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# Isolation of saccharomyces cerevisiae for quality wine preparation from pineapple and morphological, biochemical and physiochemical characterization of isolated yeast

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**Abstract**
An experiment on isolation and identification of yeast Saccharomyces cerevisiae and characterization of yeast from pineapple fruit Ananas comosus was carried out at Biotech. Laboratory at VNCAB, Yavatmal during the year 2018-2019. The experiment was carried out with an objectives to identify and isolate the yeast strains from rotten pineapple juice having good fermentation activity for quality wine preparation. In the experiment, yeasts isolated from rotten pineapple juice were inoculated and grown on YEPD media plate. The isolated yeast strains were characterized through various morphological, physiochemical and biochemical parameters for confirmation of isolated yeast strain for Saccharomyces cerevisiae. The two different strains isolated from rotten pineapple juice of S. cerevisiaePI-1 and PI-2 were also studied for fermentation quality, volatile acid and alcohol production. Based on the physiochemical tests like pH, Temp, TSS, TAA, Alcohol %, Antibiotic resistant, salt tolerant analysis. From the experimental results obtained for pH, Temp, TSS, TAA, Alcohol %, Antibiotic resistant, salt tolerant analysis, the isolate pineapple strain PI-1 of Saccharomyces cerevisiae was found to be most suitable for fermentation activity. This study put forward that isolated yeast strain PI-1 could be used to optimize the rate of fermentation for quality wine preparation.

**Keywords:** Yeast, pineapple, YEPD media, isolates, PI-1, PI-2, fermentation.

## Introduction
Yeast as classified under the kingdom fungus is a eukaryotic single-celled microorganisms varies from species to species and environment typically measuring 3–4 µm in diameter. Yeast has its own commercial importance in two major sectors as confectionery and winery.

Winery industry is paying more attention towards the yeast species that have good fermentation ability and thus have a bright scope for isolation of beneficial yeast. The strains of yeast species *Saccharomyces cerevisiae* has made it a model organism of study in both research and industrial applications, fermenting wild yeast species are being isolated from the natural sources for over decades and is being used in various fermentation processes.

The Pineapple (*Ananas comosus*) is a tropical plant with an edible fruit and the most economically significant plant in the family Bromeliaceae It contains a proteolytic enzyme called bromelain that break down protein and helps in drug digestion (Tochi *et al.,* 2008) [11]. Pineapple were used as a source for isolation of yeast Saccharomyces cerevisiae various research workers, supported by Sossou Nasir Armanul *et al.,* (2017) [4]. Liyanage W. *et al.,* (1985) [14]. and Amorim J.C. *et al.,* (2018) [1].

In the experiment carried out the for the isolation and identification of yeast strain from rotten pineapple juice and its analysis for fermentation activities is an important strategy for keeping the quality and assuring the reproducibility of quality wine. The pineapple used as source for isolation of yeast as all the year-round availability shows good quality of required nutritive value in it. The isolated yeast strains were characterized by various test at variable parameters for confirmation of isolated yeast strain of *Saccharomyces cerevisiae* and selection of one desirable high fermentative capability yeast strain. Thus the objective of this experiment carried out is to isolate, identify and characterize the isolated yeast strain followed by comparative fermentative performance study of isolated yeast to find out the most applicable and safe strain of yeast Saccharomyces cerevisiae for winery industry.

## Materials and method

In the experiment, to isolate the yeast from rotten pineapple juice and its identification and characterization following methods were adopted.

**Media:** The culture media utilized in the experiment for isolation of yeast was YEPD consisting of its constituents as Yeast extract 10g/L. Then, dextrose 20g/L and 20g of peptone was added in 1L of water. Then lastly, 15g of Agar were added in the flask make up the media of 1 lit. with added neem extract and chloramphenicol and nalidixic acid as resistant source of antibiotic.

### Sample collection and isolation

The rotten pineapple fruits of variety Giant Queen were used for juice extraction. The yeast isolates were isolated from extracted rotten pineapple juice. For the said, 1mlof extracted juice sample was inoculated on the YEPD media plates and kept for incubation at 37 °C for 72hours. The colonies grown on the media plate were studied and were to be known as two different colonies of yeast. These two isolated colonies were inoculated onto two different YEPD media plates to prepare pure culture of both colonies and the obtained pure culture was denoted as PI-1 and PI-2. Further the isolates were characterized by various tests for its nobility as yeast strain of Saccharomyces cerevisiae.

### Characterization of isolated yeast strains

**1) Morphological test**

Firstly, both the pure culture colonies PI-1 and PI-2 grown on the YEPD media plates were observed on to the inverted microscope for primary identification of yeast colony grown on the basis of shape size and colour. After that, prepared the slides by making smear of both the isolated colonies PI-1 and PI-2 followed by staining on the slides and results were observed under the microscope.

**2) Biochemical test**

**A) Citrate test:** The test was carried by inoculating both the isolated colonies PI-1 and PI-2 on the media consisting 24.8g christensen citrate agar in 1L of distilled water and allowed to grow and incubate at 37 °C for 48 hrs.

**B) Urease test:** The test was carried by inoculating both the isolated colonies PI-1 and PI-2 on the media consisting 20.30g/L of urea and allowed to grow and incubate at 37 °C for 48 hrs.

**C) Fermentation of carbohydrates:** The test was carried by inoculating both the isolated colonies PI-1 and PI-2 on the 10 different media plates consisting of 5 different sugars of 200B init and allowed to grow under incubation temperature 21OC to study the utilization of suitable carbon energy source for high cell growth.

**D) Hydrogen sulphide test:** The test was carried by inoculating both the isolated colonies PI-1 and PI-2 on the media consisting sulphur compound required for H2S production. Test was carried out to check the sulphur reduction to H2S by the isolates PI-1 and PI-2.

**E) Killer toxic test:** Test was carried out for isolate PI-1 and PI-2 against the E. coli. Presence of clear inhibition zone by microbial growth in a Petri dish is depicted as E. Coli colonies. However, presence of yeast S. cerevisiae do not produce clear zone in petri dish. The isolates as not shown clear zone confirms the presence of yeast Saccharomyces cerevisiae.

**F) Antibiotic resistance test:** The test was carried out by using isolates PI-1 and PI-2 to study the resistance of isolated strains on media containing chloramphenicol and nalidixic acid. Characteristics for resistivity for chloramphenicol and Nalidixic acid were observed to confirm the isolates to be of yeast Saccharomyces cerevisiae.

**G) Ethanol tolerance test:** The test was carried out individually with isolates PI-1 and PI-2 which were inoculated in 10ml of liquid YEPD broth added with ethanol about 5% and 10 % concentration into the 4 media plates to study the growth of isolates at variable ethanol %.

**H) Flocculation:** The test was carried out individually with isolates PI-1 and PI-2 which were inoculated in 10ml pineapple juice separately to carry out small scale fermentation setup to study the yeast cell flocculation density during fermentation.

**3) Physiochemical tests:**

**A) pH:** The test was carried out by isolates PI-1 and PI-2 to study the effect on growth of isolates by growing them on YEPD media plate at variable pH ranging from 2 to 7.

**B) Temperature:** The test was carried out by growing isolates PI-1 and PI-2 on YEPD media plate at variable temperatures 25 °C, 30 °C, 32 °C, 37 °C, 40 °C and 44 °C to study the effect of temperature on growth of isolates and confirmation of yeast.

**C) Salt concentration:** The isolates PI-1 and PI-2 were subjected to grow on YEPD media plates added with variable NaCl salt concentrations 6, 9, 12, 15, 18, and 20 to study the effect of salt concentrations on growth of yeasts isolates.

### 3.1 Results of characterization and selection of pineapple isolate.

**a. Characterization**

General technological characteristics was studied to characterize the isolated yeasts and judge the suitability of the yeasts for wine production.

**1. Fermenting power:** It was a standard criterion to check the fermenting power of isolated strain determining the maximum quantity of sugar that the isolated yeast strain were capable of fermenting in rich medium.

**2. Foam production:** The foam production by yeast was a dependent characteristic that occur during the wine fermentation depending on cell wall hydrophobicity. The absence of foam production or low foam production and fermentations in wine are considered as positive traits for yeast Saccharomyces cerevisiae.

**Technological characteristics for white vinefication**

**Capabilities to ferment highly clarified must with low level of assailable nitrogen [NFA]** White wine prepared from the isolated samples PI-1 and PI-2 from pineapple juice that resulted in stuck fermentation. It was observed that pineapple PI-1 and PI-2 both were found capable at fermentation with

100mg.L-1 nitrogen and thus found suitable for characterization.

**1. Volatile acid production:** The production of volatile acid was result of fermentation occurrence into the juice carried out by the isolates present in it. The isolates with good fermentation rate undergo production of acids and which enhance the taste of wine the isolates producing required percentage of volatile acids will be selected for wine production.

**2. Alcohol production test of pineapple isolates PI-1 and PI-2:** Alcohol was one of the most important constituent in wine. Both the isolated yeast strains PI-1 and PI-2 were kept for fermentation in pineapple juice for 48 hrs and the strain producing the high % of alcohol will be selected for comparative study of wine prepared from the isolated yeast and market yeast.

**Selection of suitable pineapple isolate for wine making**

On the basis of the physiochemical, biochemical, morphological, strain quality characterization, fermentation capability, relative toxicity absence, edible source and growth rate tests of pineapple isolate with respect to their supportive references to found that the suitability for the wine production the results were compared and presented.

**Result A and Discussion**

**1) Morphological tests**

**I. Shape:** The isolates PI-1 and PI-2 when observed under the inverted microscope showed the circular opaque dot like structures confirmed the positive test for yeast Saccharomyces cerevisiae which were isolated from rotten pineapple juice.

**II. Zone of growth:** The isolates PI-1 and PI-2 observed to be of the same size 0.1 cm however, mass of colonies were observed of varying sizes from 0.3 to 0.8 cm. These confirmed that, both the isolates from rotten pineapple juice belong to yeast Saccharomyces cerevisiae.

**III. Colour:** The isolates PI-1 and PI-2 showed the same pale-yellow off-white colour and red rust colour which confirmed the isolates belongs to yeast Saccharomyces cerevisiae. The results are supported with the findings of Jayata Saha *et al*. 2016 [9]. Who studied on the yeast cell population and morphology structures of yeast.

**2) Biochemical tests:**

**A) Citrate test:** The isolate PI-1 and PI-2 when tested for citrate test, showed colour change from actual orange colour to greenish thus confirmed presence of yeast Saccharomyces cerevisiae for both isolates as used citrate as energy source for growth.

**B) Urease test:** The isolates PI-1 and PI-2 showed that change in colour of media that turned pinkish red from actual yellow colour and thus it detected the presence of Urease activity and confirmed isolates (PIs) on media as Saccharomyces cerevisiae. The identification of yeast was carried out by using standardized Hi-Media test kit for (Saccharomyces cerevisiae).

**C) Carbohydrate fermentation:-** ThePI-1 and PI-2 isolates were allowed to grow on these 5 different type of sugars, Glucose, Sucrose, Lactose, Fructose and Maltose. Both the isolated strains utilized all the sugar but failed to grow on lactose and showed no colour change in lactose thus showing lactose as no source of energy hence, confirmed the isolates to be of Saccharomyces cerevisiae. The result is in accordance with the findings of Nasir Armanul *et al*., 2017 [4].

**D) Hydrogen sulphide test:-** The isolatesPI-1 and PI-2 when grown on YPD media plates, no production of hydrogen sulphide and no change in media colour were detected and thus confirmed the growth of isolates belongs to yeast Saccharomyces cerevisiae. The test results are supported by ONO, B *et al*., (1991) [5]. who studied the Role of hydrosulfideions (H2S) in methyl mercury resistance in Saccharomyces cerevisiae.

**E) Killer toxic test:** Killer toxic tests was carried out against the E. coli. In presence of PI-1 and PI-2 isolates. Clear inhibition zone is depicted as E. coli colonies. However, presence of yeast S. cerevisiae do not produce clear zone in Petri dish. As no clear zone were observed in treated Petri plates, confirmed the presence of yeast Saccharomyces cerevisiae. The above test results are supported by work of Nasir Armanul *et al*., 2017 [4].

**F) Antibiotic resistance test:-** Antibiotic resistance test showed that, both the (PI) strains PI-1 and PI-2 survived on YPD media containing chloramphenicol and nalidixic acid. S. Cerevisiae strain has resistant characteristics for chloramphenicol and Nalidixic acid confirmed the isolates to be of yeast Saccharomyces cerevisiae. The above test results were worked out by pre research workers Nasir Armanul *et al*., 2017 [4]. who confirmed that majority of yeast strains are antibiotic resistant.

**G) Ethanol tolerance test:** In this test, both isolates PI-1 and PI-2on 10 ml of liquid YEPD broth, with 5% ethanol showed very less microbial growth rather than present yeast growth. However, showed no growth to 10% ethanol containing YPD media plates. It may be due to more % of alcohol in 10% media resulted in inhibition of microbial growth and thus confirmed that isolated yeasts on YPD media plates were of Saccharomyces cerevisiae. The response of yeast to ethanol test is supported by Chi, Ameoborg (2006) and Guimoroes *et al*., (2006) who reported the decreased growth of yeast Saccharomyces cerevisiae by ethanol production.

**H) Flocculation test:** In this test, the pineapple isolates PI-1 and PI-2 incubated for 72 hrs at 30oCin 10 ml of liquid YPD medium, and agitated the tubes for visualization of flocculation, there observed flocculation in both the pineapple isolates (PIs) confirmed the isolates to be of yeast Saccharomyces cerevisiae. The results obtained are in accordance with the research work carried out by the Nasir *et al*. 2017 [4]. and who mediated aggregation of yeast flocculation by G. Engin, 1992 [10].

**3) Physiochemical tests**

**Effect of pH-** Table 1 revealed that, the isolates PI-1 and PI-2 showed the high cell densities at pH 4than that of other pH. This confirmed that pH 4 was suitable for the high cell growth of yeast Saccharomyces cerevisiae in YPD liquid broth while the isolates can sustain growth up to pH 7. The above results

for pH test were supported by the Narendranath and Power, 1984 who studied on relationship between pH and medium dissolved solids in terms of growth for yeast.

**Effect of temperature:** The PI-1 and PI-2. isolates showed survival at high temperature (37 °C) in incubator as shown high cell growth of yeast Saccharomyces cerevisiae in YPD media liquid broth (table 2). The above temperature test results were supported by the Shafkat Shamim Rahman, 2016.

**6) Effects of salt concentration:** From table 3, it was revealed that, isolated strains PI-1 and PI-2 have shown higher growth at the salt concentration NaCl 12% then that of other concentrations. This confirmed that the salt concentrations NaCl 12% was suitable for the high cell growth of yeast Saccharomyces cerevisiae in YPD liquid broth and can sustain growth up to NaCl 5% concentration. The above test results are supported by Ortiz-Zamora *et al*., 2009 [6]. who worked on isolation and selection of osmotolerant yeasts from regional agricultural sources in Mexico.

**b. Characterization**

**1. Fermenting power:** The pineapple isolates PI-1 and PI-2 when kept in small 100 ml flasks to undergo fermentation of 20 gm of added sugar; the results obtained for both the isolates reported the successful fermentation of sugar with formation of CO2 and alcohol. PI-1 isolate found more suitable for wine production as completely utilized the sugar and remained active up to 48 hrs. The results obtained in the above test are supported by Vaugham Martini *et al*., 1998 [12].

**2. Foam production:** For this test, both the isolate PI-1 and PI-2 were kept undisturbed for 72 hrs to produce surface foam. Visually low to medium foam were observed in the samples kept with PI-1 isolate as compared to PI-2 isolate. These findings were confirmed with the supportive statement and results confirmed by who confirmed the positivity of yeast with the low foam production.

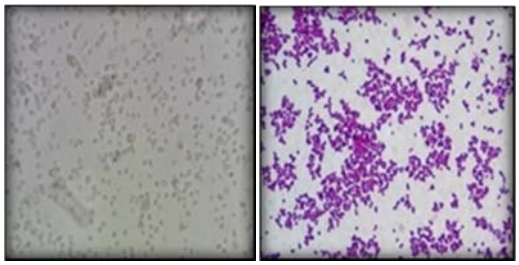
Technological characteristics for white vinefication Capabilities to ferment highly clarified must with low level of assailable nitrogen [NFA]

**1. Volatile acid production:** The isolate PI-1 was found to produced more acidity as compared to PI-2 at varying glucose levels and found superior over PI-2. The findings are in accordance with the results obtained by the research worker.

**2. Alcohol production test:** Both the isolated yeast strains PI-1 and PI-2 were kept for fermentation in pineapple juice for 48 hrs., it was observed that, PI-1 was found to be more stable for alcohol formation at varying juice concentration. However, the PI-2 was found unstable to form consistent alcohol formation at varying glucose level due to which rejected for wine production. The results are supported by the work of Nasir Armanul *et al*., 2017 [4].

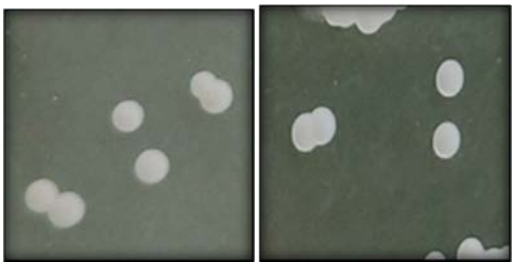
Selection of suitable pineapple isolate for wine making: From all the test conducted morphological, biochemical, physiochemical tests, edible source and quality characterization, pineapple isolate -1 of Saccharomyces cerevisiae was found of good quality.

**A) Test for Shape**



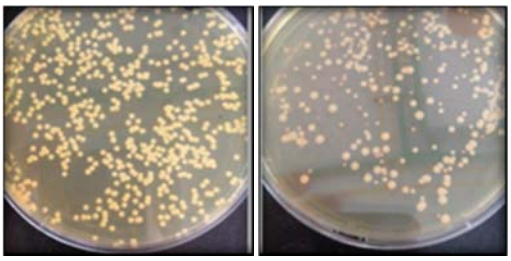
PI PI-2

**B) Test for Zone of growth**



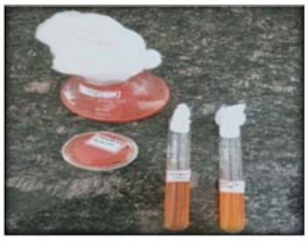
PI-1

**C) Test for Colour**



**D) Test for Ethanol Resistance**

**E) Biochemical Urease Test**



**F) Biochemical Citrate Test**

**G) Biochemical Hydrogen Sulphide Test**

**H) Flocculation Test Result**

**I) Killer Toxic Test**

**J) Foam Production Test**

**Abbreviations:** PI-Pineapple isolate, PI-1 Pineapple isolate one, PI-2 Pineapple isolate two, TSS- Total soluble solutes, TTA-Total titrable acidity, YEPD- Yeast extract peptone dextrose, SC- Saccharomyces ces cerevisiae.

**Carbohydrate fermentation by pineapple isolates PI-1 and PI-2**

| Carbohydrates | Before fermentation | After fermentation | |
|---|---|---|---|
| Sugars in YPD media | Color of the medium in (PI-1) and (PI-2) | Color of the medium in (PI-1) and (PI-2) | Gas production In (PI-1 and (PI-2) |
| Glucose | Pink | Yellow | Yes |
| Sucrose | Pink | Yellow | Yes |
| Maltose | Pink | Yellow | Yes |
| Lactose | Pink | No change | No |
| Fructose | Pink | Yellow | Yes |

**Table 1:** Growth table of pineapple PI-1 and PI-2 at varying pH

| pH level | PINEAPPLE ISOLATE 1PI-1 | | PINEAPPLE ISOLATE 1PI-2 | |
|---|---|---|---|---|
| | Initial (OD) | After 48 hrs of growth (OD) | Initial (OD) | After 48 hrs. of growth (OD) |
| 2 | 0.356 | 1.112 | 0.328 | 1.851 |
| 3 | 0.211 | 0.717 | 0.187 | 1.542 |
| 4 | 0.255 | 1.488 | 0.242 | 1.406 |
| 5 | 0.559 | 2.420 | 0.336 | 1.314 |
| 6 | 0.519 | 2.171 | 0.184 | 0.736 |
| 7 | 0.515 | 0.501 | 0.317 | 0.339 |

**Table 2:** Growth table of pineapple PI-1 and PI-2 at varying temperature

| pH level | PINEAPPLE ISOLATE 1PI-1 | | PINEAPPLE ISOLATE 1PI-2 | |
|---|---|---|---|---|
| | Initial (OD) | After 48 hrs of growth (OD) | Initial (OD) | After 48 hrs. of growth (OD) |
| 25 | 269 | 1.435 | 356 | 1.112 |
| 30 | 346 | 1.537 | 211 | 0.717 |
| 32 | 322 | 1.730 | 255 | 1.488 |
| 37 | 441 | 2.236 | 559 | 2.420 |
| 40 | 461 | 2.079 | 519 | 2.171 |
| 44 | 482 | 0.848 | 515 | 0.501 |

**Table 3:** Growth table of pineapple PI-1 and PI-2 at varying salt concentrations

| pH level | PINEAPPLE ISOLATE 1PI-1 | | PINEAPPLE ISOLATE 1PI-2 | |
|---|---|---|---|---|
| | Initial (OD) | After 48 hrs of growth (OD) | Initial (OD) | After 48 hrs. of growth (OD) |
| 6 | 0.204 | 0.877 | 0.232 | 1.372 |
| 9 | 0.237 | 0.246 | 0.219 | 0.648 |
| 12 | 0.242 | 0.248 | 0.267 | 0.443 |
| 15 | 0.197 | 0.154 | 0.202 | 0.229 |
| 18 | 0.290 | 0.184 | 0.269 | 0.263 |
| 20 | 0.257 | 0.197 | 0.254 | 0.237 |

**Table 4:** Fermenting power of isolates

| Sample Pineapple isolates | Sugar to be fermented in gm/ 100ml juice | Time required to ferment sugar | $CO_2$ | Alcohol |
|---|---|---|---|---|
| Isolate-PI-1 (2gm) | 200gm | 48hrs | Yes | Yes |
| Isolate PI-2 (2gm) | 200gm | 38hrs | Yes | Yes |

**Table 5:** Volatile acid production by pineapple isolatesPI-1 & PI-2

| Glucose concentration in 50(mg/ml) | Time interval in (hours) | Pineapple isolate -I Volatile acidity (%) | Pineapple isolate-II Volatile acidity (%) |
|---|---|---|---|
| 4.5 | 10 hrs. | 4.0 | 4.3 |
| 5.5 | 20hrs | 3.9 | 4.2 |
| 6.0 | 30hrs | 3.7 | 3.8 |
| 6.5 | 40hrs | 3.4 | 3.6 |

**Table 6:** Alcohol production by pineapple isolates PI-1 and PI-2

| Glucose concentration in 50ml | Alcohol Percentage for (PI-1) sample in (%) | Alcohol percentage for (PI-2) sample in (%) |
|---|---|---|
| 4.5 | 3.735 | 2.27 |
| 5.5 | 7.22 | 6.29 |
| 6 | 5.19 | 3.73 |
| 6.5 | 7.39 | 3 |
| 7 | 5.93 | 3.37 |
| 7.5 | 5.56 | 2.63 |

**Table 7:** Morphological, Biochemical, Physiochemical, Edible, and Quality characterization of Pineapple isolates

| Pineapple isolates [PI] | Morphological tests | Biochemical tests | Physiochemical tests | Edible source | Quality characterization |
|---|---|---|---|---|---|
| Pineapple isolate-1 [PI-1] | Round circular milky white opaque | Accurate results | Accurate results | Yes | Good quality |
| Pineapple isolate -2 [PI-2] | Round circular red rust opaque | Near to accurate results | Near to accurate results | Yes | Average quality |

## Conclusion

From the experiment, it was concluded that, based on the various tests physiochemical, biochemical, morphological tests conducted for identification of isolates from rotten pineapple juice both the two strains isolated were of saccharomyces cerevisiae. Further, tests were conducted to determine the strain quality characterization, fermentation capability, production of volatile acid and alcohol for both the isolates to obtain the best quality isolate, the isolate PI-1 of yeast Saccharomyces cerevisiae contribute maximum for fermentation ability, acidity and alcohol production and thus found most suitable for wine production than PI-2 isolate.

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