Study and Isolation of Yeast from Industrial Food Waste Peel and the Metal Effectiveness on Ethanol Production Using Various Metals Ions

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ABSTRACT

This research work explores the potential for the isolation and use of yeast from industrial food waste i.e., here pineapple and cherry peel-based ethanol bioprocesses. The process of fermentation has a long history in the production of Bio-ethanol. Bioethanol is the best alternative source of clean and renewable sources of energy and the only winner in this category. The objective of this research work was to isolate and characterize high potential ethanol producing yeast strains from industrial fruit peel waste. Two yeast isolates from (Ch) and (P) have been isolated, both yeasts have been characterized and identified on the basis of characteristics and optimized on ethanol producing capabilities using substrates. The highest production by using the yeasts was 7.12% and 5.22% for P and Ch, at pH 5.5, at 30° C temperature in medium. Metal ions helped to increase the highest rate of ethanol production is 09.56% by KH₂PO₄. This research work revealed that these yeast isolates can also be used to benefit the industrial production of alcohol and also help into the benefit of fuel demand.

KEY WORDS: BIOETHANOL, INDUSTRIAL WASTE, FERMENTATION, MICROBIOLOGY, WASTE MANAGEMENT.

INTRODUCTION

Bioethanol is the most common and most widely used type of biofuel, that is basically ethyl alcohol, the same type of alcohol found in beverage alcohol. Which can also be used as fuel or as motor fuel, it is most often used mainly as a biofuel additive for gasoline. As a form of renewable energy source that can be generated or produced using agricultural feedstock or can also be produced using food industrial waste as a feedstock. During the ethanol production or fermentation, the different types of sugars mainly glucose (in substrate) are converted into ethanol and carbon-dioxide. The basis for the production of ethanol is microbial fermentation of sugar by yeast.

Compared to other types of microorganism's yeast, specifically *Saccharomyces cerevisiae* is the most common microbe for the production of ethanol, due to its high production of ethanol capacity. In this study the *Saccharomyces cerevisiae* were selected as production microbes on account of their availability and after detailed study and characterization and optimization of parameters.

MATERIAL AND METHODS

Collection of Raw Material: Collection of Fruit waste (Peel) from Food processing industry for the isolation of Yeast from mainly Pineapple (P) and Cherry (Ch) peel which is denoted as Ch and P.

Other instruments are: Autoclave for sterilization, spectrophotometer, Laminar air flow (Biosafety Chamber), Shakin and Non-Shaking incubator for incubation, test tubes, petri plates and many more.

Isolation and screening of Yeast: Fruit samples from waste (Cherry and pineapple) were collected from local food processing industry basically from canning unit, and then

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their peels were extracted. And then the isolation is done by using the procedure from (Lee, 2018), Single colony formed was to be picked, and the cells were observed under a compound microscope. (Brooks, 2008)

Culture maintenance: The maintenance of culture was done by slants subculturing using YMM, incubating for 2 days at 28 to 30 °C, thereafter, use refrigerator for further storage at -5 to -9 °C for future use as per the requirement.

Growth of Isolates: In this current study the selected isolates, the morphology of cells of and their appearance on YEPD (yeast extract peptone dextrose) agar media was examined (Ortiz-Muñiz, 2010). The medium was then sterilized at 121 °C at 15 psi and poured on a petri dish and cooled down (Gamborg, 1995). 2 days old yeast strain than incubated at 30 °C $\pm$ 2 for 2 days. Features of the appearance of cultures were recorded.

The isolates Ch and P were cultured in YEPD (yeast extract peptone dextrose) liquid medium. Incubated at 28-32 °C for 72 hr. The culture for the growth is to be examined visually on the surface of liquid medium, and the shape of cells using compound microscope (Lorenz, 2000) (Magnus, India)

Carbohydrate's fermentation: The fermentation ability of seven different carbohydrates by selected isolates (Miller, 1959) (Ch & P) was examined anaerobically and is assessed by looking for the formation of gas (CO₂) in Durham tube and change in colour of the fermentation media.; the media colour changed from red to yellow due to the formation of gas and acid produce.. The medium was prepared by using these- peptone (10 gm), NaCl (5 gm), phenol red (Indicator) and carbohydrate (5 gm). A volume of 15 ml aliquots dispensed in different tubes were then autoclaved, after cooling 2 days old selected yeast strain.

Table 1. Fermentation of different carbohydrates using P isolate

Carbohydrate (Sugar)	Colour of the medium Before Fermentation	After Fermentation	
		Colour of the medium	Production of gas
Glucose	Pink colour	(yellow)	yes
Sucrose	Pink colour	(yellow)	yes
Maltose	Pink colour	(Yellow)	yes
Lactose	Pink colour	(No change in colour)	no
Fructose	Pink colour	(yellow)	yes
Xylose	Pink colour	Yellow	yes
Trehalose	Pink colour	Yellow	yes

Table 2. Fermentation of different carbohydrates using Ch isolate

Carbohydrate (Sugar)	Colour of the medium Before Fermentation	After Fermentation	
		Colour of the medium	Production of gas
Glucose	Pink colour	(yellow)	yes
Sucrose	Pink colour	(yellow)	yes
Maltose	Pink colour	(yellow)	no
Lactose	Pink colour	(No change in colour)	no
Fructose	Pink colour	(yellow)	no
Xylose	Pink colour	(No change in colour)	no
Trehalose	Pink colour	(yellow)	yes

Table 3. Growth of Ch yeast isolate in liquid media with different temperatures

Temperature	Initial optical density (O.D)	O.D (after 2- days of growth)
25°C	.240	1.365
30°C	.342	1.427
32°C	.328	1.650
37°C	.439	1.987
40°C	.458	2.001
45°C	.489	.681

Table 4

Temperature	Initial optical density (O.D)	O.D (after 2-days of growth)
25°C	.323	1.002
30°C	.202	.695
32°C	.265	1.322
37°C	.582	2.890
40°C	.552	2.154
44°C	.511	.496

Thermotolerance detection: YEPD liquid medium is used for thermotolerance detection and growth in liquid media of selected yeast isolates of cherry and pineapple. The medium was Sterilized and cooled. Medium was distributed into tubes and after that inoculated 2-days selected yeast isolates (Ch & P). The initial (O.D) of each tube is recorded by using spectrophotometer at 600 nm against the medium as blank (control sample). The increase in O.D in a tube was recorded as evidence of growth. The growth in YEPD agar medium at different temperature to ensure the strains thermotolerance.

Growth at different pH in liquid media: YEPD liquid media is used to find out the ability to grow on different pH. The media was sterilized by autoclaving and then cooled. YEPD broth medium is prepared at different pH which are 2–10. Then, each was inoculated with the yeast (the initial O.D at 600 nm was to be measured) and then incubated at 30 °C for 2 days. After 2 days the cell density was further recorded at 600 nm for the growth.

Table 5. P (Pineapple) isolate in liquid media with different pH growth

pH	Initial optical density (O.D)	O.D (after 2-days of growth)
2	.333	.899
3	.428	1.866
4	.345	1.870
6	.390	1.788
7	.184	1.489
8	.259	1.455
9	.277	1.219
10	.422	1.326

Table 6. Ch (Cherry) isolate in liquid media with different pH growth

pH	Initial optical density (O.D)	O.D (after 2-days of growth)
2	.342	.840
3	.389	1.457
4	.269	1.379
6	.309	1.470
7	.200	1.366
8	.220	1.270
9	.209	1.222
10	.428	1.203

Observation of Osmotolerance: YEPD broth was prepared containing with different range in between 6%- 20% of NaCl. Each tube contained 10 ml of YEPD liquid media with appropriate concentration of NaCl, and the blank media were used as a control sample. Then, each was inoculated of the yeast cell (the initial O.D at 600 nm was measured) and then incubated at 28-30 °C for 2 days. After, cell density was further recorded by spectrophotometer at 600 nm.

Molasses fermentation: Molasses fermentation is done in the conical flasks. A total of 200 ml of fermentation medium is taken into the 500 ml of flasks and then the suspension of yeast was inoculated into the medium in an aseptic condition and then incubated at different

temperatures in an incubator under non-shaking (N.S) and shaking (S) conditions.

Effect of sugar concentration: The effect of concentration of sugar in ethanol production by *S. cerevisiae* were studied, media is prepared by molasses dilution to reducing sugar concentration which ranges between 04.5% to 07%, and fermentation is carried out of 200 ml medium in to 500-ml conical flask. Inoculate is added in to the medium. After that Samples were withdrawn at different times and estimated for residual sugars as well as ethanol content in the medium (Miller, 1959).

Table 7. P isolate in liquid media with different NaCl concentration

NaCl percentage	Initial optical density (O.D)	O.D (after 2-days of growth)
6%	.235	1.365
9%	.217	.645
12%	.259	.423
15%	.208	.289
18%	.259	.271
20%	.257	.240

Table 8. Ch (cherry) isolate in liquid media with different NaCl concentration

NaCl percentage	Initial optical density (O.D)	O.D (after 2 days of growth)
6%	.209	.872
9%	.227	.239
12%	.239	.250
15%	.187	.149
18%	.285	.178
20%	.251	.189

pH effect: To study the pH effect on ethanol production by *S. cerevisiae*, fermentation media with different reducing concentration of sugar were used for the production of ethanol. Fermentation is carried out at pH 05.0 in of 200 ml media in a 500-ml conical flask. A 2-day-old inoculum is added to the media. Samples were than withdrawn at different times and estimated for residual sugars as well as ethanol content in the medium.

Effect of metals: For determination of the effects of metals on ethanol production which are MnCl₂, ZnSO₄, CuSO₄, KH₂PO₄, and were added at different concentrations and then the fermentation was carried out in the presence of different types of metals. Observation of ethanol production was done at the temperature of 30 °C, pH 5.0, and using the reducing sugar concentration of 7.0% in shaking (S) condition.

RESULTS

Growth of isolates: The morphology of yeast cells was observed grown in solid media and on liquid media according to the standard method using microscope. Six petri dishes are used to confirm the identification of the

cell morphology of cherry and pineapple yeast isolates under compound microscope.

Carbohydrate's fermentation: Here, *S. cerevisiae* shows variations in terms of utilization of different sugars in the below Tables 1 & 2. The isolated strain utilises all seven sugars but failed to grow on lactose. Change in the colour of the media confirmed the fermentation. The results were recorded after 2-days of inoculation.

Different environmental conditions to check the yeast Tolerance

Temperature effects on growth: YEPD agar plates containing yeast cell were incubated for 2-days at different temperature. Both isolates were able to grow at 25–45 °C. Thermotolerance were repeated in liquid media, for the confirmation of results obtained from the solid media, Table-3, Growth of P yeast isolate in liquid media at different temperatures From the table-3, that shows the P yeast isolate is highly thermotolerant because it is able to grow up to 45 °C, From the table-4, which shows that the Ch yeast isolate is highly thermotolerant also as it is able to grow up to 40 °C.

pH effect on growth: Both the isolates of Ch and P shows the ability to grow in a wider range of pH, from between two to ten. Both isolates with pH 10, but pH 04.0 for P isolate and pH 6 for Ch isolate shows the optimum and best result. After 2-days, the cell optical density is recorded at 600 nm and given gradually for evidence of growth in the below table-5 and 6.

Observation of Osmotolerance: The pineapple (P) isolates tolerate up to 09% NaCl successfully in the medium, and this was seen an index of osmotolerance. But at the higher concentration, the growth is reduced with accordance to time. The isolate of cherry (C) showed optimum at up to 12% NaCl concentration.

Ethanol production: The results of ethanol production and its tolerance at different concentration of sugars at 30 °C and pH 05 in shaking (S) and non-shaking (N.S) condition, Pineapple (P) yeast shows good results than the Cherry (C). Thus, some series of experiments are conducted at different-different physicochemical conditions for the optimization of ethanol production by the Pineapple isolate. For the optimum result, shaking condition experiments were carried out and the result can be seen in the below table- 9.

Metal ions effect on ethanol production: Ethanol production optimization by additional nutrient supplements as effect of metals such as MnCl₂ (Manganese (II) chloride), CuSO₄ (Copper (II) sulphate), KH₂PO₄ (Potassium dihydrogen phosphate), and ZnSO₄ (Zinc Sulphate) was analysed. Both cherry and pineapple isolates show high productivity after adding of supplements, as studied. The highest ethanol production observed is 09.56% by P isolate with KH₂PO₄ supplements in shaking condition (Nasir, 2017) shown in below graph fig.2. Ch isolate produced 8% ethanol after MnCl₂ supplementation in shaking condition as shown in below graph (fig. 1). the average of all results is shown in below graphs (fig.1 and fig.2) and in table-10.

Table 9. Under shaking and non-shaking condition ethanol production by Ch isolate at initial pH 5 and with different sugar concentration. The highest production recorded 5.22% at sugar concentration of 5.5%. The data shown are the average of 3 individual experiments under given condition. And for P isolate the highest production at 7.89% at sugar concentration of 6.5.

Concentration of glucose (%)	Cherry (shaking)	Cherry (non-shaking)	Pineapple (shaking)	Pineapple (non-shaking)
4.5	2.56	0.56	3.523	2.18
5.5	5.22	2.28	7.12	6.
6	4.56	4.22	5.19	3.49
6.5	3.22	2.27	7.89	3
7	3.55	3.56	5.55	3.08

Figure 1

Figure 2

Table 10. Production of ethanol by P and Ch isolate in presence of metals

Metals ion	Ethanol (%) by P (2-days)	Ethanol (%) by Ch isolate (2-days)
MnCl ₂	8.8	8
CuSO ₄	7.48	6.20
KH ₂ PO ₄	9.56	7.40
ZnSO ₄	8.12	6.80

DISCUSSION

Both isolates of cherry and pineapple that were found belong to *Saccharomyces* type unicellular. The P isolate shows better tolerance than the Ch isolate at up to 15% of NaCl equivalent of that osmotic pressure. (Lodder, 1970). As we know from past studies that yeasts are mesophilic with upper limit growth temperature between 30°C to 3°C. This is the reason why the temperature must be maintained between 30 to 35 °C for fermentation process. The P yeast shows far better tolerance than of the Ch peel isolate at up to 15% NaCl equivalent of osmotic pressure. This study shows that the highest ethanol production observed is 09.56% by P isolate with KH₂PO₄ in shaking (S) condition. Ch isolate produced 8% ethanol after MnCl₂ supplementation in shaking (S) condition. The isolates are tested for fermentation of carbohydrates, P (Pineapple) isolate is able to utilize six out of the seven tested total sugars, and cherry isolate is able to utilize five sugars out of seven, which indicates that both were diverse in sugar utilization that is shown in table-1 & 2.

CONCLUSION

The results reported in the present study revealed that both the P and Ch yeast isolates were highly tolerant to ethanol and as well as thermotolerant and osmotolerant, both can survive at various pH ranges. These results shows that the isolates can be used at industrial and at heigh level for the fermentation of various raw materials in reference to obtain an increased production of bioethanol.

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