farm on (MGYP) media Malt extract 0.3%, Yeast extract 0.3%, Glucose 1%, Peptone 0.5%, Agar 3%, D /W 100 ml PH 6.4-6.8 and was incubated Anaerobic ally at 25 °C for 2 days. Single colony formed was picked and the cells were observed under microscope.

Selection of Wine yeast with ethanol production activity

According to the methods the Efficiency of ethanol production of Wine yeast strain was tested. Yeast Extract peptone dextrose (YPD) liquid medium which contained 20 g glucose, 20 g Peptone and 10 g yeast extract in 1 liter water, was

used for ethanol production test. The medium was adjusted to pH 5.5 with 1 N HCl. One hundred µl cell suspensions Of the isolated Wine yeast strain taken from actively growing culture was Inoculated into 7 ml of YPD liquid medium in a test tube containing a dirham tube. Fermentation was recognized by the accumulation of CO2 gas trapped in the inner 37 Durham tube all culture was incubated at 40 °C for 3 days. The ethanol production was determined by using a gas Chromatograph equipped with capillary PE-1 column (Auto System XL, Perkin Elmer, and U.S.A.). The analysis of ethanol was operated on flame-ionization detector and an inlet system using the split less injection technique, injector and detector temperature were 250 and 300 °C, respectively. Oven program was 37 °C for 5 min and increase from 37 to 245 °C at 10 °C/ min. Helium gas was used as carrier gas, adjusted to 14 psi. The ethanol production was primarily identified by comparing the retention time of the gas chromatographic peak with ethanol standard.

Optimization of temperature for yeast growth

Following Sree *et al*. (1999), 50 ml of YPD medium was distributed into 125 ml screw cap Erlenmeyer flask were inoculated with Wine yeast from actively growing culture. All cultures were incubated at 30 °C, 37 °C, 40 °C and 45 °C for 72 h. The initial optical density of each flask was read off on spectrophotometer (Ultra spec 2000 UV/Visible Spectrophotometer, Pharmacia Biotech, England) at 660 nm against the medium as blank. The increase in optical density in a flask was recorded as evidence of growth.

Detection of yeast killer toxin

The assay of isolated Wine yeast killer toxin was performed by means of streak-plate agar diffusion assay. Approximately 105 cells/ml of the sensitive yeast strain *S. cerevisiae* EC 1118, *Escherichia coli* ATCC 25922 and *Bacillus subtitles* ATCC 6633 were suspended in each 20 ml malt 38 agar buffered at pH 4.4 (0.1 M citrate-phosphate buffer). Killer yeast strain *S. cerevisiae* K1-V1116 and isolated Wine yeast were streaked on the agar surface, incubated at 20 °C for 72 h. Killer activity of isolated Wine yeast was evident as a clear zone of inhibition to sensitive strain surrounding the streak similarly killer strain.

Detection of ethanol tolerance of Wine yeast

The medium for the detection of ethanol tolerance of Wine yeast was modified from Osho YPD liquid medium was used for detecting Wine yeasts for ethanol tolerance. The medium was sterilized at 121 °C for 15 min in an autoclave and cooled. One ml of various concentrations of absolute ethanol was varied from 5 to 25% (v/v), and then added to different flask of the same medium to constitute varying percentages of ethanol differing by 5% (v/v) from one flask to the others. Forty ml portion of the medium was distributed into 125 ml flask, and then inoculated with selected Wine yeasts. The initial optical density of each flask was read off on spectrophotometer (Ultra spec UV/Visible Spectrophotometer, Pharmacia Biotech, England) at 660 nm against the medium as blank. All cultures were incubated at 25 °C for 5 days. The increase in optical density in a flask was recorded as evidence of growth. The concentration of alcohol at which the growth of yeasts was just inhibited was assessed as the ethanol tolerance of yeasts.

B. IDENTIFICATION OF THE SELECTED WINE YEAST

Morphological characterization

According to the method of Kreger-van Rij (1984) and Kurtz man and Fell (1997), the morphology of the vegetative cells was grown in liquid and on solid media.

Growth on solid medium

The morphology of cells of Wine yeast and their appearance on solid medium, on YPD agar was examined, after incubating at 40 °C for 3 days. The following features of the appearance of cultures were recorded; texture, color and surface of colonies. Their ascosporic and pseudo mycelium formation were determined

Ascosporic formation

Wine yeast was examined for ascosporic formation applied from Kurtz man *et al*.. The culture was initially incubated for 2 days at 37 °C to facilitate growth, and then further incubated at 25 °C to induce ascosporic formation. The culture was examined for ascosporic at approximately weakly intervals for 3 weeks. Ascosporic formation was detected by staining the heat-fixed preparation (Kreger-van Rij 1984) carol-fuchsine and steamed gently for about 5 min. Slide was decolorized with 95% ethanol containing 1% concentrated hydrochloric acid. The Slide was rinsed in water and counter stained with 1% methylene blue; the mature ascosporic stain red and vegetative cells blue. 40

Pseudo mycelium formation

Following to Kroger-van Rij (1984), the formation of pseudo mycelium was investigated by slide culture technique. A Petri dish was containing a U-shaped glass rod Supporting glass slide, was sterilized by dry heat at 180 °C for 2 h. YPD agar was Melted and poured into a second Petri dish. The glass slide was quickly removed from the glass rod with a flame-sterilized, and was dipped into the agar after which it was replaced on the glass rod support. After solidification of the agar on the slide, the Wine yeast was inoculated very lightly in two lines along slide and a sterile cover slip was placed over part of the lines. Some sterile water was poured into the Petri dish to prevent the agar from drying out. The culture was incubated at 25 °C for 5 days. For observation, the slide was taken out of Petri dish and the agar was wiped off the back of the slide. The edges of the streak under and around the cover slip were examined microscopically.

Growth in liquid medium

The morphology of cells was cultured in YPD liquid medium. Cells from a young actively growing culture were inoculated into test tube containing 7 ml of medium, incubated at 40 °C for 3 days. The culture was examined the growth of Wine yeast visually on the surface of YPD liquid medium and the shape of cells by compound microscope (Alphaphot-2, Nikon, Japan) and scanning electron microscope (JSM-6400, Scanning Microscope, JEOL, Japan).

DISCUSSIONS

The production of pure ethanol apparently begins in the 12-14th century along with improvements in the art of distillation permitting the condensation of vapors of lowers boiling liquids. During the middle ages, alcohol was not only mainly used for the production or as a constituent of medical drugs, but also for the manufacture of painting pigments and other chemical industries. It was only in the 19th century that this trade became an industry with enormous production, due to economic improvements of the distilling process. Now, ethanol is an important industrial chemical with emerging potential as bio-fuels to replace vanishing fossil fuels.

RESULTS

The observation of colony characterstics and wine east characterstics are given in the table No. A, B, C, D, E and F, which are observed and calculated on the basis of grwoth of colonies in petri-plates contanining culture media as in 1, 2, 3, 4, 5 and 6 respectively. Isolation Results of Wine Yeast
Culture Identification

Figure 1: Colonies of Wine Yeast

Table A: Colonies of Wine Yeast

Sr. No.	Colony Characteristics	Wine Yeast Characteristics
1	Size	0.6mm
2	Shape	Spherical
3	Color	White
4	Margin	Entire
5	Consistency	Dry
6	Opacity	Opaque
7	Gram Nature	Gram negative rods

Isolation Results of Standard Yeast-II
Culture Identification

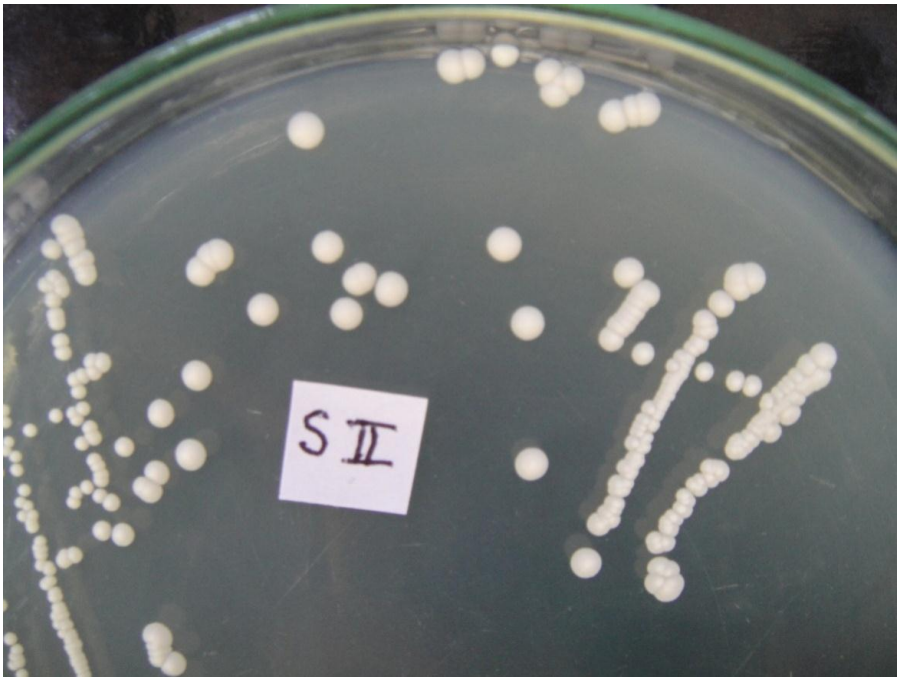

Figure 2: Colonies of Standard Yeast-II

Table B: Colonies of Standard Yeast-II

Sr. No.	Colony Characteristics	Wine Yeast Characteristics
1	Size	0.7mm
2	Shape	Spherical
3	Color	White
4	Margin	Entire
5	Consistency	Dry
6	Opacity	Opaque
7	Gram Nature	Gram negative rods

Isolation Results of Standard Yeast- I
Culture Identification

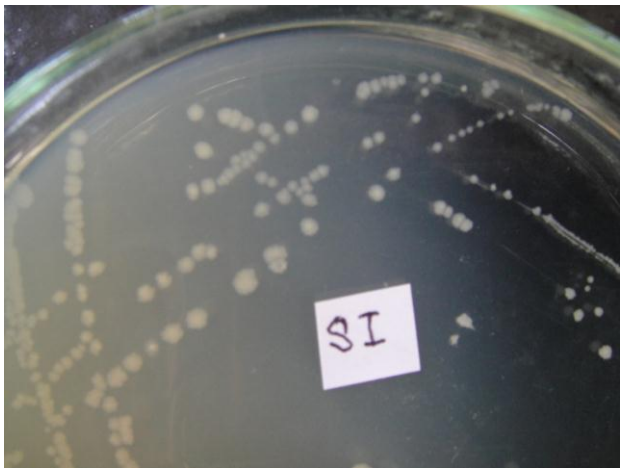

Figure 3: Colonies of Standard Yeast I

Table C: Colonies of Standard Yeast I

Sr. No.	Colony Characteristics	Wine Yeast Characteristics
1	Size	0.5mm
2	Shape	Spherical
3	Color	White
4	Margin	Entire
5	Consistency	Dry
6	Opacity	Opaque
7	Gram Nature	Gram negative rods

Isolation Results of Milk Yeast
Culture Identification

Figure 4: Colonies of Milk Yeast

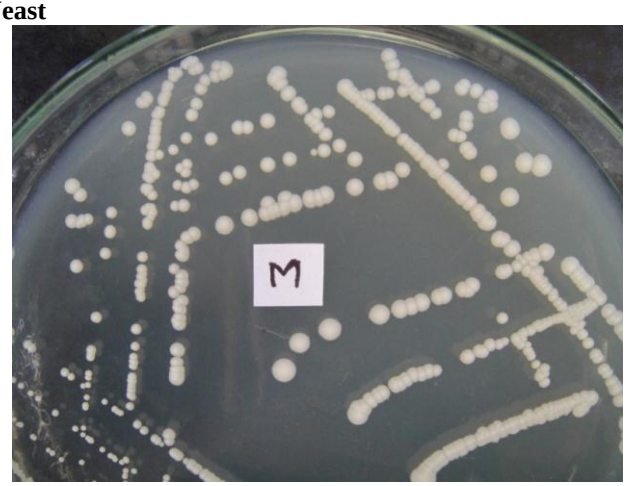

Table D: Colonies of Milk Yeast

Sr. No.	Colony Characteristics	Wine Yeast Characteristics
1	Size	0.7mm
2	Shape	Spherical
3	Color	White
4	Margin	Entire
5	Consistency	Dry
6	Opacity	Opaque
7	Gram Nature	Gram negative rods

Isolation Results of Grapes Yeast
Culture Identification

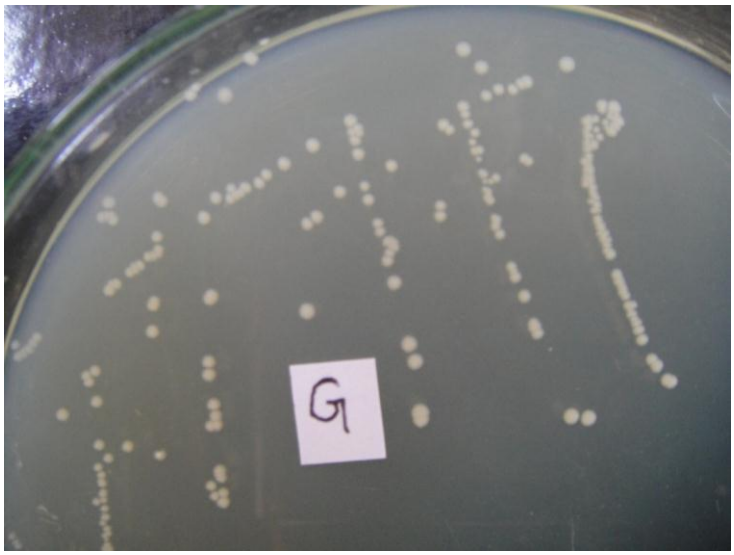

Figure 5: Colonies of Grape Yeast

Table E: Colonies of Grape Yeast

Sr. No.	Colony Characteristics	Wine Yeast Characteristics
1	Size	0.5mm
2	Shape	Spherical
3	Color	White
4	Margin	Entire
5	Consistency	Dry
6	Opacity	Opaque
7	Gram Nature	Gram negative rods

Isolation Results of Corn Yeast
Culture Identification

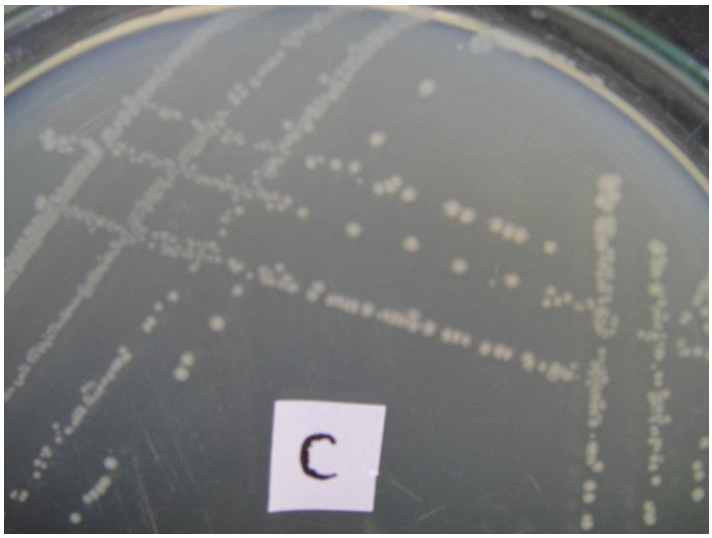

Figure 6: Colonies of Corn Yeast

Table F: Colonies of Corn Yeast

Sr. No.	Colony Characteristics	Wine Yeast Characteristics
1	Size	0.4mm
2	Shape	Spherical
3	Color	White
4	Margin	Entire
5	Consistency	Dry
6	Opacity	Opaque
7	Gram Nature	Gram negative rods

CONCLUSIONS

The present work aimed to study alternatives for the important market of common wines in Brazil that contribute to the improvement in product quality, the minimization of production costs by the use of nonconventional grapes, and the development of a technology for this fermentative process. The average ethanol formation was of 79,4 g/L, very close to an alcohol graduation of 10°GL for the skin-free wine, which is the minimum required by the Brazilian legislation for it to be considered as a table wine. Together with the low 2nd Mercosur Congress on Chemical Engineering 4th Mercosur Congress on Process Systems Engineering values for volatile acids concentration, it was verified that the production of white wine from the Italia grape variety has a large potential of being used by the winegrowers of São Roque. The development of a simple procedure for the preparation of the skin-free must and the superior results obtained in this culture medium (higher ethanol and glycerol concentrations together with a lower concentration of volatile acids) illustrate the importance of the experimentation of new procedures for the improvement of product quality. However, the good use of the must volume retained in the skins separated by flotation is essential in order to make viable the proposed procedure. Analyzing the coefficients of the adjusted empiric models, it was verified that the independent variables pH and temperature affected the studied response variables (ethanol, glycerol and volatile acids concentration), however their effects on the response variables were highly influenced by the presence or absence of skin in the fermentation. It can also be seen from the experimental results and the optimized values that even for an Italia grape wine a refrigeration system would bring considerable benefits for the quality of the product.

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