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AUTOCHTHONOUS YEAST DIVERSITY ASSOCIATED WITH FRUIT SURFACE AND FERMENTED MUST OF MINOR FRUITS

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Abstract

Indigenous yeast diversity of wild edible minor fruits exhibits distinct adaptation and specific chemical nature. In order to explore suitable autochthonous yeasts for improving the oenological characteristics of typical regional wine, it is essential to evaluate optimum strength of yeast isolates by subjecting to various biochemical analyses. In the present work autochthonous yeast diversity of wild edible minor fruits was investigated. A total of fifty five autochthonous yeasts were isolated from both the fruit surface and spontaneous fruit wine samples. The preliminary morphological identification followed by scanning electron microscopy analysis was comprehensively performed for both colonies and individual cells respectively. The biochemical characterization was performed for the efficiency of yeast isolates at various carbon and nitrogen source utilization. As a result ECRH4, GPRH10, ECRH16, SCARH23 and ZRRH8 yeast isolates were preliminarily characterized as *Saccharomyces* spp. and remaining as non-*Saccharomyces* spp. There are various oenological important autochthonous yeasts play a vital role in the wine fermentation process. The present study was focused on the preliminary identification and biochemical characterization of the autochthonous yeast populations of underutilized lesser known minor edible fruits of the Western Ghats in southern India.

Key words: Biochemical characterization, Indigenous yeasts, Minor fruit, Morphological characterization, Scanning Electron Microscopy

Introduction

Yeasts are versatile microorganisms which exhibit heterogeneity in their abilities of aromatic molecules production. The metabolic conversions may improve the production of a particular compound already produced by the microbial populations or initiate the production of completely new metabolites. Yeast metabolic activity leads the fermentation but also has an essential role in production of quality winery attributes. The fermentation potential of various yeast isolates and strains has great impact on organoleptic properties of wine increasing its complexity and sensory profiles (Ribéreau-Gayon *et al*., 2006; Capece *et al*., 2012). In fact, the composition and flavour quality of resulting wine are due to *Saccharomyces cerevisiae* strains, the different strains of non-*Saccharomyces cerevisiae* such as *Pichia*, *Hanseniaspora* can produce significantly different flavor profiles. The indigenous yeasts are better acclimated to micro area conditions of the wine producing region, therefore they can more easily dominate on the natural biota (Ebabhi *et al*., 2013; Aponte and Blaiotta, 2016). The selection of potential starter yeasts facilitates the control of fermentation and reduces differences in wine quality from one harvest to another (Capece *et al*., 2014). Presently, wine industries worldwide use commercial starter cultures of *S. cerevisiae* to assure reproducible and well controlled fermentation. However, widespread application of commercial starter cultures in wine production eliminates native microbiota, which results in wines with indistinguishable analytical and flavour profiles. Further lead to depriving their oenological potentiality and uniqueness that define wine typicity (Chovanová *et al*., 2011), this key feature provide the recognition of a wine with territory where it has been produced. Yeast isolates, such as *Pichia*, *Torulaspora*, *Zygosaccharomyces* and *Candida* are generally present on surface of the fruits (Esteve-Zarzoso *et al*., 1998; Obasi *et al*., 2014).

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Although fruit cultivar provide the basis for wine complexity, microorganisms, particularly yeasts, greatly impact on final produced wine with distant flavour composition (Jolly *et al*., 2006; Archana *et al*., 2016).

Wine is a consequence product of biochemical reactions, which begins from ripening of fruits and continues throughout the alcoholic fermentation and aging (Romano *et al*., 2003; Vigentini *et al*., 2016). Yeast plays the important role in biochemical transformation of must into fine wine. Globally, most suggested species for quality wine production is *S. cerevisiae*. Nowadays, there has been increase in use of autochthonous yeasts for wine production. The autochthonous yeast strains, in spontaneous fermentation of fruits must produce regional wines with various qualities attributes and peculiar wine flavours (Sun *et al*., 2009; Wu *et al*., 2014). Commonly, *Hanseniaspora* spp. and *Pichia* spp. initiate the fermentation process. Sometimes, species of *Kluyveromyces* and *Issatchenkia* may also contribute in the fermentation process. The survival rate of non-*Saccharomyces* spp. during fermentation is mainly regulated by ethanol production from *Saccharomyces* spp. (Desai *et al*., 2013). Species of *Pichia*, *Kluyveromyces*, *Hanseniaspora, Issatchenkia* and *Metschnikowia* isolated from fruit surfaces and must are high sensitive to increased ethanol concentrations, and this lead to decline and death. It is found necessary to protect and improve indigenous yeast populations as they better adapted to particular environmental conditions (Ndip *et al*., 2001).

Overall wine individuality and complexity is examined by relative proportions of phenolics, flavonoids, volatile and non-volatile compounds extracted from fruits and their metabolic transformation during yeast fermentation and maturation (Satora and Tadeusz, 2010). Thus evaluation of the efficacy of native yeasts on vinification is important to understand the potential variations in oenological features mainly on production of desirable wine with health benefits (Hyma *et al*., 2011; Tristezza *et al*., 2012). However, limited work has done on oenological characters of Indian native yeast isolates and its potential in wine aging (Chavan *et al*., 2009). The quality of fermented beverages is partially determined by the microorganisms used for their production (Oliveira *et al*., 2008). For instance, the secondary character of the wine is determined by sensory characteristics that arise from the direct action of microorganisms on the substrate (Raspor *et al*., 2001; Wlodarczyk *et al*., 2015).

The screening of autochthonous yeasts of enological importance was considered to be an effective strategy in exploring unique organoleptic and flavour profile of wine produced from minor fruits. In the present investigation morphological and biochemical characterization was carried out for autochthonous yeasts isolated from minor fruits surface and naturally fermented musts. Total of fifty five autochthonous yeasts were isolated and distinguished with unique features.

Materials and Methods

Collection of yeast samples

Indigenous yeasts were isolated from the wild edible minor fruits from various locations of the Uttara Kannada district of Karnataka and the Nilgiris district of Tamil Nadu. The yeast samples collected from the fruit surface using sterile swabs were immediately suspended in 0.2% of Peptone water. Meanwhile, fruits collected in the sterile polyethylene bags were squeezed and transferred to sterile conical flasks fitted with airlocks were subjected for spontaneous fermentation to isolate autochthonous yeast diversity.

Isolation of autochthonous yeasts from fruit surface and wine sample

The autochthonous microbial samples collected in the peptone water were immediately subjected to serial dilution of 10^{-1} to 10^{-8} as per the standard procedure and 100µl of each sample from each test tube were poured on MGYP agar plates (Malt extract - 3 g/L) and these plates were kept for 4 days of incubation at 28^0C. Around three to five yeasts colonies were obtained from each plate and among them, pure colonies which resemble yeast characters were subcultured using MGYP broth (0.3 g Malt extract, 1.0 g Glucose, 0.3 g of Yeast extract, and 0.5 g of Peptone) and followed by streaking on MGYP plates (Valero *et al*., 2007; Magaratham and Panneerselvam, 2011).

The fruit samples which were collected in the sterile polyethylene bags were squeezed under the sterile condition and kept for 21 days of spontaneous fermentation. The wine samples from initial, mid and final times of interval collected and subjected to serial dilution from 10^{-1} to 10^{-8} and the aliquots of each dilution were spread by spread plate technique on MGYP agar plates with the same composition and were kept for the 4-6 days of incubation at 28^0C and after the incubation around two to four yeast colonies obtained from each spontaneous wine samples and these were further subcultured in MGYP plates (Cletus *et al*., 2011). Further, all the pure culture obtained from both fruit surface and spontaneous wine samples were preserved in a mixture of 37.5% glycerol and 5% sucrose at -80^0C until further characterization.

Identification of autochthonous yeasts based on morphological features

The potential yeast colonies isolated from minor fruits were further analyzed by microscopic and Scanning Electron Microscopy (SEM). For the microscopic studies the slides were prepared by following simple staining procedure using lactophenol cotton blue stain and for scanning electron microscopy the samples were prepared as per the standard protocol where 1ml of overnight grown culture was washed with 1X PBS (pH-7.4) then 2% of 1ml glutaraldehyde was added to it and incubated for 12hrs. Further samples were centrifuged at 7000 rpm for 10 min, the pellet was washed serially with 10-100% of ethanol and centrifuged, finally 50ml of 100% ethanol was added to pellet and drop of sample was smeared on a coverslip and stored in desiccators for overnight and then subjected for SEM analysis.

Glucose fermentation test

The yeast isolates were tested for their ability to ferment glucose. For the fermentative test, isolate was inoculated into a test tube containing inverted Durham tube and peptone water containing 1-4% of glucose and a drop of Andrade's indicator. It was incubated for 48-72 hrs, change in color from pink to yellow, as well as liberation and trapping of gas in the Durham's tube indicate the result of each test; the presence of gas was taken as evidence of a reasonably high rate of fermentative activity.

Characterization of yeast isolates based on biochemical features

Carbon utilization test was performed using MYP agar supplemented with various carbon sources such as glucose, maltose, sucrose, fructose, lactose, D-xylose and D-mannitol. The yeast suspension culture was inoculated and incubated at 28°C for 6 days. Similar way nitrogen source utilization test was performed using potassium nitrate [KNO_3], ammonium sulphate [$(NH_4)_2SO_4$] and lysine as these are the key supplements used in characterization of wine yeasts. The method for growth assay is similar for both the analysis. Yeast colonies formed by the successive tests were comprehensively observed and recorded for further analysis (Lentz *et al*., 2014).

Results and Discussion

The indigenous yeast populations were successfully isolated and characterized based on morphology and biochemical features. The fruit surface and wine samples from spontaneous fermentation of minor fruits witness diverse yeast population with distinct characters. Table 1 shows source of origin of various yeast isolates. The total of fifty five yeasts were isolated and subjected to microscopic studies by examining individual colony and cell structures. Predominantly yeast isolates showed colony colours such as cream, white, light pink and distinguishable colony shapes such as globose, circular, and round. Further, all the yeast colonies were examined for individual cell morphology mainly cell shape such as ovoid, ellipsoidal and elongate. There is no filament was observed in any of yeast isolates and all the isolates exhibit budding cells (Olowonibi, 2017). Table 2 explains colony and cell morphological details of indigenous yeasts of minor fruit.

The utilization of glucose during fermentation was observed by change in color of Andrade's indicator used

Table 1: Potential autochthonous yeasts and their source of origin.

Fruit Source of Origin	Sampling Location	Isolate Code
*Glycosmis pentaphylla (*Retz.) DC.	Supa (UK)	GPRH2
		GPRH6
		GPRH10
Elaeagnus conferta Roxb.	Udhagai (N)	ECRH4
		ECRH16
Ziziphus rugosa Lam.	Yellapur (UK)	ZRRH8
		ZRRH18
Syzygium cumini (L.) Skeels	Sirsi (UK)	SCRH3
		SCRH19
*Syzygium caryophyllatum (*L.) Alston	Sirsi (UK)	SCARH5
		SCARH23
Aporosa cardiosperma (Gaertn.) Merr.	Sirsi (UK)	ACRH1
		ACRH9
Carissa spinarum L.	Haliyal (UK)	CSRH7
Syzygium jambos (L.) Alston	Siddapur (UK)	SJRH13
Bridelia retusa (L.) A. Juss.	Yellapur (UK)	BRRH11
Garcinia indica (Thouars) Choisy	Ankola (UK)	GIRH12
Rubus ellipticus Sm.	Udhagai (N)	RERH14
Flacourtia montana J. Graham	Supa (UK)	FMRH15
Rhodomyrtus tomentosa (Aiton) Hassk.	Udhagai (N)	RTRH17
Leea indica (Burm. f.) Merr.	Sirsi (UK)	LIRH20
Rubus niveus Thunb.	Udhagai (N)	RNRH24
Mangifera indica L.	Haliyal (UK)	MIRH21
Prunus domestica L.	Coonoor (N)	PDRH22

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Table 2: Colony morphology of autochthonous yeasts of minor fruits.

Yeasts Samples	Colony Colour	Colony Shape	Colony Surface	Elevation	Cell Shape	Colony Size (mm)
GPRH2	White	Circular	Smooth	Convex	Ovoid	2.0-3.1 × 1.6-1.8
GPRH10	Cream	Oval	Smooth	Flat	Ovoid	3.5-3.8 × 2.6-2.8
GPRH13	Cream	Circular	Wrinkled	Flat	Ellipsoidal	2.5-2.8 × 2.0-2.1
GPRH6	Cream	Circular	Smooth	Flat	Ovoid	2.2-2.6 × 1.5-1.7
GPRH8	Cream	Circular	Rough	Undulate	Ovoid	3.2-3.5 × 2.4-2.8
ECRH4	Cream	Circular	Smooth	Flat	Ovoid	4.1-4.2 × 2.6-2.8
ECRH9	Cream	Oval	Wrinkled	Undulate	Ovoid	3.1-3.3 × 2.2-2.4
ECRH16	Cream	Circular	Wrinkled	Flat	Ovoid	2.4-2.9 × 2.0-2.3
ECRH15	White	Circular	Smooth	Flat	Ellipsoidal	2.5-2.8 × 2.0-2.2
ZRRH8	Cream	Oval	Smooth	Flat	Ellipsoidal	3.1-3.2 × 2.7-2.9
ZRRH18	White	Circular	Rough	Flat	Ovoid	1.0-2.0 × 3.0-3.6
ZRRH12	Cream	Circular	Rough	Flat	Ellipsoidal	2.6-2.8 × 2.0-2.2
SCRH3	Cream	Circular	Smooth	Flat	Ovoid	2.0-4.0 × 4.0-6.0
SCRH19	Cream	Round	Rough	Flat	Ellipsoidal	3.5-3.7 × 2.6-2.8
SCRH15	Cream	Circular	Smooth	Flat	Ovoid	2.6-2.9 × 2.0-2.1
SCARH5	Cream	Round	Rough	Flat	Ovoid	2.0-4.0 × 4.0-8.0
SCARH9	Cream	Oval	Wrinkled	Undulate	Ovoid	2.2-3.3 × 1.5-1.9
SCARH23	White	Circular	Smooth	Flat	Ovoid	3.4-3.7 × 2.6-2.8
ACRH1	Cream	Round	Rough	Flat	Ovoid	4.0-5.0 × 5.0-8.0
ACRH3	Cream	Circular	Smooth	Flat	Ovoid	2.6-2.8 × 2.0-2.3
ACRH9	Cream	Oval	Wrinkled	Undulate	Ovoid	3.1-3.3 × 2.2-2.4
CSRH7	Cream	Circular	Wrinkled	Flat	Ovoid	2.0-3.1 × 1.6-1.8
CSRH5	White	Circular	Smooth	Flat	Ellipsoidal	3.5-3.8 × 2.6-2.8
CSRH13	White	Circular	Smooth	Flat	Ovoid	2.5-2.8 × 2.0-2.1
BRRH11	White	Circular	Smooth	Flat	Ellipsoidal	4.1-4.2 × 2.6-2.8
BRRH10	Cream	Oval	Wrinkled	Flat	Ovoid	3.1-3.3 × 2.2-2.4
BRRH8	Cream	Circular	Smooth	Flat	Ellipsoidal	2.4-2.8 × 2.0-2.2
GIRH12	White	Circular	Smooth	Flat	Ovoid	3.2-3.4 × 2.2-2.5
GIRH10	Cream	Oval	Wrinkled	Flat	Ovoid	2.4-2.8 × 2.0-2.2
GIRH7	Cream	Circular	Smooth	Flat	Ellipsoidal	3.2-3.4 × 2.2-2.6
GIRH9	Cream	Circular	Rough	Flat	Ellipsoidal	3.1-3.4 × 2.2-2.5
RERH2	Cream	Circular	Smooth	Flat	Ovoid	2.5-2.9 × 2.0-2.3
RERH8	Cream	Round	Rough	Flat	Ellipsoidal	3.1-3.5 × 2.2-2.4
RERH14	Cream	Round	Smooth	Convex	Ellipsoidal	2.4-2.9 × 2.0-2.3
FMRH15	Cream	Round	Smooth	Convex	Ellipsoidal	2.4-2.8 × 2.0-2.5
FMRH16	Cream	Oval	Wrinkled	Flat	Ovoid	3.1-3.4 × 2.2-2.6
RTRH17	Cream	Circular	Smooth	Flat	Ellipsoidal	2.4-2.8 × 2.0-2.3
RTRH12	Cream	Oval	Wrinkled	Flat	Ovoid	2.3-2.7 × 2.1-2.5
RTRH7	Cream	Circular	Smooth	Flat	Ellipsoidal	3.2-3.3 × 2.2-2.4
LIRH20	Cream	Round	Smooth	Convex	Ellipsoidal	2.0-3.1 × 1.6-1.8
LIRH12	Cream	Round	Rough	Flat	Ellipsoidal	2.4-2.8 × 2.0-2.1
LIRH8	Cream	Round	Rough	Flat	Ovoid	3.6-3.8 × 2.6-2.8
LIRH11	White	Circular	Smooth	Flat	Ovoid	2.4-2.8 × 2.0-2.1
RNRH24	Cream	Round	Smooth	Convex	Ellipsoidal	2.2-2.6 × 1.5-1.7
RNRH12	Cream	Oval	Wrinkled	Flat	Ovoid	3.3-3.5 × 2.4-2.8
MIRH21	Cream	Circular	Smooth	Flat	Ellipsoidal	2.4-2.6 × 1.5-1.7
MIRH3	White	Circular	Smooth	Flat	Ovoid	3.2-3.5 × 2.4-2.8

Table 2 continued .....

Table 2 continued .....

Yeasts Samples	Colony Colour	Colony Shape	Colony Surface	Elevation	Cell Shape	Colony Size (mm)
PGRH6	Cream	Circular	Smooth	Flat	Ellipsoidal	2.2-3.1 × 1.7-1.9
PDRH22	Cream	Round	Rough	Flat	Ovoid	3.6-3.8 × 2.5-2.9
PDRH8	White	Circular	Smooth	Flat	Ovoid	2.6-2.9 × 2.0-2.2
PDRH12	Cream	Round	Rough	Flat	Ellipsoidal	1.8-2.2 × 2.1-2.3
SJRH13	Cream	Round	Smooth	Convex	Ovoid	2.1-3.2 × 2.1-2.3
MSRH21	Cream	Circular	Smooth	Flat	Ovoid	2.2-3.3 × 1.5-1.9
MSRH6	Cream	Round	Rough	Flat	Ovoid	3.4-3.7 × 2.6-2.8
MSRH3	White	Circular	Smooth	Flat	Ovoid	2.6-2.9 × 2.0-2.2

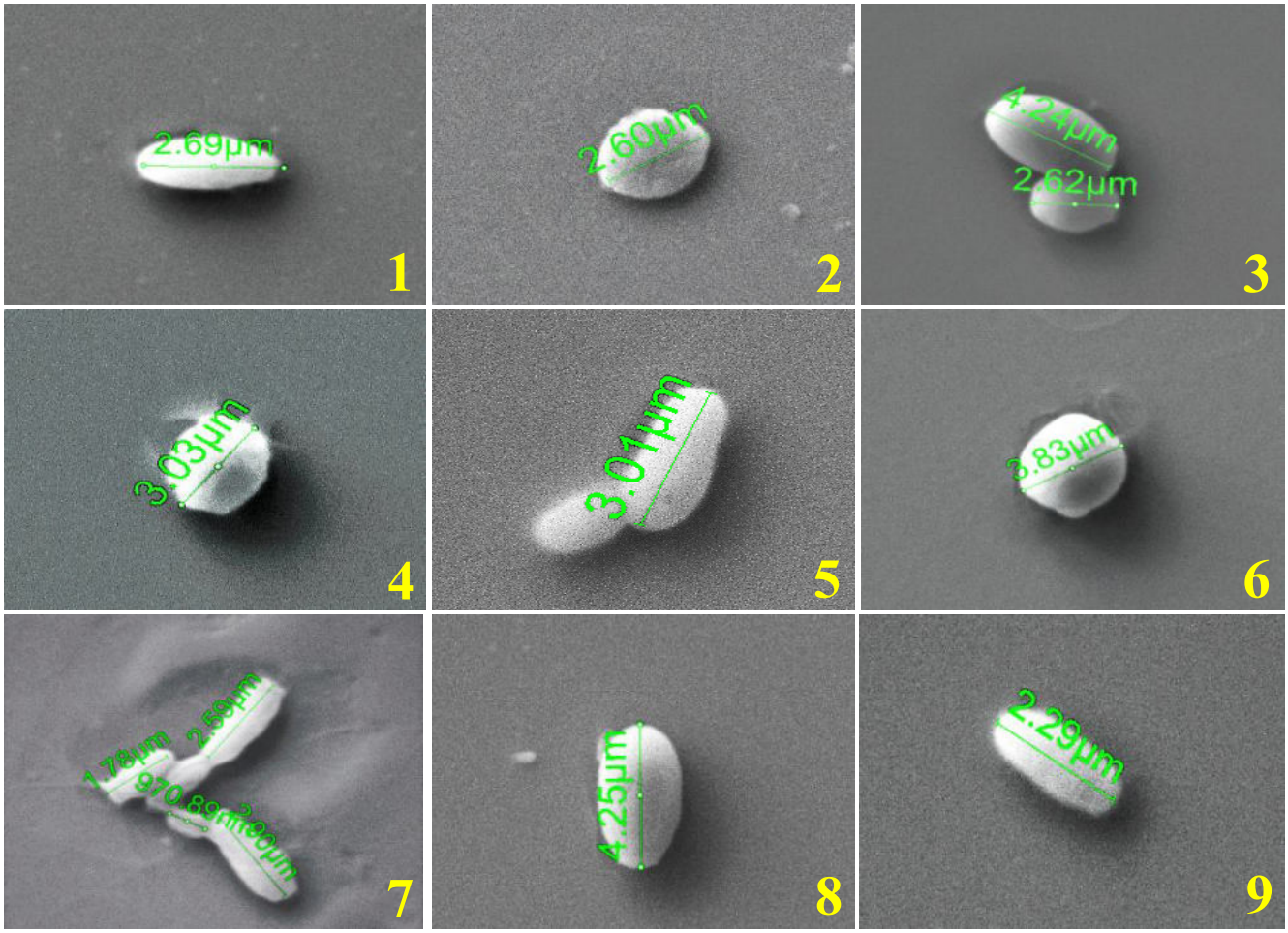

Fig. 1: SEM images of few autochthonous yeasts isolated from minor fruits. 1: ECRH16, 2: GPRH2, 3: ECRH4, 4: ZRRH8, 5: ACRH9, 6: GPRH10, 7: ZRRH18, 8: LIRH20, 9: SCRH3.

in this test, and by production of gas which was trapped in the inverted Durham's tubes. The yeast isolates GPRH2, GPRH10, ECRH4, ECRH16, ZRRH8, SCRH3, SCARH23, CSRH7, SJRH13, RERH14, LIRH20, MIRH21 and PDRH22 observed to be potential glucose fermenting yeasts. Furthermore, biochemical characterization performed by carbon and nitrogen source utilization where these tests are generally used for routine identification purpose for yeasts (Kurtzman *et al*., 2011). The yeasts are capable of utilizing wide array of carbon and nitrogen sources. However, the utilization of nitrite, nitrate, ethylamine, lysine as a sole source of nitrogen is the most routinely used tests. The ability to utilize nitrate and lysine was an important criterion in defining and distinguishing some novel genera. The fifty five yeast isolates were subjected to biochemical analysis, out of which, twenty four isolates shown good growth dynamics as they exhibited moderate to luxuriant growth at various biochemical supplements. The yeast isolates ECRH4, GPRH10, ECRH16, SCARH23 and ZRRH8 as they

Table 3: Biochemical characterizations of indigenous yeasts through carbon and nitrogen source utilization.

Yeast isolates	Carbon source							Nitrogen source		
	Glucose	Maltose	Sucrose	Fructose	Lactose	D-Xylose	D-Mannitol	KNO_3	$(NH_4)_2SO_4$	Lysine
GPRH2	++	++	++	++	+	-	+	+	++	+
GPRH10	++	+++	++	+++	+	+	+	-	+	-
GPRH13	+	-	-	+	-	-	-	-	+	+
GPRH6	++	+	++	++	-	-	+	++	+	-
GPRH8	++	+	-	+	+	-	+	+	+	-
ECRH4	+++	+++	++	+++	+++	++	++	+	-	-
ECRH9	+	+	+	+	++	+	++	+	+	+
ECRH16	++	++	+	++	++	++	++	+	++	-
ECRH15	+	+	++	+	+	+	+	-	+	+
ZRRH8	+++	++	+++	+++	++	++	+	+	+	-
ZRRH18	++	++	++	++	+	-	+	++	+	-
ZRRH12	+	+	++	-	++	+	+	+	-	+
SCRH3	++	++	+	++	-	+	+	+	++	-
SCRH19	++	++	++	+	++	++	-	++	++	-
SCRH15	+	+	+	-	-	+	-	-	+	+
SCARH5	++	++	++	-	++	+	+	++	+	+
SCARH9	+	+	-	+	-	+	+	+	+	-
SCARH23	++	++	++	-	++	+	-	++	+	-
ACRH1	++	++	++	+	-	++	+	+	++	+++
ACRH3	+	+	-	+	+	-	+	+	-	+
ACRH9	++	++	++	++	+	++	+	++	++	-
CSRH7	++	+++	++	++	+	-	++	++	++	-
CSRH5	+	+	-	+	+	-	+	+	+	+
CSRH13	++	+	++	+	+	+	+	++	++	+
BRRH11	++	++	++	++	+	+	+	+	++	-
BRRH10	+	+	++	+	-	+	-	+	+	-
BRRH8	+	+	+	-	-	-	+	-	-	+
GIRH12	++	+	++	++	+	+	-	+	++	-
GIRH10	+	+	-	+	-	+	+	+	+	++
GIRH7	+	++	+	-	+	+	+	++	+	+
GIRH9	+	-	-	+	+	+	+	-	+	-
RERH2	-	+	+	+	-	+	+	+	+	+
RERH8	+	+	-	+	-	+	+	+	+	-
RERH14	+++	+++	+++	++	+	++	+	++	++	-
FMRH15	++	+++	++	++	+	-	++	++	+	-
FMRH16	+	+	-	+	+	-	+	+	+	+
RTRH17	++	++	+	++	++	++	++	+	++	+++
RTRH12	+	-	+	++	-	+	-	+	+	-
RTRH7	+	+	+	+	-	+	+	-	-	+
LIRH20	++	++	+	++	++	++	++	++	++	-
LIRH12	+	+	++	+	-	+	-	+	+	-
LIRH8	+	+	+	-	-	-	+	-	-	+
LIRH11	+	+	++	+	-	+	-	+	+	-
RNRH24	++	+++	+	++	++	++	++	+	++	++
RNRH12	+	++	+	-	+	+	+	++	-	+
MIRH21	+++	++	+	++	+	++	++	+	++	-

Table 3 continued .....

Table 3 continued .....

MIRH3	-	+	+	+	+	-	-	+	-	+
PGRH6	+	++	+	-	+	+	+	++	-	+
PDRH22	+++	++	+	+++	+	++	++	-	++	++
PDRH8	-	+	+	+	+	-	-	+	-	-
PDRH12	+	+	-	++	-	+	+	+	+	-
SJRH13	+++	+	++	++	++	+++	+	+	++	-
MSRH21	+	+	+	+	+	+	+	-	+	-
MSRH6	-	+	+	+	+	-	-	+	-	+
MSRH3	+	+	+	++	+	-	+	+	+	+

+: normal growth; ++: moderate growth; +++: robust growth

exhibited good glucose fermentation activity and good growth dynamics in utilization of several sugars such as sucrose, fructose, maltose, xylose, and lactose, these isolates were preliminarily characterized as *Saccharomyces* spp. While other majority of the isolates which shows positive response to lysine and potassium nitrate utilization confirmed as *Pichia* and other related species. Olowonibi (2017) also found similar results while identifying palm wine yeasts.

Some of the isolates that could not utilize carbon sources, no further tests were performed on them. From the view of brewing technology, the most significant observation of survey was that most of the examined isolates were lysine-negative and that many other yeasts of known occurrence as brewery contaminants were lysine positive. These findings suggest that lysine test should be incorporated in microbiological examination of selecting wine starter cultures. Table 3 shows the biochemical characterization of indigenous yeast isolates through carbon and nitrogen source utilization. In the present study, strains which were identified in the microscopic and biochemical studies were further subjected to SEM analysis where distinct morphological features are comprehensibly observed. The length and width of the yeast cells recorded and ranges between 1.0-2.0 × 4.0-6.0 µm and similar SEM results were observed by Lentz *et al*. (2014). Fig. 1 shows SEM images of autochthonous yeast cells.

Conclusion

The current study revealed that the minor fruits from the Western Ghats region were the richest sources for winery yeast isolates. In this study, out of fifty five isolates twenty four potential yeast isolates exhibit good growth dynamics by utilizing various biochemical sources and fermentation tests. Based on morphological and biochemical studies five potential yeast isolates such as ECRH4, GPRH10, ECRH16, SCARH23 and ZRRH8 were tentatively characterized as *Saccharomyces* spp. and remaining as non-*Saccharomyces* spp. Furthermore, molecular characterization and screening for oenological importance will be carried out for their role in regional wine production. This lays ground to explore wine yeast phenotype equally important as environmental conditions.

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References

Aponte, M. and G. Blaiotta (2016). Selection of an autochthonous *Saccharomyces cerevisiae* strain for the vinification of Moscato di Saracena, a southern Italy (Calabria Region) passito wine. *Food Microbiology*, **54**:30-39.

Archana, K.M., R. Ravi and K.A. Anu Appaiah (2016). Autochthonous yeasts: role in vinification and aging of Cabernet-Sauvignon. *International Journal of Environmental and Agriculture Research*, **2(8):**53-61.

Capece, A., R. Romaniello, G. Siesto and P. Romano (2012). Diversity of *Saccharomyces cerevisiae* yeasts associated to spontaneously fermenting grapes from an Italian heroic vine-growing area. *Food Microbiology*, **31(2)**:159-166.

Capece, A., R. Romaniello, R. Pietrafesa, and P. Romano (2014). Indigenous *Saccharomyces cerevisiae* yeasts as a source of biodiversity for the selection of starters for specific fermentations. In: BIO Web of Conferences, 3: 02003. EDP Sciences. doi.org/10.1051/bioconf/20140302003

Chavan, P., S. Mane, G. Kulkarni, S. Shaikh, V. Ghormade, D.P. Nerkar and M.V. Deshpande (2009). Natural yeast flora of different varieties of grapes used for wine making in India. *Food Microbiology*, **26(8)**:801-808.

Chovanová, K., L. Kraková, K. Ženišová, V. Turcovská, B. Brežná, T. Kuchta and D. Pangallo (2011). Selection and identification of autochthonous yeasts in Slovakian wine samples using a rapid and reliable three-step approach. *Letters in Applied Microbiology*, **53(2)**:231-237.

Desai, M.V., K.V. Dubey, K.V. Vakil and V.V. Ranade (2013). Isolation, identification and screening of the yeast flora

from Indian Cashew Apple for sugar and ethanol tolerance. *International Journal of Biotechnology for Wellness Industries*, **1(4)**:259-265.

Ebabhi, A.M., A.A. Adekunle, W.O. Okunowo and A.A. Osuntoki (2013). Isolation and characterization of yeast strains from local food crops. *Journal of Yeast and Fungal Research*, **4(4)**:38-43.

Esteve-Zarzoso, B., P. Manzanares, D. Ramon and A. Querol (1998). The role of non-*Saccharomyces* yeasts in industrial winemaking. *International Microbiology*, **1(2)**:143-148.

Hyma, K.E., S.M. Saerens, K.J. Verstrepen and J.C. Fay (2011). Divergence in wine characteristics produced by wild and domesticated strains of *Saccharomyces cerevisiae*. *FEMS Yeast Research*, **11(7)**:540-551.

Jolly, N.P., O.P.H. Augustyn and I.S. Pretorius (2006). The role and use of non- *Saccharomyces* yeasts in wine production. *South African Journal for Enology and Viticulture*, **27(1)**:12-18.

Kurtzman, C.P., J.W. Fell, T. Boekhout and V. Robert (2011). Methods for isolation, phenotypic characterization and maintenance of yeasts. In *The yeasts*, Elsevier. pp. 87-110.

Lentz, M., T. Putzke, R. Hessler and E. Luman (2014). Genetic and physiological characterization of yeast isolated from ripe fruit and analysis of fermentation and brewing potential. *Journal of the Institute of Brewing*, **120(4)**:559-564.

Maragatham, C. and A. Panneerselvam (2011). Isolation, identification and characterization of wine yeast from rotten papaya fruits for wine production. *Advances in Applied Science Research*, **2(2)**:93-98.

Ndip, R.N., J.F. Akoachere, L.L. Dopgima and L.M. Ndip (2001). Characterization of yeast strains for wine production. *Applied Biochemistry and Biotechnology*, **95(3)**:209-220.

Obasi, B.C., C.M.Z., Whong, S.A. Ado and I.O. Abdullahi (2014). Isolation and Identification of yeast associated with fermented orange juice. *The International Journal of Engineering and Science*, **3(9)**:64-69.

Oliveira, V.A., M.A. Vicente, L.G. Fietto, I. de Miranda Castro, M.X. Coutrim, D. Schüller and P.H.A. da Silva (2008). Biochemical and molecular characterization of *Saccharomyces cerevisiae* strains obtained from sugar-cane juice fermentations and their impact in cachaça production. *Applied and Environmental Microbiology*, **74(3)**:693-701.

Olowonibi, O.O. (2017). Isolation and characterization of palm wine strains of *Saccharomyces cerevisiae* potentially useful as bakery yeasts. *European Journal of Experimental Biology*, **7**:2-11.

Raspor P., S.S. Mozina, N. Cadez (2001) Identification of yeasts from the grape/must/wine system. In: Spencer J.F.T., de Ragout Spencer A.L. (eds), *Food Microbiology Protocols*, **Vol. 14**. Humana Press. pp. 243-257.

Ribéreau-Gayon, P., D. Dubourdieu, B. Donèche and A. Lonvaud (2006). *Handbook of enology: The microbiology of wine and vinifications*, **vol. 1**. John Wiley & Sons. pp. 12-45.

Romano, P., C. Fiore, M. Paraggio, M. Caruso and A. Capece (2003). Function of yeast species and strains in wine flavour. *International Journal of Food Microbiology*, **86(2)**: 169-180.

Satora, P. and T. Tuszyñski (2010). Influence of indigenous yeasts on the fermentation and volatile profile of plum brandies. *Food Microbiology*, **27(3)**:418-424.

Sun, H., H. Ma, M. Hao, I.S. Pretorius and S. Chen (2009). Identification of yeast population dynamics of spontaneous fermentation in Beijing wine region, China. *Annals of Microbiology*, **59(1)**:69-76.

Tristezza, M., C. Vetrano, G. Bleve, F. Grieco, M. Tufariello, A. Quarta and G. Spano (2012). Autochthonous fermentation starters for the industrial production of Negroamaro wines. *Journal of Industrial Microbiology and Biotechnology*, **39(1)**:81-92.

Valero, E., B. Cambon, D. Schuller, M. Casal and S. Dequin (2007). Biodiversity of *Saccharomyces* yeast strains from grape berries of wine-producing areas using starter commercial yeasts. *FEMS Yeast Research*, **7(2)**:317-329.

Vigentini, I., D. Maghradze, M. Petrozziello, F. Bonello, V. Mezzapelle, F. Valdetara and R. Foschino (2016). Indigenous Georgian wine-associated yeasts and grape cultivars to edit the wine quality in a precision oenology perspective. *Frontiers in Microbiology*, **7**:352. doi: 10.3389/fmicb.2016.00352

Wlodarczyk, S.R., F. Gaensly, R. Calegari-Santos, F. Deffert, G.A. da Silva and T.M.B. Bonfim (2015). Characterization of autochthonous wine yeasts isolated in vineyards of the State of Paraná, Brazil. *Acta Scientiarum*, **37(3)**:361-365.

Wu, Q., J, Ling and Y. Xu (2014). Starter culture selection for making Chinese sesame-flavoured liquor based on microbial metabolic activity in mixed-culture fermentation. *Applied and Environmental Microbiology*, **80(14)**:4450-4459.