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Characterization and fermentation potentiality of optimal yeast species derived from *Madhuca indica* flowers

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Abstract
Madhuca indica flowers are widely acclaimed for possessing a substantial nutritional content, yet another of its unusual distinctive traits is the existence of wild yeasts on their surface. The investigation looks into the fermentation potentiality of yeast species isolated from *Madhuca indica* flowers for ethanol generation. Twenty yeast isolates were initially derived from the Madhuca flowers and assessed regarding their aptitude to yield ethanol. Four isolates manifested over 1% ethanol were chosen for comprehensive characterization and recognized as *Saccharomyces fibuligera*, *Saccharomyces boulardii*, *Pichia anomala*, *and Saccharomyces cerevisiae*. In-depth fermentation assessments with these specific yeast species uncovered that *Saccharomyces cerevisiae* can ingest around 18.06%w/v of reducing sugar over the 16-day fermentation process to yield maximum of 7.05% v/v of ethanol. Furthermore, it had been noted that during 18 days of fermentation, *Saccharomyces fibuligera* needed 2.98% w/v of reducing sugar to produce 1.29% v/v of ethanol. Utilizing 4.34% w/v of reducing sugar, *Saccharomyces boulardii* yielded 1.88% v/v of ethanol following 22 days of fermentation. *Pichia anomala* needed 8.34% w/v of reducing sugar in 20 days to generate 3.68% v/v of ethanol. This finding underscores the significant potential of *Saccharomyces cerevisiae* in optimizing ethanol production processes. The strain's robustness and high ethanol yield suggest its suitability for industrial-scale fermentation especially in Ayurvedic fermented formulations like asava and arista, presenting a sustainable and efficient alternative to traditional methods. The direct utilization of *Saccharomyces cerevisiae* in such formulations not only improves the fermentation process but also enhances the quality and consistency of the final product. This approach also reveals the untapped biotechnological potential of yeast strains associated with *Madhuca indica* flowers. This research paves the way for innovative and eco-friendly approaches in bioethanol production and traditional beverage fermentation, emphasizing the value of exploring local microbial diversity to address global sustainability challenges and advance biotechnological processes.

Keywords: *Madhuca indica*, fermentation, ethanol, *Saccharomyces cerevisiae*

Introduction

Usually discovered in distinct regions of India, the *Madhuca indica*, frequently recognized as the Mahua tree, is a member of the *Sapotaceae* family (Srinivasa Reddy, 2022) [22]. This tree's flowers are widely acknowledged for sustaining substantial amounts of sugar and an abundance of nutritional composition. Substantial levels of protein molecules, dietary fibers, amino acids, vitamins, and calcium also reside within them. Phytochemical vetting of Madhuca flowers points out the existence of substantial secondary plant metabolites encompassing tannins, anthocyanins, flavonoids, essential oils, alkaloids, saponins, and sterols (Lungade and Karadbhajne, 2022) [11]. The prevalence of wild yeasts on the surface of Madhuca flowers is one of their atypical distinctive features. By processing the sugars into ethanolic content and carbon dioxide, these yeasts contribute significantly to the fermentation procedure (Wagh, 2016) [24]. Despite Mahua trees being widespread, people rarely eat their flowers. Rather, among India's tribal populations, they occupy a major traditional and commercial role. Madhuca flowers are predominantly employed for producing distilled liquors, where the sugar in flowers aids in fermentation. Additionally, dried Mahua flowers have been revealed to be employed for preparing sugar syrup, utilizing their inherent sweetness (Patel *et al*., 2012) [20]. Mahua flowers are naturally abundant in sugar (40–70%), which is a suitable base for the growth of natural yeasts. Since these naturally existing yeasts are adaptable to a variety of environmental circumstances, these organisms are certainly essential to indigenous tribes' traditional production of liquor but also offer a possible source for the production of bioethanol (Gavankar and Chemburkar, 2016) [6].

A noteworthy methodological approach is the incorporation of natural fermentation intermediaries, like Dhataki or Madhuca flowers in Sandhana Dravya. To begin and sustain the fermentation process, these natural fermentation initiators rely upon microflora prevalent within these flowers. Yeast species recognized in Madhuca flowers express their role in conventional fermentation procedures for producing asava and aristas, which are ancient medicinal formulations. These techniques are acknowledged in older publications like the Indian Materia Medica and Bhaishajya Kalpana Vijnana. The precise scientific roles of these flowers in Sandhana Kalpana, have not been adequately investigated despite this ancient knowledge (Mallya Suma V *et al*., 2017) [12]. Findings of published sources have shown that Madhuca flowers are home to multiple kinds of yeast species, encompassing Saccharomyces, Pichia, Torulopsis, Kloeckera, and several anonymous genera (Gavankar and Chemburkar, 2016) [6]. Flies, social wasps, and bees are drawn to certain volatiles generated through yeast, usually

alcoholic compounds and their esters. These insects use these flowers as a dietary supplement, which aids in their growth and survival. Additionally, these insects carry yeast to new environments, giving an advantage of efficient outbreeding and dispersal of yeast species [9].

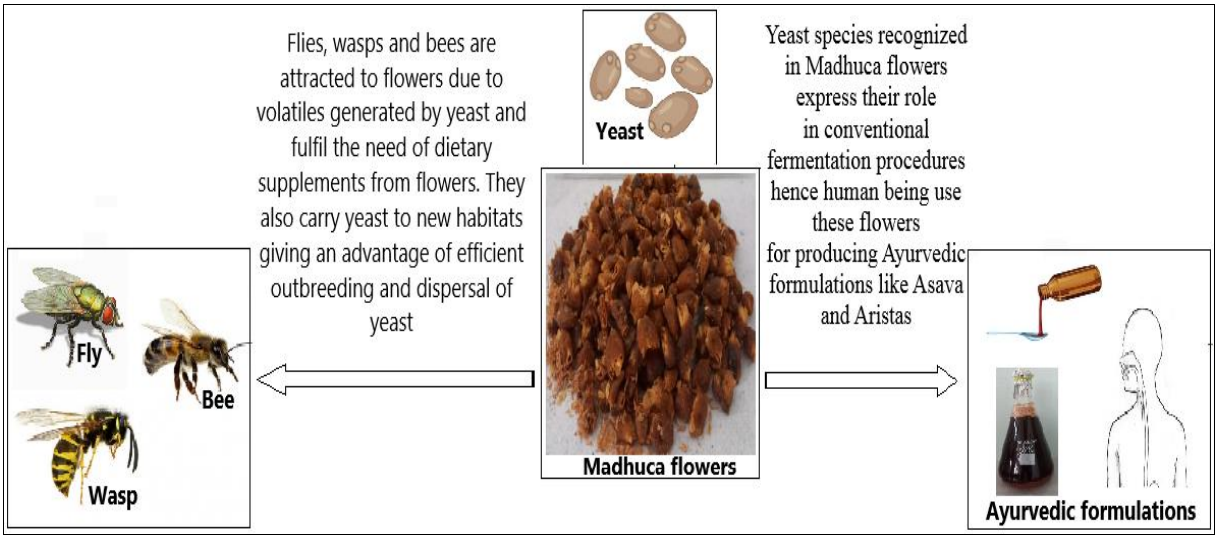

Fig 1: Ecosystem benefits of Madhuca flowers and their yeast species for humans and insects

Nevertheless, yet to thorough assessment of these innately occurring wild yeasts' aptitude to yield alcohol has not been conducted. To bridge this knowledge deficit, yeast species from Madhuca flowers must be identified and assessed more precisely with their potentiality for the generation of alcohol and fermentation duration. These indigenous yeasts can adapt adequately to volatile circumstances within the environment, which renders them viable bioethanol-producing inocula. The current vetting intended to investigate the importance of Madhuca flowers in fermentation methods by examining the particular yeasts involved and their fermentation capabilities. This comprehension may furnish pertinent facts regarding their possible applications in both conventional and contemporary scenarios.

Material and methods

Materials

Madhuca Indica flowers were gathered from the Sakoli taluka in Maharashtra, and the Botanical Department of RTM Nagpur University approved the authenticity of the specimens. Jaggery was bought from the area's stores. Every chemical deployed to execute the investigation, encompassing solvents, had a sufficient analytical level. Every microbiological medium was made under the established protocol.

Isolation of Yeasts

Yeast isolates were separated from the *Madhuca indica* flowers. Two grams of flowers were incorporated into a sanitized vessel holding 200 mL of sterile malt extract-glucose-yeast extract-peptone (MGYP) broth and then maintained for twenty-four hours at 32°C whilst shaken at 180 rpm using a rotary shaker. A small part of around 500 µL of this obtained blend was equally put onto an MGYP agar dish and thereafter retained at 32°C for a full 24 hours in an incubation chamber. When each colony acquired maturity, it was appropriately examined, and the sterility was examined by perusing the yeast implementing a microscope. Unique isolates with morphological alterations, especially variances in dimensions, shape, and hue, had been chosen and subsequently streaked onto MGYP agar dishes for a minimum of three instances within the course of a purification operation. Because beginning yeast specimens might have contamination, each one was submitted towards streaking onto MGYP agar plates bearing 100 ppm streptomycin. Twenty unique sorts of yeast were isolated from *Madhuca indica* flowers, cultivated through MGYP agar, and allocated the identities AY-1 to AY-20. Afterwards, these yeast varieties were upheld for subsequent usage at 4°C by settling on MGYP agar slants (Manwar *et al*., 2013[a]; Kurtzman *et al*., 2011) [14, 10].

Fermentation and Screening for Ethanol Production

A fermentation protocol was implemented with the isolated yeast species. During this approach, jaggery was dissolved in demineralized water to create 50% w/v jaggery media. Nitrogen sources comprising carbamide and diammonium phosphate, were added at 0.01% w/v (Manwar *et al*., 2013[b]; Hawaz *et al*., 2022) [15, 7]. To establish an inoculum specific to the obtained isolate, one loop of yeast was transferred to a hundred millilitres of sterile jaggery media. Each inoculum was maintained in a shaking incubator with a 180-degree rotational velocity for two days. Subsequently, 90 mL of prepared jaggery medium was topped off with about 10 mL of the incubated inocula per flask, and the flasks were allowed to incubate for ten days at ambient temperature (Bajaj *et al*., 2003; Manwar *et al*., 2013[b]) [2, 15]. Thereafter 50 mL of demineralized water was infused with a millilitre of fermentation broth, proceeded to be distilled and 20 mL of obtained distillate was admixed with 25 mL of potassium dichromate reagent. The resultant mixture was placed over an electric water bath at 60°C for half an hour. Demineralized water was employed for setting the final quantity to 50 mL, and identical water was deployed as a reference for analyzing the optical density at 620 nm. A linear equation computed from the standard calibration curve was applied to figure out the level of alcohol (Zoecklein *et al*., 1990; Miah *et. al*., 2017) [27, 17]. A series of diluted versions at 2%, 4%, 6%, 8%, and 10% v/v were

implemented to derive a calibration curve for ethanol (99.99% v/v).

Characterization of Selected Yeast Species

Metabolic traits serve as vital for defining, differentiating, and classifying yeast strains. In recognition of characterization, AY-7, AY-9, AY-15, and AY-19 yeast types that yielded ethanol at levels of not less than 1% v/v were opted for. These yeast varieties were recognized according to morphological traits in addition to particular physiological and biochemical test outcomes regarding every individual type. Following that, the findings gleaned were scrutinized deploying the data from reference yeast strains published in the text of relevant literature (Kurtzman *et al*., 2011; Barnett *et al*., 2000) [10, 3].

Comparative Fermentation Study of Selected Yeast Species

A 50 mL of inoculum from selected yeast sorts, namely AY-7, AY-9, AY-15, and AY-19, were infused into distinguish flasks, each of which carried 500 mL of 50% w/v decontaminated jaggery media. These specific flasks were admitted for incubation at the ambient temperature unless the course of fermentation had finished. The necessary quantity of samples was taken from the flask every 48 hours. The nonexistence of further alcoholic output and subsequent intake of substrate signalled the termination of the course of fermentation within every flask. The dichromate oxidation protocol was implemented for estimating the alcohol, and the substrate intake was acquired by subtracting the leftover reducing sugar from the initial level. The titrimetric protocol implementing the Fehling solutions approach was adopted to ascertain the level of reducing sugar (Bajaj *et al*., 2003; Manwar *et al*., 2013[b]) [2, 15]. The burning stick trial, effervescence testing, and vetting with a saturated solution of slaked lime were executed to validate the completion of the course of fermentation (Moharana *et al*., 2019) [18].

Result

Isolation of Yeasts

Using the flowers of *Madhuca indica*, twenty unique kinds of yeast were recognized. Differentiable colonies received consideration through variations in morphological features encompassing dimension, shape, and hue. Twenty unique sorts of yeast were isolated from *Madhuca indica* flowers, cultivated through MGYP agar, and allocated the identities AY-1 to AY-20. The morphological details of yeast colonies are demonstrated in Table 1.

Table 1: Morphological characteristics of yeast colonies

Colony	Colour	Size (mm)	Opacity	Elevation	Margin	Consistency	Shape of cells
1	Creamy	2.0	Opaque	Convex	Irregular	Rough	Hyphae
2	-do-	2.0	-do-	-do-	-do-	-do-	-do-
3	-do-	2.0	-do-	-do-	-do-	-do-	-do-
4	-do-	0.9	-do-	-do-	-do-	-do-	-do-
5	-do-	2.0	-do-	-do-	-do-	-do-	-do-
6	-do-	1.0	-do-	-do-	-do-	-do-	-do-
7	-do-	1.5	-do-	-do-	-do-	-do-	Pseudohyphae
8	-do-	2.0	-do-	-do-	-do-	-do-	Hyphae
9	Creamy white	1.5	-do-	-do-	-do-	-do-	Oval
10	Creamy	2.0	-do-	-do-	-do-	-do-	Hyphae
11	-do-	0.5	-do-	-do-	-do-	-do-	-do-
12	-do-	0.5	-do-	-do-	-do-	-do-	-do-
13	-do-	0.5	-do-	-do-	-do-	-do-	-do-
14	Red	1.0	-do-	-do-	Irregular	smooth	Oval
15	Creamy	1.0	-do-	-do-	Regular	Rough	Ovoid
16	Red	2.0	-do-	-do-	-do-	-do-	-do-
17	-do-	1.0	-do-	-do-	Regular	smooth	Oval
18	Creamy	2.0	-do-	-do-	Irregular	Rough	Hyphae
19	Creamy	2.0	-do-	-do-	Regular	Smooth	Oval
20	Creamy	2.0	-do-	-do-	Regular	Smooth	-do-

Screening for Alcohol Production by all Isolated Yeast Species

The dichromate oxidation strategy, an acknowledged analytical methodology for alcohol vetting, was employed for assessing the level of alcohol formed by each of the twenty distinct yeast varieties. The facts acquired clarified that the alcohol yielded by divergent yeast species differed considerably. Evidently, isolates AY-7, AY-9, and AY-15 have laid out the alcoholic output throughout the entire screening operation exhibiting levels of 1.08% v/v, 1.63% v/v, and 2.80% v/v, respectively. With an alcoholic output of 6.52% v/v, yeast AY-19 delivered the highest level among the varieties tested. The 16 leftover yeast types performed poorly, with an ethanolic level of no more than one percent v/v. The notable significant disparity in alcoholic output points out that the distinguishing *Madhuca indica* yeast species has a diverse spectrum of fermentation perspectives. Recognizing that the isolates of AY-19, AY-15, AY-9, and AY-7 yielded well over 1%v/v of alcohol, they were deemed noteworthy and could potentially employed for the formation of alcohol. Figure 2 illustrates a graphical representation of the relative alcoholic output, delivering an explanation of the disparities in productivity for each species.

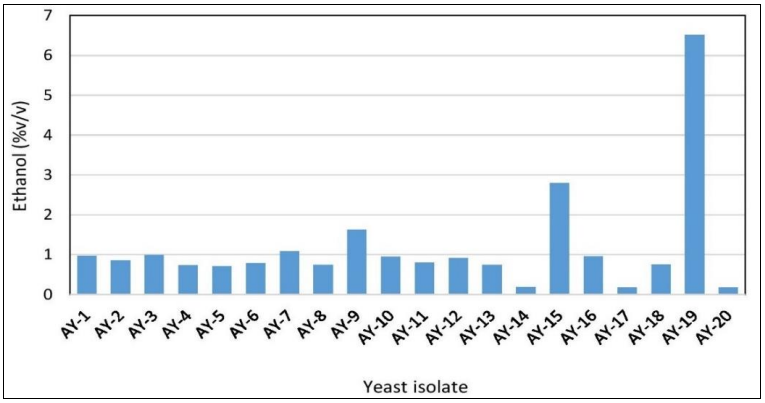

Fig 2: Comparability of the ethanol yield through the various yeast isolates

Characterization and Comparative Fermentation Study of Selected Yeast Species

The yeast species AY-7, AY-9, AY-15, and AY-19 which highlighted an ethanol yield of 1% v/v or above received consideration to further examination. The identification procedure relied on typical morphology-based and metabolic/biochemical testing that had been employed to the strain level as well as these species admitted for an in-depth examination of their fermentation aptitudes.

Characterization of Yeasts

To appraise the morphology-based attributes, a slide culture protocol was executed. Fig. 4 focuses on depictions of the assigned yeasts acquired with a phase-contrast microscopy instrument (Leica-DM2000). Each scrutinized isolate had unique traits that were uncovered by the investigation. For instance, cells with filaments alongside a profusion of pseudohyphae were turned out by the AY-7 isolate. On the converse hand, the AY-9, AY-15, and AY-19 isolates had an oval pattern. The AY-15 yeast colony vetting implied a whitish-creamy hue, whereas the AY-7, AY-9, and AY-19 strains disclosed a creamy colouration. All of the isolates chosen emerged to be opaque, convex and had rough exteriors, except the notable distinction of AY-19, which featured a smooth exterior.

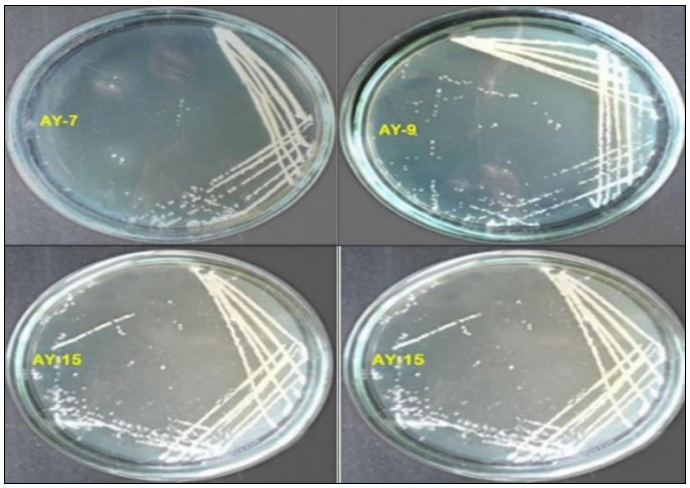

Fig 3: Selected yeast isolates when cultured on MGYP agar

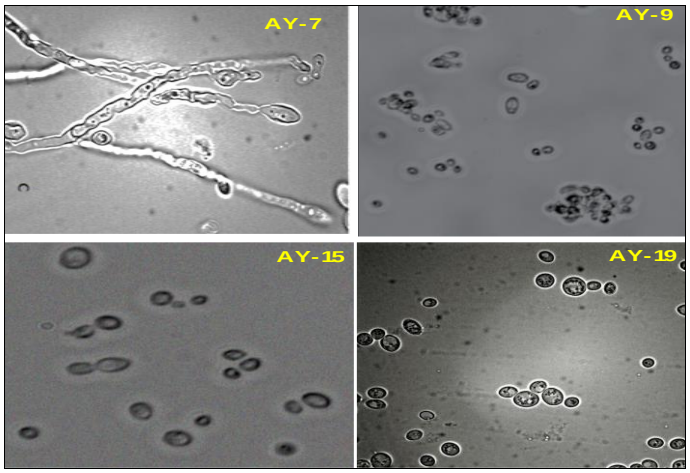

Fig 4: Investigation of selected yeast isolates using phase contrast light microscopy

Trials for selected isolates concerning biochemical and physiological function were additionally accomplished. Every tested variant appeared capable of fermenting sucrose, maltose, and dextrose, yet not trehalose, melibiose, or lactose. AY-19 and AY-15 laid out the fermentation of raffinose and galactose, however only AY-15 vented the fermentation of cellobiose and D-xylose. Regarding carbon assimilation, each selected species disclosed a knack for assimilating sucrose, malt sugar, dextrose, and ethanol. However, neither within the isolates exhibited melibiose, lactose, L- and D-arabinose, or inulin assimilation. Moreover, AY-7 and AY-15 revealed favourable assimilation performance with glycerol and cellobiose, whereas AY-9 and AY-19 established unfavourable findings with these carbon-based substances. AY-15 specimen was noticed distinct among the tested ones owing to its superior assimilation outputs towards galactose, D-ribose, D-sorbitol, and D-xylose. Furthermore, only AY-15 and AY-19 specimens gave unambiguous confirmation of trehalose and raffinose assimilation. Every isolate revealed a tendency to assimilate soluble starch, excluding the AY-19. Such variable assimilation patterns point out the diverse metabolism outputs among the investigated yeast sorts, especially with an emphasis on the utilization of varying materials containing carbon. During assimilation investigation with nitrogen-carrying materials, AY-19 specimen was incapable of assimilating anyone among the nitrogen-based substances tested, while the AY-9 variant implied that it could assimilate lysine only. The species AY-7 and AY-15 disclosed an ability to assimilate apart nitrogenous substances, notably the nitrate of potash, ethanamine hydrochloride, and L-lysine. To recognize specific yeast types, rigorous biochemical screening was undertaken. Concerning tolerability for 1% ethanoic acid, generation of ammonia utilizing urea, citric-based consumption, and nitrate reduction, every isolate disclosed unsuccessful findings. They succeeded in demonstrating significant glucose-to-acid synthesizing. However, each of the isolates revealed favorable outcomes regarding hydrolyzing the starch as well as cycloheximide resistance, excluding the AY-19 specimen. Tables 2 through 5 deliver an exposition of the substantial outputs of varied metabolic and biochemical-based attributes.

The morphology-based, as well as metabolic, and biochemical-based vetting of selected isolates including AY-7, AY-9, AY-15, and AY-19, comply with the typical characterizations established by Barnett J.A. *et al*., and Kurtzman C.P. *et al*., and confirm their recognition as *Saccharomyces fibuligera*, *Saccharomyces boulardii*, *Pichia anomala*, and *Saccharomyces cerevisiae*, respectively. The findings were additionally compared against the characterization scrutiny disclosed in scientific articles by Suzuki *et al*. and Yang *et al*. which implies significant similarities between the approved attributes for these acknowledged yeast species and the characteristics that were detected within the tested specimens (Kurtzman *et al*., 2011; Barnett *et al*., 2000; Suzuki *et al*., 1987; Yang *et al*., 2019) [10, 3, 23, 26].

Table 2: Outcomes of fermentation of saccharides by selected yeast isolates

Sugar	Yeast Isolate			
	AY-7	AY-9	AY-15	AY-19
Dextrose	+	w/+	+	+
D-Galactose	-	-	+	+
Sucrose	w/+	w/+	+	+
Raffinose	w/-	w/-	+	+
Lactose	-	-	-	-
Maltose	w/+	w/+	+	+
Trehalose	-	-	-	-
D-Xylose	-	-	+	-
Cellobiose	-	-	+	-
Melibiose	-	-	-	-

Responses: + = positive; - = negative; w/+ = weak or positive; w/- = weak or negative

Table 3: Outcomes of assimilation of nitrogen-based compounds by selected yeast isolates

Nitrogen-based compound	Yeast Isolate			
	AY-7	AY-9	AY-15	AY-19
Potassium Nitrate	+	-	+	-
Sodium Nitrite	-	-	-	-
Ethylamine HCl	+	-	+	-
L-Lysine	+	+	+	-

Responses: + = positive; - = negative

Table 4: Outcomes of assimilation of carbon-based compounds by selected yeast isolates

Carbon-based compound	Yeast Isolate			
	AY-7	AY-9	AY-15	AY-19
Dextrose	+	+	+	+
D-Galactose	-	-	+	w/-
Lactose	-	-	-	-
D-Sucrose	+	+	+	+
Trehalose	w/-	w/-	+	+
Maltose	+	+	+	+
Raffinose	w/-	-	+	+

Cellobiose	+	-	+	-
Melibiose	-	-	-	-
Soluble Starch	w/+	+	+	-
Inulin	-	-	-	-
D-Arabinose	-	-	-	-
L-Arabinose	-	-	-	-
D-Xylose	-	-	+	-
D-Ribose	-	-	+	-
Ethanol	+	+	+	+
Glycerol	+	-	+	-
D-Sorbitol	w/-	w/-	+	-

Responses: + = positive; - = negative; w/+ = weak or positive; w/- = weak or negative

Table 5: Outcomes from Biochemical testing by selected yeast isolates

Test Executed	Yeast Isolate			
	AY-7	AY-9	AY-15	AY-19
Ammonia generation using carbamide	-	-	-	-
Starch hydrolysis	+	+	+	-
Nitrate reduction	-	-	-	-
Cycloheximide resistance (0.1%)	+	+	+	-
Growth on 50% glucose-yeast extract agar	w/-	w/+	w/-	w/-
Growth in 10% NaCl plus 5% dextrose solution	w/+	w/-	w/+	w/-
Tolerance of 1% acetic acid	-	-	-	-
Acid generation using dextrose	+	w/+	+	+
Citrate utilization	-	-	-	-
Gelatin liquefaction	w/+	-	w/+	-
Growth at 37°C	w/+	+	w/+	w/-
Growth at 20°C	+	+	+	+
Growth at 25°C	+	+	+	+
Growth at 40°C	-	-	-	-

Responses: + = positive; - = negative; w/+ = weak or positive; w/- = weak or negative

Comparative Fermentation Study of Selected Yeast Strains

In the course of the entire fermentation procedure, yeast species which gave an ethanol output of at least 1% v/v, notably AY-7, AY-9, AY-15, and AY-19, were assessed. The disappearance of bubbling, the flame extinguishment, and a negative lime water appraisal altogether signified that the course of fermentation had terminated (Moharana *et al*., 2019) [18]. According to this investigation, *Saccharomyces cerevisiae* (AY-19) can form a maximum of 7.05% v/v of ethanol across the span of the 16-day fermentation process. During 16 days, the tested strain generated the mentioned ethanol quantity by consuming about 18.06%w/v of reducing sugar. Additionally, it had been recorded that *Saccharomyces fibuligera* (AY-7) needed 2.98% w/v of reducing sugar to create 1.29% v/v of ethanol upon 18 days of fermentation. After 22 days of fermentation, *Saccharomyces boulardii* (AY-9) yielded 1.88% v/v of ethanol by employing 4.34% w/v of reducing sugar. Within 20 days, *Pichia anomala* (AY-15) needed 8.34% w/v of reducing sugar to consummate the fermenting process and yield 3.68% v/v of ethanol. The depiction of alcohol creation and usage of the substrate is sequentially displayed in Fig. 5 and Fig. 6.

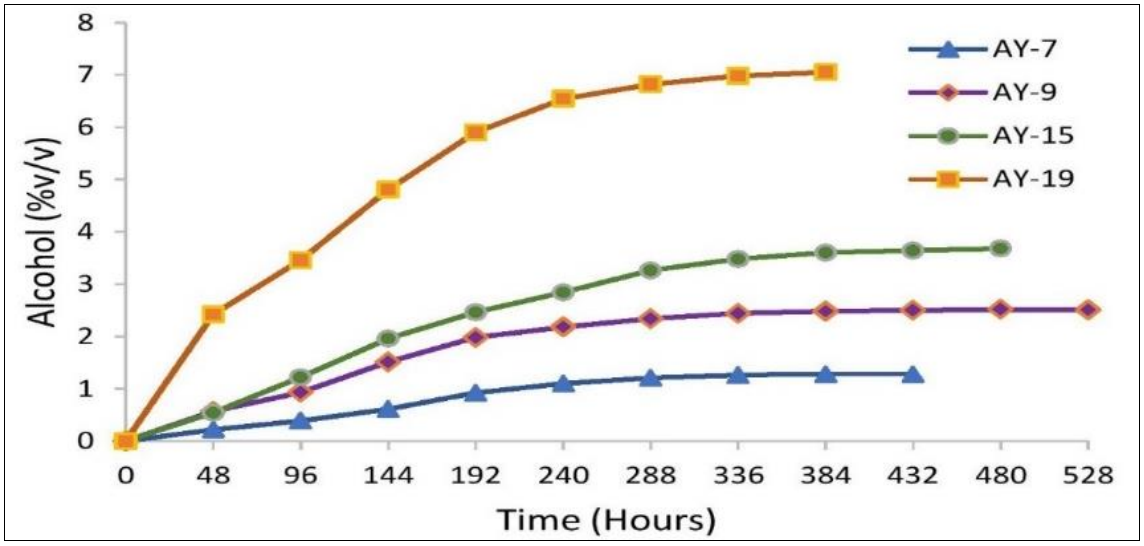

Fig 5: Alcoholic output by selected yeast isolates throughout fermentation

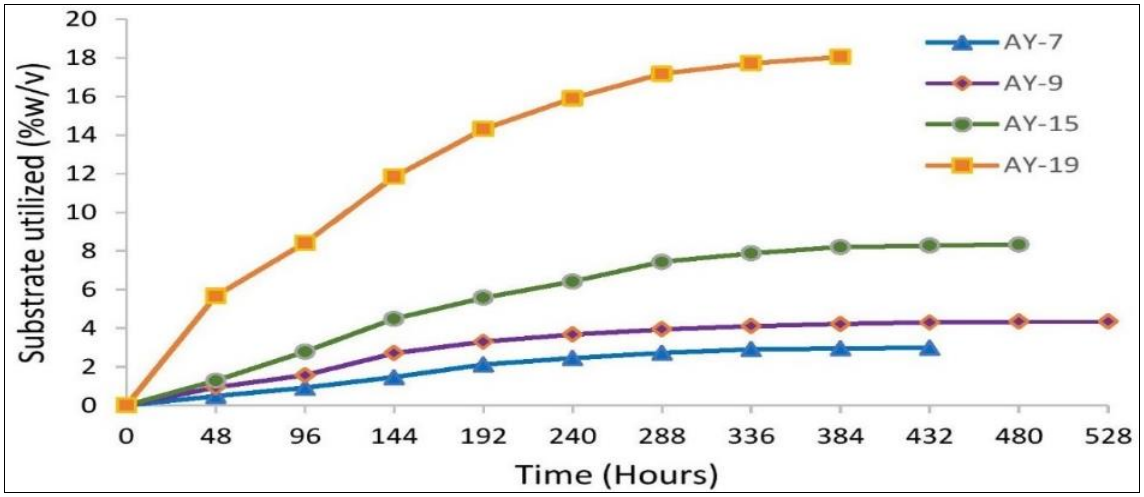

Fig 6: Substrate consumed by selected yeast isolates throughout fermentation

Discussion

In terms of a knack for generating alcohol, the fermentation perspective of four distinct yeast species was systematically analyzed. *Saccharomyces fibuligera* (AY-7) strain, is recognized by its unique morphology, which includes dimorphism and the formation of discrete circular or oval-shaped budding cells in tandem with mycelia. Furthermore, the AY-7 strain's authentication as a *Saccharomyces fibuligera* strain is reinforced by physiological and biochemical profiling, which offers relevant data about the strain's morphological and metabolic attributes (Xie *et al*., 2021) [25]. Findings compiled from the AY-9 strain were analogous to those of *Saccharomyces boulardii*. Although preceding studies hinted that it could possibly belong to a type of *Saccharomyces cerevisiae*, more in-depth analyses of its genetic information and enzyme-based content uncovered substantial discrepancies. Furthermore, unusual microsatellite allele patterns set aside this from remaining yeast strains. This strain has proven to strengthen immune and alimentary enzyme output, which facilitates dietary breakdown and absorption through the digestive tract and concurs with the strain's usefulness as a probiotic. Particularly this strain grows optimally at 37°C, while strains of *Saccharomyces cerevisiae* typically thrive around 30°C. (Hossain *et al*., 2020) [8]. The published evidence points out that the assimilation of lysine and starch in the experimentation, alongside an optimum development temperature of 37°C, revealed the differentiation of AY-9 from *Saccharomyces cerevisiae* and substantially imitates *Saccharomyces boulardii*. Results of investigations from morphological assessment and multiple confirmation evaluations, such as the strain's adaptability towards the assimilation of varied carbon-based and nitrogen-based resources, corroborate the recognition of the AY-15 species as *Pichia anomala*. It can survive in specifically anaerobic habitats, with a temperature range within 3° to 37°C and a pH encompassing 2 to 12.2 (as demonstrated during laboratory experiments and tracked in literature). These characteristics enable for recognizing of species as the *Pichia anomala* strain (Fredlund *et al*., 2002) [5].

AY-19, which was perceived to yield greater quantities of alcohol in early fermentation assessments, is most certainly a *Saccharomyces cerevisiae* strain. This is corroborated by a broad spectrum of similarities with the standard descriptions of previously published findings about *Saccharomyces cerevisiae*. In response to numerous sugars, especially maltose, raffinose, galactose, sucrose, and dextrose, the standard strain of *Saccharomyces cerevisiae* and AY-19 demonstrated succeeding fermentation effects. However, lactose, xylose, trehalose, melibiose, and cellobiose weren't fermented by it. Certain enzymes have to exist in microorganisms that are able to convert certain resources of carbon towards other molecules through metabolism. *Saccharomyces cerevisiae* is incapable of fermenting lactose in this specific case since it has no lactase. However, it has mannase, which allows maltose to be converted into glucose molecules. Prior research has indicated that there is an elevated likelihood that yeast species that are competent to ferment glucose, galactose, maltose, sucrose, and raffinose are likely to be categorized as *Saccharomyces cerevisiae*. The accomplished examination's findings demonstrated that AY-19 is a Saccharomyces cerevisiae (Abdulla *et al*., 2022) [1].

Investigation about fermentation revealed that, isolated yeast AY-7, later recognized as *Saccharomyces fibuligera*, represented the only one among four yeast species that yielded reduced ethanol across screening. It ferments much more sluggish compared to other established yeast species, yet it can hydrolyze and degrade a broad range of carbohydrates, as shown by previous studies. As pointed out in the preceding research this yeast can form ethanol with a lower rate, between 0.8 to 1.2% v/v. Therefore, *Saccharomyces fibuligera* is optimal for the creation of products with unique flavor profiles and low alcohol content (Methner *et al*., 2022) [16]. The knack to generate alcohol was also assessed by employing *Saccharomyces boulardii* (AY-9). Based on a past investigation, *Saccharomyces boulardii* has a prolonged fermentation period and expresses substandard fermentation performance. This prolonged fermentation period during the current laboratory study is noted for being 22 days long and producing 1.88% v/v alcohol (Manshin *et. al*., 2022) [13]. The isolated yeast AY-15 which was determined as *Pichia anomala*, produced the second-highest concentration of alcohol. Previous research found that *Pichia anomala* may ferment carbon-based substrates under aerobic development and create a small quantity of ethanol (Passoth *et al*., 2006) [19]. Data from earlier reports imply that *Saccharomyces cerevisiae* and *Pichia anomala* both metabolize dextrose at a similar rate during fermentation. *Saccharomyces cerevisiae* creates less biomass on dextrose than *Pichia anomala*, but it yields ethanol at a faster pace. (Fletcher *et al*., 2015) [4]. During

fermentation, *Saccharomyces cerevisiae* (AY-19) revealed the highest level of ethanol yield (Abdulla R., *et. al*., 2022) [1]. Past investigation has documented the significance of *Saccharomyces cerevisiae* as the yeast of choice and its pivotal significance in the realm of biotechnology, particularly in the context of commercial manufacture of ethanol. *Saccharomyces cerevisiae* are distinguished through a multitude of advantageous characteristics, such as their quick growth, effective anaerobic glucose processing, high ethyl alcohol productiveness, increased output, and remarkable resistance to a range of natural stressful circumstances, such as ethanolic presence, low pH, and confined oxygen availability (Ruchala *et al*., 2020) [21].

Conclusion

The study's findings emphasize the untapped potential of *Madhuca indica* flower-associated yeast in biotechnological processes. Specifically, the utilization of *Saccharomyces cerevisiae* for ethanol production presents a promising avenue to improve the efficiency and sustainability of fermentation processes. Furthermore, its application in asavarista formulations introduces exciting possibilities for the improvement of the fermentation process. Future research efforts should be directed towards optimizing fermentation conditions and scaling up production, thereby unlocking the full industrial and medicinal benefits of identified yeast. This could lead to more sustainable and innovative options during fermentation processes for scaling up ethanol-based production.

References

1. Abdulla R, Ahmad NH, Sabullah MK, Gansau JA. Characterization of wild type yeast isolated from Sabah soil for environmental friendly biofuel production. IOP Conf. Series: Earth Environ Sci,2022:1103:012024.
2. Bajaj BK, Taank V, Thakur RL. Characterization of Yeasts for Ethanolic Fermentation of Molasses with High Sugar Concentarations, J Sci Ind Res,2003:62: 1079-1085.
3. Barnett JA, Payne RW, Yarrow D. Yeasts: Characteristics and identification. Third Edition, Cambridge University Press, Cambridge, UK, 2000.
4. Fletcher E, Feizi A, Kim S, Siewers V, Nielsen J. RNA-seq analysis of *Pichia anomala* reveals important mechanisms required for survival at low pH. Microb Cell Fact,2015:14:143.
5. Fredlund E, Druvefors U, Boysen ME, Lingsten KJ, Schnürer J. Physiological characteristics of the biocontrol yeast Pichia anomala J121. FEMS Yeast Res,2002:2(3):395-402.
6. Gavankar R, Chemburkar M. Isolation and Characterization of Native Yeast from Mahua Flowers. Int J Curr Microbiol Appl Sci,2016:5(11):305-314.
7. Hawaz E, Tafesse M, Tesfaye A, Beyene D, Kiros S, Kebede G, *et al*. Isolation and characterization of bioethanol producing wild yeasts from bio-wastes and co-products of sugar factories. Ann Microbiol,2022:72:39.
8. Hossain MN, Afrin S, Humayun S, Ahmed MM, Saha BK. Identification and growth characterization of a novel strain of Saccharomyces boulardii isolated from Soya paste. Front Nutr,2020:7:27.
9. Jouhten P, Ponomarova O, Gonzalez R, Patil KR. Saccharomyces cerevisiae metabolism in ecological context. FEMS Yeast Res,2016:16(7):fow080.
10. Kurtzman CP, Fell JW, Boekhout T, Robert V. Methods for Isolation, Phenotypic Characterization and Maintenance of Yeast, in The Yeasts: A Taxonomic Study (eds Kurtzman C.P., Fell J.W. and Teun B.), Fifth Edition, Elsevier Publishers, Amsterdam, 2011, 87-110.
11. Lungade P, Karadbhajne SV. Mahua flower (*Madhuca Indica*): Approach of functional, nutritional characteristics and an accompaniment to food products. J Pharm Negat Results,2022:13(Sp. Issue 9):9093-9104.
12. Mallya Suma V, Castelino JE, Seema MB. Role of Madhuka Pushpa in Sandhana Kalpana; microbiological pharmaceutico-analaytical study. Journal of Scientific and Innovative Research,2017:6(2):68-72.
13. Manshin D, Meledina TV, Britvina T, Davydenko SG, Shelekhova NV, Andreev V, *et al*. Comparison of the yeast Saccharomyces cerevisiae var. boulardii and top-fermenting brewing yeast strains during the fermentation of model nutrient media and beer wort. Agron Res,2022:20(3):625–636.
14. Manwar J, Mahadik K, Paradkar A, Sathiyanarayanan L, Vohra M, Patil S. Isolation, biochemical and genetic characterizations of alcohol-producing yeasts from the flowers of *Woodfordia fruticosa*. Integr Med Res,2013[a]:2(2):56-61.
15. Manwar J, Mahadik K, Paradkar A. Plackett-Burman Design: A Statistical Method for the Optimization of Fermentation Process for the Yeast Saccharomyces cerevisiae Isolated from the Flowers of *Woodfordia fruticosa*. Ferment Technol,2013[b]: 2(1):1000109.
16. Methner Y, Magalhaes F, Raihofer L, Zarnkow M, Jacob F, Hutzler M. Beer fermentation performance and sugar uptake of Saccharomycopsis fibuligera–A novel option for low-alcohol beer. Front Microbiol,2022:13:1011155.
17. Miah R, Siddiqa A, Tuli JF, Barman NK, Dey SK, Adnan N, Yamada M, *et al*. Inexpensive Procedure for Measurement of Ethanol: Application to Bioethanol Production Process. Adv Microbiol,2017:7:743-748.
18. Moharana S, Das AK, Jena R, Meher RK. A comparative pharmaceutico-analytical evaluation of Dhatryarista prepared with different fermentative initiators. Int J Ayurveda Pharm Chem,2019:10(1):16-22.
19. Passoth V, Fredlund E, Druvefors UA, Schnürer J. Biotechnology, physiology and genetics of the yeast Pichia anomala. FEMS Yeast Res,2006:6(1):3-13.
20. Patel PK, Prajapati NK, Dubey BK. *Madhuca indica*: A review of its medicinal property. Int J Pharm Sci Res,2012:3(5):1285-1293.
21. Ruchala J, Kurylenko OO, Dmytruk KV, Sibirny AA. Construction of advanced producers of first- and second-generation ethanol in *Saccharomyces cerevisiae* and selected species of non-conventional yeasts (Scheffersomyces stipitis, Ogataea polymorpha). J Ind Microbiol Biotechnol,2020:47(1):109-132.
22. Srinivasa Reddy IV. Madhuca indica: An untapped forest tree for its medicinal uses. The Pharma Innovation Journal,2022:11(3):1747-1751.

23. Suzuki M, Nakase T, Daengsubha W, Chaowsangket M, Suyanandana P, Komagata K. Identification of yeasts isolated from fermented foods and related materials in Thailand. J Gen Appl Microbiol,1987:33:205-220.
24. Wagh PM. Characterization and utilization of yeast isolates from flowers of Madhuka indica from Nasik locale for preparation of wine. Journal for Advanced Research in Applied Sciences,2016:3(Sp. Issue):1-10.
25. Xie ZB, Zhang KZ, Kang ZH, Yang JG. Saccharomycopsis fibuligera in liquor production: A review. Eur Food Res Technol,2021:247(7):1569–1577.
26. Yang X, Shu G, Lei Z, Du G, Liu Z, Cao J. Effect of carbon sources, nitrogen sources and prebiotics on growth of *Saccharomyces boulardii*. Acta Univ Cibiniensis, Ser. E: Food Technol,2019:XXIII(2):101-108.
27. Zoecklein BW, Fugelsang KC, Gump BH, Nury FS. Production Wine Analysis. First Edition, AVI Book, New York, 1990, 58-59.