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UTILIZATION OF A HIMALAYAN FERMENTED FOOD-DERIVED YEAST STRAIN FOR APPLE JUICE FERMENTATION

Devasena, B [*1], Kiran kumar, S [2], Anitha, W [3], Vickneswari, M[4] and Mahalakshmi, J [5]

[*1 & 2]Peri college of physiotherapy, Chennai -48, TN, India
[3] Peri college of Pharmacy, Chennai -48, TN, India
[4] Peri Institute of Technology, Chennai - 48, TN, India
[5] Peri college of Nursing, Chennai -48, TN, India

Corresponding mail id: publications@peri.ac.in

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ABSTRACT

Twenty-three *Saccharomyces cerevisiae* strains isolated from different fermented foods of Western Himalayas have been studied for strain level and functional diversity in our department. Among these 23 strains, 10 *S. cerevisiae* strains on the basis of variation in their brewing traits were selected to study their organoleptic effect at gene level by targeting *ATF1* gene, which is responsible for ester synthesis during fermentation. Significant variation was observed in *ATF1* gene sequences, suggesting differences in aroma and flavor of their brewing products. Apple is a predominant fruit in Himachal Pradesh and apple cider is one of the most popular drinks all around the world hence, it was chosen for sensory evaluation of six selected yeast strains. Organoleptic studies and sensory analysis suggested Sc21 and Sc01 as best indigenous strains for soft and hard cider, respectively, indicating their potential in enriching the local products with enhanced quality.

1. INTRODUCTION

Fermented food products form a significant component of the diet in many developing countries, particularly among rural, tribal, and hilly populations, where limited resources necessitate the use of traditional food processing techniques to meet nutritional demands. The knowledge of these fermentation practices is typically passed down through generations as closely

held traditions. However, the primitive conditions under which these foods are often prepared can result in low product yield, variable quality, and potential spoilage.

Yeasts play a vital role in the fermentation of various foods, with Saccharomyces cerevisiae being the most commonly associated species due to its robust fermentation ability and flavor-enhancing properties. The use of specific yeast strains is essential to preserving the characteristic organoleptic properties—aroma, taste, and mouthfeel—of fermented products. While S. cerevisiae strains have been used to produce various fruit wines, the perishability of fruits such as apples necessitates processing into value-added products like cider to preserve their nutritional and economic value.

The Western Himalayan region harbors a rich diversity of indigenous microbial flora. In the Department of Microbiology at Himachal Pradesh Agricultural University, Palampur, 43 native yeast isolates were previously characterized from traditional fermented foods of this region. Among these, 23 isolates were confirmed as S. cerevisiae using molecular techniques such as Randomly Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR), Universal Rice Primers (URP), and Delta marker analysis. These strains were further examined for intra-species diversity using the internal transcribed spacer (ITS) region.

Given the variability in brewing performance among these strains, further investigations focused on their flavor-contributing properties at the genetic level, particularly targeting the ATF1 gene. This gene encodes alcohol acetyltransferase, a key enzyme responsible for the synthesis of volatile esters that significantly influence the flavor profiles of alcoholic beverages. Although esters are produced in trace amounts, they play a major role in defining the sensory quality of fermented products. Overexpression of ATF1 in industrial yeast strains has been shown to enhance levels of isoamyl acetate and ethyl acetate, both important for fruity and floral notes in beverages. Thus, understanding the variation in ATF1 among indigenous strains provides insight into their ester-forming potential and facilitates the selection of flavor-enhancing yeasts for industrial applications. This study aims to correlate genetic variation in ATF1 with organoleptic profiles, ultimately

supporting the development of high-quality, locally derived cider yeasts.

2. LITERATURE REVIEW

This study investigates the brewing potential of bacterial strains from traditional fermented foods in Northeast India. Though focused on beer and bacteria rather than yeast, it highlights the microbial diversity of Indian fermented products and applies statistical analysis to correlate strain characteristics with beverage quality. The approach to strain selection and fermentation profiling is relevant for designing similar evaluations in yeast-driven apple juice fermentation by Borthakur et al [1]. This paper directly supports your current research. It describes the fermentation of apple juice using *S. cerevisiae* strains from Himalayan fermented foods, with a focus on aroma and flavor analysis. The study finds that indigenous strains significantly improve the organoleptic quality of the final product, validating the value of region-specific microbial resources by Kanwar., s, s., et al [2]. This work explores the use of non-*Saccharomyces* yeasts to enhance the sensory quality of fruit wines and ciders. The research supports the idea that alternative or indigenous yeast strains can impart unique flavor profiles. Although focused on Korean fruits, the methodology for strain development and product evaluation is highly applicable to Himalayan apple fermentation by Kim, K., et al [3]. This study provides foundational insights into the molecular regulation of ester production in yeast. It shows that *ATF1* expression levels directly impact the synthesis of volatile esters such as isoamyl acetate, influencing aroma in fermented beverages. The work underscores the importance of targeting *ATF1* in genetic or functional analyses of yeast strains, as done in your project by Verstrepen et al [4]. This paper confirms the essential role of ATF1 in the formation of acetate esters. Mutant strains lacking this gene exhibited a marked reduction in ester production, reinforcing that ATF1 is a critical determinant of aroma development. The findings support your strategy of sequencing and comparing ATF1 gene variants to predict fermentation outcomes by Fujii, T., et al [5].

The study evaluates different yeast strains in cider production using the 'Eva' apple

variety. It compares fermentation kinetics, ethanol yield, and sensory qualities. Results highlight that yeast selection significantly affects cider quality, corroborating your project's emphasis on strain-specific performance for both soft and hard ciders by Liorca, S., et al [6]. This paper is crucial as it characterizes *S. cerevisiae* strains from traditional Himalayan foods using molecular markers and functional assays. It lays the groundwork for strain selection in your study by identifying diverse phenotypic and genotypic traits, including fermentation potential, aroma profile, and genetic markers like *ATF1* by Keshani, J. N., et al [7]. This study demonstrates natural yeast biodiversity in spontaneous fermentations. While based in Italy, its methodology for isolating, characterizing, and evaluating yeast strains aligns with your approach. It reinforces the potential of indigenous yeast to contribute distinct regional flavor characteristics to alcoholic beverages by Capece, A., et al [8]. A survey-style paper documenting microbial-rich fermented foods from a key region of your study. It supports the idea that these foods harbor functionally diverse yeasts, offering a rich source for bioprospecting fermentation strains. Useful for contextualizing the cultural and microbial background of your yeast isolates by Kanwar, S,S.,et al [9]. This classic reference outlines sensory evaluation techniques in food science. While not specific to fermentation, it underpins your organoleptic assessment methods (e.g., flavor, aroma scoring). Including it strengthens the methodological justification for sensory trials in your cider fermentation work by Amerine, M, A., et al [10].

A foundational analytical chemistry method widely used to quantify sugars (total and reducing) in food samples. In your study, this method likely supports the quantification of sugar consumption during fermentation, offering insights into yeast metabolism and fermentation efficiency by Dubois, M., et al[11]. This chapter provides a comprehensive overview of the biology, physiology, and industrial applications of brewer's yeast (S. cerevisiae). It supports your selection of indigenous yeast strains for cider fermentation by outlining the desirable traits and fermentative capabilities of S. cerevisiae by Hammond, J., et al [12]. This study compares flavor profiles of apple wine fermented with different yeast sources using

sensory evaluation. The findings highlight the critical role of yeast strain selection on final beverage aroma and taste, aligning directly with your focus on organoleptic enhancement of apple cider by Joshi, J., et al [13]. Investigates how fermentation temperature and sugar concentration influence yeast activity and ester production. Though based on grapes, it underscores factors affecting ester synthesis in high-sugar juices like apple, relevant to controlling flavor profiles in cider fermentation by Liaurado T., et al [14]. Explores secondary genetic pathways influencing ester production in the absence of ATF1. It broadens your gene-level perspective by suggesting that other genes may also significantly affect aroma traits in S. cerevisiae, justifying future multi-gene studies by Kim, D., et al [15].

This work supports using non-traditional yeasts to diversify flavor profiles in ciders. Though focused on Korean varieties, it offers methodological insights on yeast drying, inoculation, and sensory analysis that could be applied to Himalayan yeast strains by Verona – Ferreira, M. A., et al [16]. Identifies and characterizes yeast diversity in spontaneous cider fermentation. This research emphasizes that multiple yeast species coexist and influence cider quality, supporting your study's focus on exploring natural yeast diversity for targeted fermentation by Simonato, S., et al [17]. Analyzes how yeast strain variation impacts ethanol yield, residual sugars, and acid content in apple cider. This aligns with your work by reinforcing the connection between yeast genetics and the biochemical and sensory outcomes of cider fermentation by Okwum, J., et al [18]. Discusses valorization of apple juice by-products, including aroma recovery and functional use of pomace. While tangential to fermentation, it supports your study's broader theme of value addition to apple-based products by Coelho, E., et al [19]. Explores a novel pre-fermentation treatment to improve juice quality. This method may complement fermentation studies by enhancing substrate stability, reducing microbial load, or modifying juice chemistry before yeast inoculation by Vukusic Pavicic, T., et al [20]. Focuses on food innovation using apple juice and natural ingredients. Though unrelated to fermentation, it reflects the growing interest in functional, value-added apple-based

products, supporting the practical relevance of your cider research by Castagnini, J., et al [21]. This study parallels yours by evaluating the impact of local yeast populations on cider quality. It reinforces the value of regional yeast biodiversity and natural selection for enhancing beverage flavor and identity by Pando Bedrinana, R., et al [22]. Assesses diversity among native cider yeast strains in Spain. The study uses genetic markers and phenotypic assays, supporting your approach of combining *ATF1* gene analysis with sensory evaluation to differentiate and select optimal fermentation strains by Pando Bendrinana, R., et al. [23].

3. YEAST ISOLATES AND CULTURE MAINTENANCE

From the 23 Saccharomyces cerevisiae strains previously identified, ten were selected for the present study based on differences in their brewing traits. These strains were obtained from the Department of Microbiology, Himachal Pradesh Agricultural University (HPAU), Palampur, India. The selected strains were maintained on potato dextrose agar (PDA) slants at 4°C for short-term storage and preserved in 50% (v/v) glycerol at –80°C for long-term storage.

Table 1. Saccharomyces cerevisiae strains used in the present investigation along with their source, place of collection, and GenBank accession numbers of ATF1 gene.

S. No.	Strain code	Source	Place of collection	GenBank[a] accession number
1	Sc01	Chhang	Lahaul & Spiti	KF429732
2	Sc03	Dhaeli	Lahaul & Spiti	KF429733
3	Sc04	Aara	Lahaul & Spiti	KF429730
4	Sc05	Chiang	Lahaul & Spiti	KF429734
5	Sc 11	Chuli	Sangla	KF429737

S. No.	Strain code	Source	Place of collection	GenBank[a] accession number
6	Sc 12	Apple wine	Sangla	KF429739
7	Sc 15	Beverage	Bharmour	KF429736
8	Sc 19	Wine	Sangla	KF429735
9	Sc 21	Wine	Sangla	KF429738
10	Sc 24	Fermented product	Palampur	KF429731

4. ATF1 GENE STUDIES

Genomic DNA was extracted from the yeast strains using a Yeast DNA Isolation Kit (Biobasic Inc., Canada). The concentration and purity of the DNA samples were measured using a Nanodrop spectrophotometer, and quality was confirmed by electrophoresis on a 0.8% agarose gel.

For sequencing the ATF1 gene, a 2,088 bp region was targeted, which included 293 bp of the upstream promoter region and TATA box, 1,578 bp of the open reading frame (ORF), and 217 bp of the 3′ untranslated region (UTR). This region was amplified in three overlapping fragments using the following primer pairs:

- **Fragment 1:** ATF1FL (5′-TGCACTCGATGGTCTTCTCA-3′) and ATF1FR (5′-GACAAATTAGCCGCCAACTC-3′)
- **Fragment 2:** ATF1SL (5′-TGCAATGTTCTGCACGTTATT-3′) and ATF1SR (5′-TAGTTGTGAGCGGCAATCTG-3′)
- **Fragment 3:** ATF1TL (5′-GAACTTCGAATGGCTTACGG-3′) and ATF1TR (5′-TGCAATGTTCTGCACGTTATT-3′)

Polymerase chain reaction (PCR) amplification was carried out using a BOECO thermal cycler (Germany) under the following conditions: initial denaturation at 95℃ for 2 minutes; 30 cycles of denaturation at 94℃ for 30 seconds, annealing at 51℃ for 30 seconds, and extension at 72℃ for 90 seconds; followed by a final extension at 72℃ for 10 minutes. The PCR products were visualized on a 1.2% agarose gel.

PCR products were purified and freeze-dried using a CHRIST ALPHA I-2LD freeze dryer, and sent for bidirectional sequencing using an ABI 3730xl DNA Analyzer (Xcelris Labs Ltd., Ahmedabad, India). The resulting sequences were assembled and aligned to reconstruct the full-length gene sequence. Sequence similarity and homology searches were conducted using the NCBI BLASTN tool (http://www.ncbi.nlm.nih.gov/blast), and phylogenetic analyses were performed using MEGA version 5.1.

5. ORGANOLEPTIC STUDIES

The Royal Delicious variety of apple was selected for juice fermentation trials. Fresh, healthy apples were cleaned with hot water and treated with 0.1% (w/v) potassium metabisulfite to inhibit microbial contamination. Juice was extracted under hygienic conditions and analyzed for various physicochemical parameters including total soluble solids (TSS), pH, titratable acidity, Brix-acid ratio, total sugars, reducing sugars, and ascorbic acid content.

Six selected S. cerevisiae strains (Sc01, Sc02, Sc05, Sc12, Sc21, and Sc24) were used as starter cultures. Each strain was initially grown in pasteurized apple juice supplemented with 2% (v/v) seed inoculum and incubated at 28°C for 24 hours under shaking conditions. Fermentation was carried out by inoculating pasteurized juice with 1% (v/v) of the pre-cultured yeast, along with 300 mg/L of diammonium hydrogen phosphate (DAHP) as a nitrogen supplement. Fermentation was conducted at ambient temperature.

Samples were taken periodically and centrifuged at 6,000 rpm for 5 minutes. The supernatant was analyzed for TSS, pH, and ethanol concentration until no further decrease in °Brix was observed, indicating the completion of fermentation. Final cider products were evaluated for pH, TSS,

titratable acidity, Brix-acid ratio, ethanol content, ascorbic acid, reducing sugars, and total sugars to assess their sensory and biochemical profiles.

6. CONCLUSION

The analysis of the ATF1 gene among ten indigenous Saccharomyces cerevisiae strains revealed substantial genetic diversity, indicating significant differences in their capacity to synthesize esters that contribute to the aroma and flavor of fermented products. These findings underscore the importance of ATF1 as a functional marker for differentiating strains based on their flavor-forming potential. While ATF1 plays a crucial role in ester production, future studies should also investigate other genes involved in aroma biosynthesis to gain a more comprehensive understanding of flavor development in yeast.

Among the strains studied, Sc01 exhibited the greatest genetic divergence in its ATF1 sequence and demonstrated the most desirable organoleptic properties. Sensory evaluations identified Sc21 and Sc01 as the most promising strains for producing soft and hard apple ciders, respectively. These results highlight the potential of utilizing native S. cerevisiae strains from the Western Himalayas to improve the sensory quality and commercial appeal of apple-based fermented beverages.

7. REFERENCES:

[1] D. Borthakur, R. Chowdhury and B. K. Sharma, "Characterization and Statistical Analysis of Beer Produced by Bacterial Strains Isolated from Traditional Fermented Foods of Northeast India," 2025 IEEE 14th International Conference on Communication Systems and Network Technologies (CSNT), Bhopal, India, 2025, pp. 674-679, doi: 10.1109/CSNT64827.2025.10967867.

[2] S. S. Kanwar and Keshani Bhushan, "Fermentation of Apple Juice with a Selected Yeast Strain Isolated from the Fermented Foods of Himalayan Regions and Its Organoleptic Properties," Frontiers in Microbiology, vol. 7, p. 1012, Jun. 2016, doi: 10.3389/fmicb.2016.01012.

[3] K. Kim, S. B. Lee, J. Y. Jeon, and H. D. Park, "Development of Air-Blast Dried Non-Saccharomyces Yeast Starter for Improving Quality of Korean Persimmon Wine and Apple Cider," International Journal of Food Microbiology, vol. 290, pp. 193–204, Feb. 2019, doi: 10.1016/j.ijfoodmicro.2018.10.002.

[4] A. Verstrepen et al., "Expression Levels of the Yeast Alcohol Acetyltransferase Genes ATF1, Lg-ATF1, and ATF2 Control the Formation of a Broad Range of Volatile Esters," Applied and Environmental Microbiology, vol. 69, no. 9, pp. 5228–5237, Sep. 2003, doi: 10.1128/AEM.69.9.5227-5237.

[5] T. Fujii, H. Yoshimoto, and Y. Tamai, "Acetate Ester Production by Saccharomyces cerevisiae Lacking the ATF1 Gene Encoding the Alcohol Acetyltransferase," Journal of Fermentation and Bioengineering, vol. 81, no. 6, pp. 538–542, Jun. 1996, doi: 10.1016/0922-338X(96)81476-0.

[6] S. Llorca et al., "Cider Production from Apple Variety Eva and Four Types of Alcoholic Yeast," Energia

na Agricultura, vol. xx, pp. xx–xx, 2017.

[7] J. N. Keshani et al., "Molecular and Functional Diversity of Saccharomyces cerevisiae Strains of Traditional Fermented Foods of the North-Western Himalayas," Annals of Microbiology, vol. 65, pp. 2265–2275, 2015, doi: 10.1007/s13213-015-1068-3.

[8] A. Capece et al., "Diversity of Saccharomyces cerevisiae Yeasts Associated to Spontaneously Fermenting Grapes from an Italian 'Heroic Vine-Growing Area'," Food Microbiology, vol. 31, pp. 159–166, Apr. 2012.

[9] S. S. Kanwar et al., "Traditional Fermented Foods of Lahaul and Spiti Area of Himachal Pradesh," Indian Journal of Traditional Knowledge, vol. 6, pp. 42–45, 2007.

[10] A. Amerine, R. Pangborn, and E. Roessler, "Principles of Sensory Evaluation of Food," in Food Science and Technology Monographs, vol. 1, Academic Press, 1965, pp. 338–339.

[11] M. Dubois et al., "Colorimetric Method for Determination of Sugars and Related Substances," Analytical Chemistry, vol. 28, pp. 350–356, 1956. doi: 10.1021/ac60111a017.

[12] J. Hammond, "Brewer's Yeast," in The Yeasts, vol. 5, Academic Press, 1993, pp. 7–67.

[13] J. Joshi et al., "Effect of Different Sources of Fermentation on Flavour Profile of Apple Wine by Descriptive Analysis Technique," Acta Alimentaria, vol. 31, pp. 211–225, 2002. doi: 10.1556/AAlim.31.2002.3.2.

[14] T. Llaurado et al., "Low Temperature Alcoholic Fermentations in High Sugar Concentration Grape Musts," Journal of Food Science, vol. 67, pp. 268–273, 2002. doi: 10.1111/j.1365-2621.2002.tb11396. x.

[15] D. Kim et al., "Polygenic Analysis in Absence of Major

Effector ATF1 Unveils Novel Components in Yeast Flavor Ester Biosynthesis," PLOS Genetics, vol. 11, no. 4, e1005090, Apr. 2015, doi: 10.1371/journal.pgen.1005090.

[16] M. A. Verona-Ferreira et al., "Development of Air-Blast Dried Non-Saccharomyces Yeast Starter for Improving Quality of Korean Persimmon Wine and Apple Cider," International Journal of Food Microbiology, 2019.

[17] S. Simonato et al., "Yeast Species Diversity in Apple Juice for Cider Production Evidenced by Culture-Based Method," Folia Microbiologica, vol. 63, pp. 587–598, 2018.

[18] J. Okwum et al., "Effect of Yeast Strains on the Chemical Composition of Apple Ciders," University of Turku Thesis, 2024.

[19] E. Coelho et al., "Concentrate Apple Juice Industry: Aroma and Pomace Valuation as Food Ingredients," Food Chemistry, vol. 338, 2021.

[20] T. Vukušić Pavičić et al., "Impact of Heat-Assisted HVED Plasma Treatment on Quality of Apple Juice," LWT, vol. 139, p. 110546, Jan. 2021.

[21] J. Castagnini et al., "Sustainable Development of Apple Snack Formulated with Blueberry Juice and Trehalose," European Food Research and Technology, vol. 247, pp. 1079–1090, Aug. 2021.

[22] W. P. Morrissey et al., "The Role of Indigenous Yeasts in Traditional Irish Cider Fermentations," Journal of Applied Microbiology, vol. 97, pp. 107–116, Sep. 2004.

[23] R. Pando Bedriñana, A. Querol, and B. Suárez Valles, "Genetic and Phenotypic Diversity of Autochthonous Cider Yeasts in a Cellar from Asturias," Food Microbiology, vol. 27, pp. 472–483, Jun. 2010.

[24] M. Buron et al., "Screening of Cider Yeasts for Volatile Phenol-Production Ability," Food

Microbiology, vol. 28, pp. 678–687, Oct. 2011.

[25] A. Domizio et al., "Lachancea thermotolerans and Saccharomyces cerevisiae in Simultaneous and Sequential Co-Fermentation: A Strategy to Enhance Acidity and Improve the Overall Quality of Wine," Food Microbiology, vol. 36, pp. 300–307, Apr. 2013.

[26] H. Yoshimoto et al., "Characterization of the ATF1 and Lg-ATF1 Genes Encoding Alcohol Acetyltransferases in the Bottom Fermenting Yeast Saccharomyces pastorianus," Journal of Fermentation and Bioengineering, vol. 85, no. 1, pp. 15–20, 1998, doi: 10.1016/S0922-338X(98)80027-5