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Development of fermented rice-based beverages from wild strains of *Saccharomyces cerevisiae* isolated from fresh litchi and Jamun fruits of Assam, India

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Abstract

The isolates *Saccharomyces cerevisiae* L1 and *Saccharomyces cerevisiae* J7 were isolated from Litchi and Jamun fruits of Assam, India respectively. These were applied for beverage production using rice as substrate. The fermented beverages were analyzed physiochemically using parameters like pH, total soluble solids, and antioxidant properties. The beverages were also observed for ethanol content, carbohydrate, energy, and protein content using FSSAI (Food Safety and Standards Authority of India). The study revealed that the developed fermented rice-based beverages have shown some differences and similarities in different criteria. The strain *Saccharomyces cerevisiae* L1 was found to be potent in comparison to the strain *Saccharomyces cerevisiae* J7. The pH was found to be 3.5, Total soluble solid was 7.8° Brix and 58.6% antioxidant properties for *Saccharomyces cerevisiae* L1 whereas, for *Saccharomyces cerevisiae* J7, pH was found to be 3.7, total soluble solids was 8° Brix and 55.6% antioxidant properties. The ethanol content was measured to be 9% in the case of *Saccharomyces cerevisiae* L1 and 5.2% in the case of *Saccharomyces cerevisiae* J7. A sensory evaluation study revealed that the fermented rice beverage by *Saccharomyces cerevisiae* L1 was more satisfactory than *Saccharomyces cerevisiae* J7.

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Introduction

Fermentation is an ancient technique adopted by humans. A century ago, people were not aware of the science behind it but they used it for the preservation of grains and for the cultural heritage (Scholes *et al.,* 2021). Science and research activities established the relationship between microbial metabolism with fermentation. Fermented foods occupy one-third of the total foods in the world. Thus, fermentation influences the economy globally as well as locally (Tamang *et al.,* 2016). *Saccharomyces cerevisiae* is the principal organism that plays a very important role in fermentation. Whereas other organisms also develop during the process of the addition of organoleptic characteristics of fermented foods. The home for yeasts is the sugar-rich ecosystem. Naturally, different parts of plants are the preferred habitation for yeasts because of the presence of different sugars (Parapouli *et al.,* 2020, Vadkertiová *et al.,* 2012). Geologically, the raw materials for example different cereals, the grape must use for fermentation consists of a fraction of naturally present microbes. Their presence is treasured by the presence of carbon-rich compounds. Among the microbes, *Saccharomyces cerevisiae* is dominant over other *Saccharomyces* genera (Mandakovic *et al.,* 2020). The outcompete nature of *Saccharomyces cerevisiae* in the fermentation process is due to its stress tolerance behaviors.

The stress includes low pH, high ethanol concentration, osmotic pressure in presence of different sugar concentrations, an anaerobic environment, and during a shortage of nutrients. Their survival in such conditions makes them superior in fermentation (García-Ríos and Guillamón, 2019). Considering all these factors, industrialists make use of starter culture in the fermentation process. The contemporary brewing style evolved from the differences in regional brewing design (Scholes *et al.,* 2021). As extreme exploitation of starter culture assures accurate fermentation without any mishappening, the producers make use of specific *S. cerevisiae* strains as starter culture in the process that could not be able to aid new flavors and aromas to the beverages (Albergaria and Arneborg, 2016). Although these commercially available strains or their hybrids are inadequate for the aid of unique characteristics of beer or wine. For the novelty of the products, the necessity arises to search for a unique superior strain for the fermentation process (Gibson *et al.,* 2020). In recent times, brewers' selection of yeast as a critical parameter in brewing is considered to be a demanding task (Scholes *et al.,* 2021). Therefore, currently, the search for wild strains from different habitats for the development of novel beer, and wine with new organoleptic characteristics is a new trend in research (Pontes *et al.,* 2020).

Therefore, this study has been designed to apply the identified wild strains of *Saccharomyces cerevisiae* from natural sources such as Litchi and Jamun fruits. The development of beverages from these strains and the estimation of different parameters to introduce them as industrial strains for brewers. A small attempt has been made to search for a suitable strain that can provide a novel aroma and taste to fermented rice-based beverages.

Materials and methods

Rice-based beverage production

The rice-based beverages were prepared using the strains *Saccharomyces cerevisiae* L1 (GenBank accession number OK326788) and *Saccharomyces cerevisiae* J7 (GenBank accession number OK326764) in the month of April. During fermentation, the temperature maintained was 28-30°C. The inoculum was prepared by inoculating 48-hour-old culture in potato dextrose broth. This was incubated at 28±2°C for 24 hours. The preparation of beverages started with washing rice. Then autoclaved rice was cooled down to room temperature. 3% of inoculum was added to the cooked rice. It was incubated for 5 days at 28±2°C temperature. Then, different parameters of the beverages were estimated. The strains *Saccharomyces cerevisiae* L1 (GenBank accession number OK326788) and *Saccharomyces cerevisiae* J7 (GenBank accession number OK326764) were isolated from local fruits of Assam, India. The work was reported by the authors in a previously published paper (Senapati *et al.*, 2021).

Physicochemical properties of rice-based beverages developed by the strains L1 and J7

Physicochemical parameters include pH, total soluble solids, and antioxidant properties. The pH of the beverages was estimated using a pH electrode compared with the standard buffer solutions (Pakuwal and Manandhar, 2020).

Total soluble solids were calibrated by the use of a refractometer. First, distilled water was used for calibration, and then the sample was placed for the reading that appeared on the screen as 'Brix' (Pakuwal and Manandhar, 2020).

The antioxidant activity of fermented rice beverages was determined by DPPH (2,2-diphenylpicrylhydrazyl) assay.

Antioxidant activity (%) = $[(a_{dpph} - a_{sample})/ a_{dpph}] \times 100$

Where A_{DPPH} is the absorbance of DPPH at 517 nm and a_{sample} is the absorbance of beer samples at 517 nm (Deka *et al.,* 2018).

Proximate analysis of fermented rice beverages developed by L1 and J7

The ethyl alcohol content of fermented rice beverages was estimated using a clean and dry pycnometer. The fat was extracted using the Soxhlet apparatus with petroleum ether. Then the desiccated residue was weighed and the total fat was calculated. The total protein of the beverages was determined by the Kjeldahl method (FSSAI, 2015).

The total Carbohydrate and energy of the beverages were calculated using the formulae (FSSAI, 2015)

$$\text{Total carbohydrate} = 100 - (\text{Moisture} + \text{Ash} + \text{Fat} + \text{Protein})$$

$$\text{Total energy} = (\text{Carbohydrate} \times 4) + (\text{Protein} \times 4) + (\text{Fat} \times 9)$$

Sensory evaluation of fermented rice beverages developed by L1 and J7

The developed fermented rice beverages by L1 and J7 were evaluated for organoleptic tests by a panel of 30 judges with nine points hedonic scale. The age of judges ranges from 20-40 years. These samples were assessed for taste, aroma, appearance, and overall impression according to the hedonic scale of nine categories: extremely dislike = 1; very much dislike = 2; moderately dislike = 3; slightly dislike = 4; neither like nor dislike = 5; slightly like = 6; moderately like = 7; very much like = 8 and extremely like = 9 (Miguel *et al.,* 2017).

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the differences among various groups. A value of $p < 0.05$ was considered to be statistically significant.

Result and discussion

The physicochemical properties of the beverages developed by *Saccharomyces cerevisiae* L1 and *Saccharomyces cerevisiae* J7 were observed in table no 1. The pH of the fermented rice-based beverage from isolate *Saccharomyces cerevisiae* L1 and *Saccharomyces cerevisiae* J7 was found to be 3.5 and 3.7 respectively shown in table 1. Lee *et al.,* 2007; Seo *et al.,* 2008 observed in their study, that the final pH of the rice-based alcoholic beverages was within the range of 3.4 to 4.5 (Lee *et al.,* 2007, Seo *et al.*, 2007).

The result of our experiment was similar to the literature. The total soluble solids of the fermented rice-based beverages by the isolates *Saccharomyces cerevisiae* L1 and *Saccharomyces cerevisiae* J7 were found to be 7.8 and 8 respectively shown in table no1. Chay *et al.*, observed in their experiment that the TSS of the rice-based beverage was within the range of 9-18°Bx (Chay *et al.,* 2017). Our result showed less TSS than the range cited by the literature. This could be because of the substrate used for the developed products. In the case of the antioxidant properties of the beverages, the isolates *Saccharomyces cerevisiae* L1 and *Saccharomyces cerevisiae* J7 were found to be 58.6% and 55.6% respectively shown in table no 1. For Korean rice wine, the antioxidants observed were 35% to 66% by Jeong *et al,* (Jeong *et al.,* 2011). Our observation of the antioxidant properties of the produced beverages was in this range, similar to the literature cited. Antioxidant properties rely on the substrate used as suggested by Kushwaha, 2016 (Kushwaha, 2016).

Table 1. Physicochemical properties of beverages produced by *S. cerevisiae* L1 and *S. cerevisiae* J7.

Isolates	pH	TSS	Antioxidant (% Inhibition)
L1	3.5 ± 0.001	7.8 ± 0.001	58.6 ± 0.015
J7	3.7 ± 0.001	8.0 ± 0.001	55.6 ± 0.041

The proximate analysis of the products developed by *Saccharomyces cerevisiae* L1 and *Saccharomyces cerevisiae* J7 as shown in table no 2. The fermented beverages were analyzed for fat content, protein content, carbohydrate, energy, and ethanol content after 5 days of fermentation.

It was found that the ethanol content of the beverages produced by the isolates *Saccharomyces cerevisiae* L1 and *Saccharomyces cerevisiae* J7 was found to be 9% and 5.2% respectively shown in table no 2. Bhuyan *et al.*, reported that ethanol production from the beverages was in the range of 10.5% to 11.28%. our observation was close to the reported literature (Bhuyan *et al.,* 2014). The reason could be the substrate used, the glassware used, or the environmental factors during the process of fermentation.

Table 2. Proximate analysis of prepared rice beer from *S. cerevisiae* L1 and *S. cerevisiae* J7.

Properties	L1	J7
Total fat	0.12 ± %	2.30 ±%
Total protein	Nil	Nil
Total carbohydrate	2.53%	1.13%
Total energy (Kcal/100g)	11.20	25.22
Ethyl alcohol content	5.20%	9.0%

Table no 3 shows the sensory evaluation for the products by *Saccharomyces cerevisiae* L1 and *Saccharomyces cerevisiae* J7. The sensory evaluation of the beverages was found to be satisfactory in terms of taste, appearance, and aroma shown in table no 3. The colour of the beverages was found to be straw yellow. The colour may vary in the case of the use of coloured rice varieties. But in the case of our experiment, we used normal white coloured rice, so no such colour developed.

Table 3. Sensory evaluation of rice beverages developed by *S. cerevisiae* L1 and *S. cerevisiae* J7.

Isolates	Appearance	Aroma	Taste	Overall acceptability
J7	7.73±0.868	7.36±0.668	7.93±0.739	7.73±0.739
L1	7.96±0.668	7.6±0.770	7.6±0.663	8.03±0.556

Statistical Analysis

Different physiochemical properties of the beverages produced by *S. cerevisiae* L1 and *S. cerevisiae* J7 are shown in figure 1. It was observed that the ethanol content (%v/V) and total energy content of the two beverages were significantly different. However, the other parameters like pH, TSS, and antioxidant properties did not show any difference.

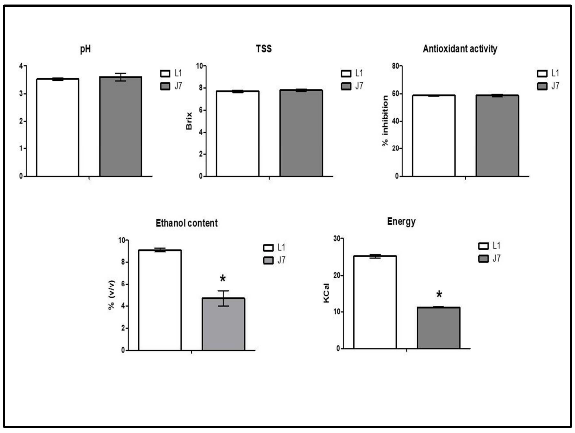

Fig. 1. Physicochemical properties of the beverages produced by the strains *S. cerevisiae* L1 and *S. cerevisiae* J7. * Represents the statistical difference between two parameters ($P<0.05$).

Conclusion

The isolation and application of wild yeast strains could help brewers for the development of beverages with novel tastes and aromas. This study concludes that the *Saccharomyces cerevisiae* L1 strain isolated from Litchi fruits, Assam, India was observed more potent strain in the production of ethanol than the isolate from Jamun fruits. The substrate used for the beverage production was rice in this experiment. To enhance the quality and quantity of ethanol production, the substrate and fermentation conditions could be standardized in further studies. On the other hand, the adaptation and fermentative environment of wild strains are the most challenging task. Bioprospecting is a solution to this challenge. The search for new wild strains may be inter or intra-species hybrids could dig out new possibilities and opportunities to acquire potent strains for brewers.

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References

Albergaria H, Arneborg N. 2016. Dominance of *Saccharomyces cerevisiae* in alcoholic fermentation processes: Role of physiological fitness and microbial interactions. Applied Microbiology and Biotechnology **100(5),** 2035-2046.

Bhuyan DJ, Barooah MS, Bora SS, Singaravadivel K. 2014. Biochemical and nutritional analysis of rice beer of North East India **13(1),** 142-148.

Chay C, Elegado FB, Dizon EI, Hurtada WA, Norng C, Raymundo LC. 2017. Effects of rice variety and fermentation method on the physiochemical and sensory properties of rice wine. International Food Research Journal **24(3).**

Deka AK, Handique P, Deka DC. 2018. Antioxidant-activity and physicochemical indices of the rice beer used by the Bodo community in north-east India. Journal of the American Society of Brewing Chemists **76(2),** 112-116.

FSSAI M. 2015. Manual of Methods of Analysis of Foods. Method **16,** 56-61.

García-Ríos E, Guillamón JM. 2019. Sulfur dioxide resistance in *Saccharomyces cerevisiae*: beyond SSU1. Microbial Cell **6(12),** 527.

Gibson B, Dahabieh M, Krogerus K, Jouhten P, Magalhães F, Pereira R, Vidgren V. 2020. Adaptive laboratory evolution of ale and lager yeasts for improved brewing efficiency and beer quality. Annual Review of Food Science and Technology **11,** 23-44.

Jeong JW, Nam PW, Lee SJ, Lee KG. 2011. Antioxidant activities of Korean rice wine concentrates. Journal of Agricultural and Food Chemistry **59(13),** 7039-7044. https://doi.org /10.1021 /jf200901j.

Kushwaha UKS. 2016. Black rice: Research, history and development. Springer.

Lee SJ, Kwon YH, Kim HR, Ahn BH. 2007. Chemical and sensory characterization of Korean commercial Rice wines (Yakju). Food Science and Biotechnology **16(3),** 374-380.

Mandakovic D, Pulgar R, Maldonado J, Mardones W, González M, Cubillos FA, Cambiazo V. 2020. Fungal diversity analysis of grape musts from central valley-Chile and characterization of potential new starter cultures. Microorganisms **8(6),** 956. https://doi.org /10.3390/microorganisms8060956.

Miguel MGDCP, de Castro Reis LV, Efraim P, Santos C, Lima N, Schwan RF. 2017. Cocoa fermentation: Microbial identification by MALDI-TOF MS and sensory evaluation of produced chocolate. LWT **77,** 362-369.

Pakuwal E, Manandhar P. 2020. Production of rice based alcoholic beverages and their quality evaluation. Journal of Food Science and Technology Nepal **12(12),** 37-48. https://doi.org/10.3126 /jfstn.v12i12.30133.

Parapouli M, Vasileiadis A, Afendra AS, Hatziloukas E. 2020. Saccharomyces cerevisiae and its industrial applications. AIMS microbiology **6(1),** 1. https://doi.org/10.3934%2Fmicrobiol.2020001.

Pontes A, Hutzler M, Brito PH, Sampaio JP. 2020. Revisiting the taxonomic synonyms and populations of *Saccharomyces cerevisiae*- phylogeny, phenotypes, ecology and domestication. Microorganisms **8(6),** 903. https://doi.org/10.3390 /microorganisms8060903.

Scholes AN, Pollock ED, Lewis JA. 2021. A wild yeast laboratory activity: from isolation to brewing. Journal of Microbiology & Biology Education **22(2),** e00186-21 https://doi.org/10.1128 /jmbe.00186-21.

Senapati SS, Saikia D, Bhagawati P. 2021. Isolation and Characterization of Amylolytic Yeast Strains Isolated from Indigenous Fruits of Assam, India.

Seo DH, Jung JH, Kim HY, Kim YR, Ha SJ, Kim YC, Park CS. 2007. Identification of lactic acid bacteria involved in traditional Korean rice wine fermentation. Food Science and Biotechnology **16(6),** 994-998.

Tamang JP, Watanabe K, Holzapfel WH. 2016. Diversity of microorganisms in global fermented foods and beverages. Front Microbiol **7(377),** 1-28. https://doi.org/10.3389/fmicb.2016.00377.

Vadkertiová R, Jana M, Dana V, Elena S. 2012. "Yeasts and yeast-like organisms associated with fruits and blossoms of different fruit trees. Canadian Journal of Microbiology **58(12),** 1344-1352. https://doi.org/10.1139/cjm-2012-0468.