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ATF1 GENE STUDIES FOR AROMA AND FLAVOUR OF BREWING YEASTS OF NORTH WESTERN HIMALAYAS

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ABSTRACT

Twenty-four yeast strains isolated from alcoholic beverages in the North-Western Himalayas were systematically screened for their brewing characteristics. Among these, four strains—designated ABB, GP4, CH1, and PH1—demonstrated remarkable alcohol production, exceeding 11% v/v, with strains ABB, GP4, SC02, and SM2 exhibiting the highest alcohol tolerance above 14% v/v. Additionally, significant attenuation rates greater than 90% w/v were recorded for ABB, CH1, GP4, and PH1. DNA barcoding confirmed the identification of all yeast strains as *Saccharomyces cerevisiae*, except for one strain, classified as *Pichia membranifaciens* (SMLC). Based on the observed variations in brewing traits, fifteen strains were selected for further investigation of the *ATF1* gene. Sequencing revealed a coding sequence of 1578 base pairs in the open reading frame (ORF), encoding a protein of 525 amino acids. Multiple sequence alignment against the Saccharomyces genomic database (SGD) identified 108 nucleotide substitutions within the coding region. Notably, twelve strains exhibited variations in their amino acid sequences, with strain ABB showing the greatest variation of four amino acids, while a minimum of one amino acid substitution—specifically asparagine (N) to lysine (K)—was noted in seven strains. These significant differences in the *ATF1* gene correlate with variations in the aroma and flavour profiles of the fermented products, suggesting that *ATF1* expression plays a critical role in shaping the sensory characteristics of brewing products. This study highlights the potential of these indigenous yeast strains to enhance the quality and distinctiveness of fermented beverages.

Keywords: Allele mining, *ATF1* gene, Brewing, North Western Himalayas, *Saccharomyces cerevisiae*

The Western Himalayan region serves as a rich repository of microbial genetic diversity. Its ecosystem, characterized by harsh conditions, differs significantly from more temperate agricultural environments. Speciation in these challenging habitats is largely influenced by climatic and ecological changes (Kanwar and Bhushan, 2020). Fermented foods are particularly prevalent among rural populations, especially among hill and tribal communities. Numerous studies have documented a wide array of traditional fermented foods and beverages across various countries (Anal, 2019; Keshani *et al*., 2015). The production of these indigenous fermented foods relies on specific microflora, involving a complex interplay of bacteria, yeast, and fungi. The quality of the final product is determined not only by the initial starter culture but also by the dynamic presence of microbial flora throughout the fermentation process (Kanwar and Keshani, 2014).

Yeasts are the primary organisms involved in fermentation processes, and understanding the natural diversity of yeast strains in a given area—or those specifically associated with particular products—is crucial for identifying the most effective strains (Anju and Keshani, 2023). Among various yeast isolates, *Saccharomyces cerevisiae* is the dominant genus, playing a pivotal role in the fermentation of numerous traditional foods and beverages. The flavours of fermented beverages such as beer, cider, sake, and wine are significantly influenced by the raw materials and primary fermentation yeasts employed in their production (Anju *et al*., 2023). The flavour compounds that contribute to the yeast bouquet—such as ethyl esters, acetate esters, fusel alcohols, carbonyls, and volatile fatty acids—are secondary metabolites produced by a diverse range of microbial species. Notably, the synthesis of many aroma-active compounds is highly dependent on the specific yeast strain utilized during fermentation (Kaur *et al*., 2021).

The genome of each yeast strain is unique and plays a crucial role in defining the final aroma profile of the product. Although esters represent only trace elements among the myriad of metabolites in beverages, they are the most significant aromatic compounds produced by yeast (Pires *et al*., 2014). The primary enzymes responsible for ester synthesis are alcohol acetyltransferases (AATases; EC 2.3.1.84). These enzymes are encoded by the *ATF1* gene, its homologue *Lg-ATF1*, and the *ATF2 gene*. Several studies have demonstrated that the overexpression of *ATF1* in commercial brewing strains results in a significant increase in the concentrations of isoamyl

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acetate and ethyl acetate in the final product. These findings suggest that the expression level of *ATF1* is a critical limiting factor for ester synthesis (He *et al.*, 2014; Parapouli *et al.*, 2019). Consequently, this gene was selected for investigating functional diversity from an aromatic and flavor perspective at the molecular level, aiming to understand and characterize the range of flavour phenotypes among brewing strains from the North-Western Himalayas.

MATERIALS AND METHODS

Yeast isolation and maintenance of cultures

Samples were collected from the Lahaul and Spiti, and Bharmour regions of the North-Western Himalayas and processed using serial dilution on YEPDA plates. A total of eight yeast isolates were successfully isolated, purified, and preserved. Additionally, sixteen *Saccharomyces cerevisiae* strains previously isolated and available in the laboratory were included in this study. Table 1 provides an overview of the microbial cultures, including their strain codes, regions of origin, and sources of isolation.

Morphological, biochemical and molecular studies on yeast isolates

Yeasts were analyzed in terms of their morphological characteristics, including colony features, cell shape, and growth behaviors in broth cultures. Biochemical assessments were conducted to evaluate growth at various temperatures, fermentation patterns of different sugars, and growth in the presence of 10% ethanol (Barnett *et al.*, 1983). Molecular identification of the strains was performed through ITS region sequencing, utilizing a commercial sequencing facility (Xcelris Labs Ltd., Ahmedabad, India). Universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) were employed to amplify the ITS region (ITS1-5.8S-ITS2). The editing and analysis of the ITS sequences for the yeast isolates were conducted using the NCBI BLASTN program (http://www.ncbi.nih.gov/blast; Altschul *et al.*, 1997).

Brewing Traits

Yeast isolates were evaluated for their brewing potential using sugarcane juice, a cost-effective substrate that serves as a carbon source for ethanol production.

Alcohol production by fermentation of the sugarcane juice

The initial degree Brix (°B) of the sugarcane juice was measured using a Brix hydrometer. The juice was pasteurized at 62.5°C for 30 minutes before being inoculated with 7% (v/v) of starter cultures. Fermentation of the sugarcane juice, conducted at a scale of 300 mL, was carried out at 28°C and 200 rpm in a BOD incubator. Observations continued until stable °Brix values were achieved. Periodic samples (every 24 hours) were analyzed for ethanol concentration (% v/v), total soluble solids (TSS, °B), total sugars (g/100 mL), and reducing sugars (g/100 mL).

Alcohol tolerance

The method described by Keo (1967) was employed to assess the alcohol tolerance of the yeast isolates. A standard sugarcane juice concentration of 20 °B was prepared and distributed into ten flasks to determine the maximum alcohol content tolerated by the yeasts. Various doses of alcohol were added to each flask to achieve initial concentrations of 0% in flask 1, 2% in flask 2, and so on. Each flask was then inoculated with 7% (v/v) yeast cultures prepared in GYE media. The flasks were incubated at 28 °C in a BOD incubator until stable °Brix values were reached. The method of Caputi *et al*. (1968) was utilized to estimate the percentage of alcohol produced.

Fermentation efficiency and attenuation

Fermentation efficiency and Attenuation was done by using the following formulae. Attenuation is measured by specific gravity, it can be defined as the percentage of conversion of sugars to alcohol and carbon dioxide.

Attenuation= [(OG-FG) / (OG-1)] X 100

where, FG is final gravity (specific gravity at the end of the fermentation), OG is original gravity (specific gravity before pitching).

Fermentation efficiency is defined as the actual amount of alcohol produced, in relation to the theoretical production.

Fermentation efficiency (%) of the process was determined by the following formula:

$$\text{Fermentation efficiency (\%)} = \frac{\text{Actual ethanol recovery (\%v/v)}}{\text{Theoretical ethanol recovery (\%v/v)}} \times 100$$

Allele mining of *ATF1* genes

Fifteen out of twenty-four yeast strains were selected for allele mining of the *ATF1* gene based on their variation in brewing traits, specifically those exhibiting significant differences in alcohol production, tolerance, and sensory profiles. Literature indicates that the gene comprises a 1578 bp protein-coding region; when including the preceding promoter and TATA box, the total length of the sequence is 1988 bp. The gene was custom sequenced using an ABI 3730xl automated sequencer, employing both forward and

reverse primers (Xcelris Labs Ltd., Ahmedabad, India). Homology searches were conducted using the online NCBI BLASTN program (http://www.ncbi.nih.gov/blast; Altschul et al., 1997). Multiple sequence alignment was performed to compare the gene sequences of the fifteen indigenous yeast strains with reference sequences of *Saccharomyces cerevisiae*. The corresponding amino acid sequences were deduced using the ExPASy tool and subsequently analyzed with MEGA X software. All phylogenetic analyses were conducted within the MEGA X program. The *ATF1* gene sequences were submitted to GenBank at the National Center for Biotechnology Information (NCBI), USA.

Sensory analysis

Consumer approval of the product was assessed using a nine-point Hedonic scale, as described by Amerine *et al.* (1965). Five panelists, all regular consumers or occasional drinkers aged between 35 and 60 years, were selected for the sensory evaluation. The panelists evaluated the sensory profile of the fermented beverage based on its appearance, colour, flavour, mouthfeel, and overall acceptability. Each panelist completed a 9-point Hedonic scale form, where ratings were defined as follows: 1 = Disliked extremely, 2 = Disliked very much, 3 = Disliked moderately, 4 = Disliked slightly, 5 = Neither liked nor disliked, 6 = Liked slightly, 7 = Liked moderately, 8 = Liked very much, and 9 = Liked extremely.

Statistical Analysis

The IBM Check Processing Control System (CPCS1) and Microsoft Excel were utilized for the statistical analysis of the experimental data. Fisher's analysis of variance (ANOVA) technique, as described by Panse and Sukhatme (1961), was employed to assess differences in the brewing traits among the various yeast strains. Results are presented as the mean values from three independent experiments.

RESULTS AND DISCUSSION

Identification of yeasts isolated from alcoholic beverages

Yeast isolates were identified based on their morphological, biochemical, and molecular characteristics. Eight morphologically distinct yeast isolates, coded GP2, GP4, ABB, SB, SM2, SMSC,

Table 1. Strain code of yeasts used and their source of isolation

Sr. no.	Strain code	Strain	Sources	Region
1.	GP2	Streptomyces cerevisiae	Gundaji	Bharmour
2.	GP4	S. cerevisiae	Gundaji	Bharmour
3.	GP6	S. cerevisiae	Gundaji	Bharmour
4.	SB	S. cerevisiae	Sura	Bharmour
5.	SMSC	S. cerevisiae	Sura	Bharmour
6.	SMLC	*Pichia membranifaciens*	Sura	Bharmour
7.	SM2	S. cerevisiae	Sura	Bharmour
8.	ABB	*S. cerevisiae*	Alcoholic beverage	Bada Bhangal
9.	CH3*	S. cerevisiae	Chatki	Lahaul & Spiti
10.	CH2*	S. cerevisiae	Chatki	Lahaul & Spiti
11.	MK1*	*S. cerevisiae*	Milk	Lahaul & Spiti
12.	MK2 *	*S. cerevisiae*	Milk	Lahaul & Spiti
13.	PH1*	S. cerevisiae	Phab	Lahaul & Spiti
14.	PH2*	S. cerevisiae	Phab	Lahaul & Spiti
15.	MK6 *	*S. cerevisiae*	Milk	Lahaul & Spiti
16.	CH1*	S. cerevisiae	Churpe	Lahaul & Spiti
17.	B1*	S. cerevisiae	Boti	Lahaul & Spiti
18.	C1 *	*S. cerevisiae*	Curd	Lahaul & Spiti
19.	Sc02*	S. cerevisiae	Lugari	Lahaul & Spiti
20.	Sc06*	S. cerevisiae	Chilra	Lahaul & Spiti
21.	Sc07*	S. cerevisiae	Bhaturu	Lahaul & Spiti
22.	Sc08*	S. cerevisiae	Babru	Lahaul & Spiti
23.	Sc09*	S. cerevisiae	Khameer	Lahaul & Spiti
24.	Sc10*	S. cerevisiae	Faasur	Sangla

*Available from Department of Microbiology, PAU, Ludhiana

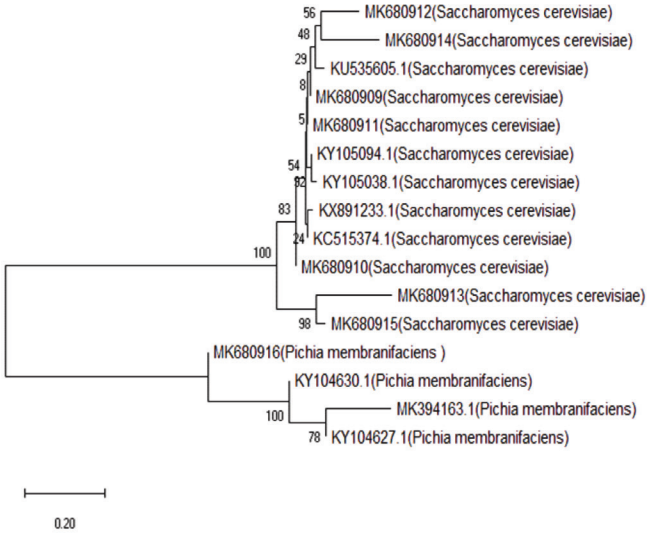

Fig. 1. Phylogenetic tree showing the clustering behavior of different yeast isolates based on ITS region sequencing

SMLC, and GP6, were obtained from alcoholic beverages (*Gudanji* and *Sura*) collected from the North-Western Himalayan region. Microscopic examination revealed that the yeast colonies predominantly exhibited a spherical coccus shape, with the exception of GP4, which displayed a spherical to oval coccus shape. For fermentation tests, various sugars—including dextrose, fructose, xylose, lactose, maltose, and mannitol—were utilized. Most yeast strains produced both acid and gas when fermenting these sugars. All isolates demonstrated the ability to grow at temperatures ranging from 15ºC to 37ºC, except for GP2, GP4, B1, and MK6, which did not grow at 15ºC. Moderate growth in the presence of 10% ethanol was observed across all yeast isolates. A study by Qvirist *et al.* (2016) focused on the isolation, identification, and characterization of yeasts from fermented goat milk, and reported that all strains studied showed growth in 6% ethanol.

ITS region sequencing is a widely accepted method for molecular systematics, applicable at both inter- and intra-species levels (Peay *et al*., 2008). In this study, it was employed for the identification of yeast isolates. All seven yeast isolates demonstrated over 98% similarity to *Saccharomyces cerevisiae*, except for SMLC, which exhibited more than 96% similarity to *Pichia membranifaciens* (Fig. 1). Thakur *et al*. (2015) isolated yeast strains, including *Saccharomyces cerevisiae*, *Saccharomyces fibuligera*, *Pichia kudriavzevii*, and *Candida tropicalis*, from traditional fermented alcoholic beverages such as chaang, jauchaang, and sura in the North-Western Himalayas of India. This study concluded that *S. cerevisiae* and *S. fibuligera* are the principal yeasts associated with these alcoholic beverages, a trend that aligns with our findings highlighting the prevalence of *S. cerevisiae*.

Alcohol production and tolerance

A high degree of alcohol production, coupled with alcohol tolerance in yeast strains, is of significant relevance to the brewing industry. Consequently, it is essential to evaluate high alcohol producers for their alcohol tolerance capabilities as well. In this study, the maximum alcohol production was observed with strains GP4 (11.32% v/v) and ABB (11.2% v/v), while strain GP6 exhibited nearly negligible production at 9.05% v/v. The highest alcohol content tolerated by the yeasts without compromising their production ability was found to be 14.96% (v/v) for the ABB strain, whereas the lowest tolerance was recorded for B8 at 10.47% (v/v) (Table 2). Notably, the highest alcohol producer, ABB, also displayed the highest alcohol tolerance. These results indicate a correlation between alcohol production ability and alcohol tolerance in yeast strains. Most previous studies on alcohol tolerance have primarily focused on yeast survival (Mehdikhani *et al*., 2011) rather than examining alcohol production in the presence of high ethanol concentrations, as recommended by Keshani *et al*. (2015).

Attenuation and fermentation efficiency

Attenuation is a crucial parameter for assessing the fermentation efficiency of brewing strains. Among the 24 yeast strains studied, percent attenuation varied from 79.71% to 92.4% w/v, with strains GP4 and ABB exhibiting attenuation rates exceeding 90%, identifying them as highly effective attenuators. The fermentation efficiency ranged from 76.33% v/v for GP6 to 94.50% v/v for GP4, demonstrating significant differences in brewing potential among the strains (Table 2).

Allele variation in the *ATF1* gene

The *ATF1* gene is the primary gene responsible for ester synthesis in *S. cerevisiae*, and it is present in both ale and lager yeast strains (Keshani *et al*., 2015). Allele mining of the *ATF1* gene was conducted to determine whether differences in ester synthesis traits are linked to variations in the gene's sequence. Fifteen yeast strains were selected for this analysis. Our study revealed a gene fragment of 1578 bp encoding 525 amino acids. In contrast, Kanwar and Keshani (2016) reported that the *ATF1* gene in native *S. cerevisiae* strains consisted of 1566 bp in the open reading frame (ORF) and encoded 522 amino acids. However, our results are consistent with previous findings, indicating a 1578 bp ORF that encodes 525 amino acids in *S. cerevisiae* strains (Fuji *et al*., 1994; Verstrepen *et al*., 2003). The protein-coding regions of the *ATF1* gene displayed considerable variation, with 108 nucleotide

Table 2. Brewing potential of various yeast isolates in terms of fermentation efficiency, attenuation and alcohol tolerance

Sr. no.	Strain code	Fermentation efficiency (% v/v)	Attenuation (% w/v)	Alcohol tolerance (% v/v)	Alcohol production (% v/v)
1	GP4	94.50^{a}	92.40^{a}	14.26^{b}	11.32^{a}
2	GP2	86.47^{gh}	83.47^{i}	13.27^{cde}	10.30^{fgh}
3	GP6	76.33^{n}	79.71^{m}	13.06^{cdefg}	9.05^{o}
4	SB	81.50^{k}	81.04^{k}	13.37^{cd}	9.50^{mn}
5	SMLC	82.20^{jk}	82.19^{j}	12.90^{defg}	9.73^{klm}
6	SMSC	88.53^{e}	85.99^{e}	13.43^{c}	10.55^{ef}
7	SM2	87.30^{fg}	84.80^{f}	14.16^{b}	10.40^{fg}
8	ABB	93.96^{ab}	91.10^{b}	14.96^{a}	11.20^{ab}
9	CH3	78.73^{m}	80.30^{l}	12.23^{ij}	9.30^{no}
10	CH2	78.20^{m}	80.33^{l}	12.37^{hij}	9.23^{no}
11	MK1	91.43^{cd}	89.77^{c}	13.17^{cdef}	10.94^{bcd}
12	MK2	90.63^{d}	88.57^{d}	11.90^{jk}	10.82^{cde}
13	PH1	92.00^{c}	91.10^{b}	12.87^{efg}	11.04^{abcd}
14	PH2	88.93^{e}	89.77^{c}	11.67^{k}	10.7^{6de}
15	MK6	81.50^{k}	82.22^{j}	11.17^{l}	9.70^{jklm}
16	CH1	93.13^{b}	91.10^{b}	11.97^{jk}	11.10^{abc}
17	B1	88.20^{ef}	86.06^{e}	10.47^{m}	10.50^{ef}
18	C1	83.07^{ij}	83.47^{i}	12.77^{fgh}	9.90^{ijkl}
19	Sc02	83.10^{ij}	82.22^{j}	14.17^{b}	10.00^{hijk}
20	Sc06	79.80^{l}	81.04^{k}	12.28^{ij}	9.60^{lmn}
21	Sc07	83.83^{i}	83.43^{i}	11.65^{k}	10.10^{ghij}
22	Sc08	85.43^{h}	83.47^{i}	12.60^{ghi}	10.20^{fghi}
23	Sc09	78.06^{m}	80.26^{l}	12.17^{ij}	9.30^{no}
24	Sc10	81.50^{k}	83.47^{i}	12.11^{ijk}	9.80^{jklm}
CD ($p \leq 0.05$)		0.72	0.29	0.26	0.22

Results are presented as mean of three replications.
Within a column means followed by different letters differ significantly at *p≤0.05*.

substitutions identified at different locations through multiple sequence alignments, without any deletions or insertions in the sequence. This variation suggests that *ATF1* sequences may be less conserved among these indigenous isolates. No differences were observed in the promoter region of the gene. Amino acid sequences were deduced from the coding region of the *ATF1* gene using the ExPASy tool. Out of the fifteen strains, twelve exhibited variations in amino acids. Analysis of the amino acid sequences of the *ATF1* genes revealed differences of approximately 14 amino acids among the strains, indicating significant variation in the aroma and flavour profiles of the fermented products. Notably, most nucleotide substitutions resulted in codon degeneracy. The ABB strain showed four amino acid variations, while GP6 and PH1 exhibited three variations. Strains SC06, SC09, SC10, and CH2 displayed variations of two amino acids compared to the reference strain (Table 3). Additionally, seven strains—SC02, SC06, SC10, CHK4, GP6, GP2, and SC09—showed a variation of one amino acid at the same site, with asparagine (N) replaced by lysine (K) (Table 3). Three strains, SMSC, SM2, and SC07, exhibited no variation at the protein level.

Based on nucleotide sequence analysis, the phylogenetic tree (Fig. 2) revealed a mixed clustering pattern among native and exotic yeast strains, with most differences in nucleotide sequences attributed to silent mutations. The *ATF1* sequences of yeast strains MK689377 (ABB), MK689389 (GP6), and MK689379 (PH1) displayed significant dissimilarity compared to the other strains in the study. Among these, the ABB

Table 3. Variation in amino acids among different strains based on amino acid substitution

Yeast	Original	Replaced	Site no. (nucleotide)	Amino acid original	Replaced	Site no. (amino acid)
GP6	CGG	GGG	79	R	G	27
Sc09	ATG	AGG	86	M	R	29
ABB	GTT	GGT	95	V	G	32
ABB	TAT	GAT	106	Y	D	36
ABB	TGT	TGG	174	C	W	58
Sc10, CH2	ATA	ACA	251	T	I	84
ABB	CAA	CCA	338	Q	P	113
GP4	GAA	GCA	341	E	A	114
GP6	CTG	ATG	349	L	M	117
P7, Sc08	ATC	GTC	556	I	V	186
P7	TAC	TGC	1094	Y	C	365
Sc02,Sc06,Sc10,CH2, GP6,GP2,Sc09	AAT	AAA	1173	N	K	391
Sc06	AAT	AAG	1470	N	K	490
P7,SB,Sc08	TCC	TTC	1547	S	F	516

(MK689377) strain exhibited the highest degree of dissimilarity in its amino acid sequence as well. These findings suggest that the ABB (MK689377) strain may have a distinct variation in ester formation compared to the other strains analyzed. The *ATF1* gene sequences of these yeast strains have been submitted to GenBank at the National Center for Biotechnology Information (NCBI), USA. In a related study involving GC-MS analysis of these native yeast strains to compare their ester synthesis profiles, the ABB (MK689377) strain demonstrated an ester profile of 36.55 mg/L for ethyl acetate and 940 mg/L for isoamyl acetate (Kaur *et al*., 2021).

Sensory analysis

Sensory evaluation involves utilizing human senses—specifically smell and taste—to analyze products intended for commercialization or laboratory-scale studies. Sensory characteristics such as colour, aroma, and mouthfeel are closely linked to product quality. Aroma, in particular, is a major factor influencing product quality and plays a crucial role in differentiating various products (Yang *et al*., 2020). In this study, five wine samples, selected based on heterogeneity in the *ATF1* gene of the chosen yeast strains, were subjected to sensory analysis to assess their acceptability. A panel of five judges evaluated these samples using a 9-point Hedonic scale. The results indicated that the product prepared using the ABB yeast strain was the most acceptable compared to the others, making it the best among all strains analyzed. These findings align with the *ATF1* gene analysis, which revealed the maximum differences in amino acid sequences in the ABB yeast strain.

This study provided valuable insights into the role of the *ATF1* gene in ester synthesis; however, a more comprehensive investigation into the ATF gene cluster is necessary to fully understand the contributions of these genes to the aroma and flavour of fermented products

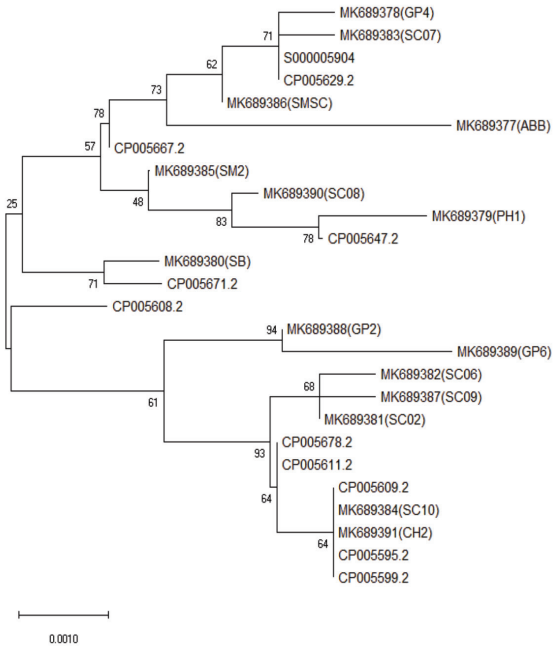

Fig. 2. Phylogenetic tree depicting variation in *ATF1* gene sequences, with a scale of 0.001 substitutions per nucleotide position

produced by yeast strains during fermentation. Among all the strains studied, the ABB (MK689377) strain exhibited the highest fermentation potential and flavour profile, indicating its perspective use in the industrial sector.

The results of this study contribute significantly to our understanding of the genetic and functional diversity of yeast strains used in fermentation, particularly focusing on the role of the *ATF1* gene in ester synthesis. The variation in the *ATF1* gene sequences among the analyzed yeast strains suggests a substantial genetic diversity that could impact the production of aromatic compounds, essential for the quality of fermented beverages. Our findings indicate that the ABB (MK689377) strain not only exhibited the highest alcohol production and tolerance but also demonstrated a distinct amino acid profile in the *ATF1* gene compared to other strains. This correlation implies that the genetic makeup of ABB may be closely linked to its fermentation efficiency and aromatic profile. The substantial number of nucleotide substitutions observed, particularly in the coding region, points to the potential for selective breeding or biotechnological applications aimed at enhancing ester production. The absence of variation in the promoter region across strains suggests a conserved regulatory mechanism, emphasizing the importance of the coding region in determining functional outcomes. The sensory evaluation results reinforce the molecular findings, revealing that the ABB strain was preferred for its flavour profile, which aligns with the predicted aromatic contributions from its unique *ATF1* gene composition. This dual approach—integrating molecular genetics with sensory analysis—highlights the potential of native yeast strains in developing high-quality fermented products that appeal to consumer preferences.

Moreover, the study highlights the necessity for further exploration of the ATF gene cluster. While the *ATF1* gene is a critical component in ester synthesis, understanding the interactions with other genes in the cluster could provide a more comprehensive picture of how these pathways influence flavour and aroma. Future studies should consider examining other key genes involved in fermentation, as well as conducting larger-scale fermentation trials to evaluate the industrial applicability of these findings. This study not only sheds light on the genetic basis for flavour variation in fermented products but also emphasizes the potential for utilizing native yeast strains, such as ABB, in the brewing industry. The insights gained here could inform strategies for optimizing fermentation processes, thereby enhancing the sensory attributes of alcoholic beverages and contributing to the preservation of microbial diversity in traditional fermentation practices.

CONCLUSION

This study highlights the significant role of the *ATF1* gene in ester synthesis among various yeast strains sourced from the North-Western Himalayas. Through comprehensive morphological, biochemical, and molecular characterization, we identified key yeast isolates, with the ABB (MK689377) strain demonstrating superior fermentation potential and flavour profile. The variations observed in the nucleotide and amino acid sequences of the *ATF1* gene suggest a relationship between genetic diversity and aromatic compound production, highlighting the importance of this gene in enhancing the quality of fermented products.

Sensory evaluations corroborated these findings, with the ABB strain receiving the highest acceptability scores among the evaluated wine samples, reflecting its potential for industrial applications. While this research provides valuable insights, further investigation into the broader ATF gene cluster is essential to elucidate the precise mechanisms by which these genes contribute to the aroma and flavour profiles of fermented products. Overall, the findings support the use of native yeast strains, particularly ABB, in brewing and fermentation industries, offering opportunities for the development of high-quality alcoholic beverages.

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Authors' contribution

Conceptualization and designing of the research work (MG,K, GSK); Execution of lab experiments and data collection (MG,K); Analysis of data and interpretation (MG,K); Preparation of manuscript (MG, K).

LITERATURE CITED

Amerine M A, Pangborn R M and Roessler E B 1965. Principles of sensory evaluation of food. Academic Press, London, pp. 72-77.

Anal A K 2019. Quality ingredients and safety concerns for traditional fermented foods and beverages from Asia: A review. *Fermentation* **5**: 8. doi:10.3390/fermentation5010008.

Anju and Keshani 2023. Role of conventional and non-conventional yeasts in fermentation and flavour profile of beer. **In**: *Recent trends in microbiology,* Shriyanshi Prakashan, Agra, India, pp. 29-38.

Anju, Bhushan K and Kocher G S 2023. Preliminary research on the analytical and volatile profiles of beers produced

with raw malt and roasted specialty malts. *JASBC* doi: 10.1080/03610470.2023.2213604

Altschul S F, Madden T L, Schäffer A A, Zhang J, Zhang Z, Miller W and Lipman D J 1997. Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res* **17**: 3389-402.

Barnett J A, Payne R W and Yarrow D 1983. **In**: *Yeast: Characterization and identification*. 2nd Edn, Cambridge University Press, Cambridge, pp. 642-44.

Caputi A, Ueda M and Brown T 1968. Spectrophotometric determination of ethanol in wine. *Am J Enol Viticul* **19**: 160-65.

Fujii T, Nagasawa N, Iwamatsu A, Bogaki T, Tamai Y and Hamachi M 1994. Molecular cloning, sequence analysis, and expression of the yeast alcohol acetyltransferase gene. *Appl Environ Microbiol* **60**: 2786-92.

He Y, Dong J, Yin H, Zhao Y, Chen R, Wan X, Chen P, Hou X, Liu J and Chen L 2014. Wort composition and its impact on the flavour-active higher alcohol and ester formation of beer – A review. *J Inst Brew* **120**: 157-63.

Kanwar S S and Keshani 2014. Bio-prospecting of indigenous yeast isolates of fermented foods of North-Western Himalayas. **In**: *Advances in industrial biotechnology,* IK International Publishing House Pvt. Ltd., India, pp. 180-95.

Kanwar S S and Keshani 2016. Fermentation of apple juice with a selected yeast strain isolated from the fermented foods of Himalayan regions and its organoleptic properties. *Frontiers Microbiol* **7**: 1-6.

Kanwar S S and Bhushan K 2020. Ethnic fermented foods and beverages of Himachal Pradesh **In**: *Ethnic fermented foods and beverages of India: Science, history and culture,* Springer Nature Singapore Pte Ltd, pp. 189-208.

Kaur H, Keshani and Kocher G S 2021. Flavour profiling of red wine with respect to different strains of yeast. *Indian J Hort* **78**: 325-29. doi:10.5958/0974-0112.2021.00047.5

Keo L J 1967. Process improvement for alcohol manufacture. (1965 - En: Congress of the International Society of Sugarcane Technologists, 12, San Juan, Puerto Rico). Proceedings, Amsterdam, Elsevier, pp. 1820-26.

Keshani, Sharma P N, Sharma K D and Kanwar S S 2015. Molecular and functional diversity of *Saccharomyces cerevisiae* strains of traditional fermented foods of the North-Western Himalayas. *Ann Microbiol* **65**: 2265-75.

Mehdikhani P, Bari M R and Hovsepyan H 2011. Screening of *Saccharomyces cerevisiae* for high tolerance of ethanol concentration and temperature. *Afr J Microbiol Res* **5**: 2654-60.

Panse V G and Sukhatme V G 1967. Statistical methods for agricultural workers 2nd Edn, Indian Council of Agricultural Research, New Delhi, 381p.

Parapouli M, Sfakianaki A and Monokrousos N 2019. Comparative transcriptional analysis of flavour-biosynthetic genes of a native *Saccharomyces cerevisiae* strain fermenting in its natural must environment, vs. a commercial strain and correlation of the genes' activities with the produced flavour compounds. *J Biol Res-Thessaloniki* **26**: 5. doi:10.1186/s40709-019-0096-8

Peay K G, Kennedy P G and Bruns T D 2008. Fungal community ecology: A hybrid beast with a molecular master. *BioScience* **58**: 799-810.

Pires E J, Teixeira J A, Branyik T and Vicente A A 2014. Yeast: The soul of beer's aroma - A review of flavor-active esters and higher alcohols produced by the brewing yeast. *Appl Microbiol Biotechnol* **98**: 1937-49.

Qvirist L A, De Filippo C, Strati F, Stefanini I, Sordo M, Andlid T, Felis G E, Mattarelli P and Cavalieri D 2016. Isolation, identification and characterization of yeasts from fermented goat milk of the Yaghnob Valley in Tajikistan. *Front Microbiol* **7**: 1690.

Thakur N, Savitri, Saris P E J and Bhalla T C 2015. Microorganisms associated with amylolytic starters and traditional fermented alcoholic beverages of North Western Himalayas in India. *Food Biosci* **11**: 92-96.

Verstrepen K J, Derdelinckx G, Dufour J P, Winderickx J, Thevelein J M, Pretorius I S and Delvaux F R 2003. Flavour-active esters: Adding fruitiness to beer. *J Biosci Bioeng* **96**: 110-18.

Yang J and Lee J 2020. Current research related to wine sensory perception since 2010. *Beverages* **6**: 47.