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Isolation Of Saccharomyces Cerevisiae From Vitis Vinifera And Comparing Its Ethanol Fermentation Efficiency With Commercial Brewer's Yeast

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Abstract
In view of the anticipated shortage of traditional fossil fuel supplies, there is a rising demand for the production of ethanol as an alternative biofuel. The main objective of this research was to isolate Saccharomyces cerevisiae from a natural source. Vitis vinifera (grape) was used to isolate natural Saccharomyces cerevisiae colonies. The total ethanol production by natural Saccharomyces cerevisiae and commercial Saccharomyces cerevisiae (brewer's yeast) was estimated through a simple distillation method. Although there is a potential risk of ethanol loss with this method, efforts were made to ensure the results were as precise and accurate as possible. Our study found that commercial Saccharomyces cerevisiae (brewer's yeast), under anaerobic conditions, had a higher ethanol fermentation efficiency (74.62%) compared to the natural isolate, which showed an efficiency of (65.6%). The fermentation studies were conducted at 30°C at a pH of 6.5.
Keywords: *Saccharomyces cerevisiae, fermentation, ethanol fermentation efficiency, Vitis vinifera (grapes)*

INTRODUCTION

There is a rising demand for ethanol due to its use for various purposes such as fuel additives, industrial solvents, preservatives in many pharmaceutical preparations like rubbing compounds, lotions, and tonics, sanitizers, hand washes etc. Currently ethanol is primarily produced through the fermentation of sugars or starches derived from various sources like sugarcane, corn and the other grains. First these materials are broken down into simple sugars thereafter, yeast is used to ferment these sugars, which produces carbon dioxide and ethanol. Petrochemical processes, which include hydration of ethylene, can also be used in the synthesis of ethanol. An ideal microorganism used for ethanol production must have rapid fermentative potential. Mostly commercial S.cerevisiae (Brewer's yeast) is used for ethanol production in industries. But natural S.cerevisiae also have an ethanol production efficiency, if it is isolated from their natural habitat and provided with a suitable environment to grow. Generally S.cerevisiae is found on ripe fruits, particularly grapes and berries, vegetables, soil, and oak trees. Most industrial microorganisms are patented and may not be available for use outside their country of origin. Thus, through our study, we are trying to find the ethanol production and ethanol fermentation efficiency of natural S.cerevisiae and commercial S.cerevisiae (Brewer's yeast). This study will help us evaluate whether we can use natural S.cerevisiae for fermentation instead of commercial yeast. Vitis vinifera (grapes) are easily available in India and contain several wild yeasts including S.cerevisiae. Grape waste in India, primarily in the form of pomace (skins, seeds, stems, and pulp after juice extraction), is a significant issue for fruit processing industries.Their skin contains many useful wild yeasts which can be very helpful to produce biofuels like ethanol. Therefore the aim of this study is to isolate indigenous S.cerevisiae from ripe grapes and compare its ethanol fermentation efficiency with the commercial S.cerevisiae.

METHODOLOGY

The inoculation of grape media was done on a YPD agar plate in the following way:
250g of ripe black grapes (Vitis vinifera) was taken. We mashed the grapes, and placed them into a sterile glass jar. 25ml of sterile distilled water along with 5g of sugar was added into the jar. The total volume of grape media was 160ml. We added 20mg of Chloramphenicol to reduce bacterial growth.Then the jar was loosely closed with a jar cap. We kept the jar in the incubator for 48 hours at 30°C. S.cerevisiae consumes sugar and produces alcohol and co2. As time passes in the fermentation media, its population increases rapidly. One of its best features is its alcohol-withstanding property. Thus, after 48 hours our prepared grape media had a high number of S.cerevisiae along with a good amount of alcohol content.

Preparation of YPD agar media for inoculation of grape media

Yeast extract (1g) , peptone (2g), dextrose (3g), and sterile distilled water 150ml were used to prepare our YPD agar media in a 500ml beaker. The beaker was sterilized in an autoclave at 121° C for 20 minutes. Then YPD was added to it along with 3g of agar. The prepared mixture was heated until all ingredients were mixed completely. The mixture was poured into a sterile petri dish inside the laminar airflow to maintain the sterility. When the YPD agar media gets solidified at room temperature it was inoculated with 48 hour old grape media. The Perti dish was covered and kept inside the incubator at 30°C for 2 days. In microbiology, petri dishes are essential for cultivating and observing microorganisms like bacteria and fungi. They provide a controlled, sterile environment for microbial growth, often with a nutrient-rich agar medium .After 2 days, round, creamy and white colonies of wild yeast were observed on our inoculated petri dish, which were similar to the morphology of S.cerevisiae.

Confirmation of natural S. cerevisiae colonies:

Morphology

Shape: Typically round to oval was observed

Size: Small to medium, usually 2–5 mm in diameter after 48 hours on standard YPD media.

Microscopic observation

Later, we observed the colonies using a microscope. Round and oval cells were observed under the microscope at 40X. Oval/elliptical larger budding cells (4–10µm) were also observed.

Fermentation test to confirm natural S.cerevisae

The S.cerevisiae ferments sugar and produces ethanol along with carbon dioxide. So, we prepared 100ml of YPD fermentation media. (Yeast extract: (3g), peptone: (2g) and dextrose (12g) in 100 ml of sterile distilled water along with 0.1g of Mgso4 for slightly acidic medium.These ingredients were added to a sterile 500 ml beaker and stirred to dissolve every ingredient properly. The prepared YPD fermentation media was inoculated with round and creamy yeast colonies that appeared on the YPD agar media. The pH of the prepared media was found to be 6.5. We kept the YPD fermentation media in an incubator at 30° C for 2 days under anaerobic conditions. After 2 days, we took out our fermented YPD media and extracted the ethanol through simple distillation. After the distillation and centrifugation, a total of 5.1ml extract was obtained (we kept the temperature between 80°C to 95°C to get more accurate results).

Confirmation test for ethanol

Oxidation Test

A brown precipitate is formed when ethanol reacts with Kmno4, in the presence of NaOH. Make 0.02M Kmno4 solution for 50ml distilled water. Molarity (M) = moles / vol (L) , Vol = 50 mL = 0.050 L , Molarity = 0.02 M Moles of KMNO4 = 0.02× 0.050 = 0.001molar

Mass of KMNO4 = 0.001×158.4g/mol = 0.158g So, 0.158g of KMNO4 is added to 50ml of distilled water and dissolved by continuous stirring.

Make 0.1M NaOH solution for 20ml distilled water:

Molarity (M) = moles / vol (L) ,Vol= 20mL = 0.020 L , Molarity= 0.1 M

Moles Naoh needed = 0.1 mol/L × 0.020 L = 0.002 mol

Molar mass of Naoh = 40.00 g/mol

Mass of Naoh needed = 0.002 mol × 40.00 g/mol = 0.08 g

Thus, 0.08g of NaOH will be dissolved in 20ml water. Now In test tube1, take 1ml of 0.02M KMNO4 solution along with 2ml of 0.1M NaOH solution and mix them together. In test tube 2, add 1ml of ethanol obtained from the Fermentation media 1 (produced from the natural S.cerevisiae colonies). Add the mixture of NAOH & KMNO4 from test tube1 into test tube 2. On heating the mixture, a brown precipitate was formed, which confirms the presence of ethanol in our extract.

Chemical reaction: $3CH_3CH_2OH + 2KMnO_4 + 4H_2O \rightarrow 3CH_3COOK + 2MnO_2 + 5H_2O + 2KOH$.

In a basic medium, KMNO4 in the presence of ethanol is reduced to form manganese oxide (MnO2). MnO2 forms a brown precipitate in the reaction, which confirms the presence of ethanol. KMNO4 oxidizes the ethanol (CH3CH2OH) to potassium acetate (CH3COOK) . Potassium hydroxide (KOH) and water are also byproducts in the reaction above. So, through morphology, microscopy and sugar fermentation tests, we can conclude that the round, creamy yeast colonies which appeared on our petri dish after the inoculation of grape media belongs to S.cerevisiae. The urease test was carried out to check the ability of the isolate to hydrolyze urea. The culture was inoculated on Christensen's urea agar slant and incubated at 30°C for 48–72 hours. Development of a pink color indicates a positive reaction due to

ammonia production, while no color change (yellow) indicates a negative result which is a characteristic feature of S.cerevisiea

Preparation of fermentation media (2)

To check the ethanol fermentation efficiency we will prepare the YPD fermentation media for commercial S.cerevisiae (Brewer's yeast). So, in a beaker take 50 ml of sterile distilled water and add (yeast extract (3g), peptone (2g), dextrose (12g) and MgSO4 (0.1g). Stir the ingredients properly to mix them all.

Take Beaker 2: In this beaker take 50ml of sterile distilled water and add 1g of commercial S.cerevisiae (Brewer's yeast) to protect yeast from shock. Now, stir properly and keep the mixture untouched for 5 minutes. After 5 minutes, add this 50ml yeast containing mixture to Beaker 1, which contains YPD. This will form 100ml of YPD fermentation media. The pH of the media was 6.5. We covered the beaker with aluminum foil and kept it in an incubator at 30°C for 2 days for fermentation. We Used simple distillation and centrifugation to extract our ethanol from the prepared fermentation media. The ethanol has a boiling point of 78.37° C. Thus, it evaporates before water.The total ethanol yield we obtained was 5.8ml.

RESULT

Table 1. Ethanol volume (mL) produced by S.cerevisiae from natural and commercial sources in Fermentation Media 1 and 2, respectively.

Ethanol produced in fermentation media 1	Natural S. Cerevisiae	5.1 ml ethanol
Ethanol produced in fermentation media 2	Commercial S. Cerevisiae	5.8 ml ethanol

Calculation of Maximum theoretical yield

Determining the theoretical maximum ethanol Yield from dextrose:

The fermentation of glucose (dextrose) by yeast follows this balanced reaction: C6H12O6C6H12O6 → 2C2H5OH + 2CO2. From the chemical reaction we can say: 1 mole of glucose produces 2 moles of ethanol.

Relating Moles to Mass: Molar mass of glucose ($C_6H_{12}O_6$): 180 g/mol, Molar mass of ethanol (C_2H_5OH): 46 g/mol so, one mole of glucose (180 g) yields 2 moles of ethanol (2 × 46 g = 92 g). Calculating theoretical yield per gram of glucose: 92g ethanol / 180g glucose = 0.511 g ethanol per gram. In both fermentation media (1 and 2) we used 12g of glucose thus, theoretical ethanol yield is 12g×0.511g = 6.132g (7.77ml).

Fermentation efficiency of Natural S.cerevisiae

Ethanol produced = 5.1ml, density of ethanol is = 0.78g/ml, actual alcohol in (g) : 5.1 ml×0.789 g/mL= 4.0239 g. So actual ethanol yield = 4.023g , theoretical ethanol yield is = 6.132g, fermentation efficiency = Actual yield/ Theoretical yield × 100, 4.0239g / 6.132g ×100 = 65.6%

Thus, fermentation efficiency of natural S. cerevisiae colonies is = 65.6%.

Fermentation efficiency of commercial S. cerevisiae (Brewer's Yeast)

Ethanol yield of fermentation media 2 containing Brewer's yeast is = 5.8ml. Actual ethanol yield in gram is = 5.8ml×0.789g/ml= 4.5762g

Theoretical yield is = 6.132g (7.77ml)

Thus, fermentation efficiency of commercial S.cerevisiae is :

fermentation efficiency = Actual yield/ Theoretical yield × 100 = 4.5762/6.132×100 = 74.62%.

CONCLUSION

The demand for ethanol as a biofuel is high in the current scenario, so the production of ethanol through fermentation by yeast can be very useful for us. Through this experiment, we tried to find out the alcohol production efficiency of natural and commercial S.cerevisiae. We have come to the conclusion that commercial S.cerevisiae has better fermentation efficiency as compared to the natural S.cerevisiae . While wild strains retain the natural ability to ferment sugars, their performance in terms of ethanol yield and conversion efficiency is limited, likely due to lack of selective adaptation for industrial use. However in the case of overripe grapes, a significant portion of which is discarded as waste in India, this waste contains a variety of wild yeast including S.cerevisiae, which can be used in the biofuel production through fermentation. The ethanol fermentation efficiency calculated in this study is based on the volume of

ethanol recovered through simple distillation, which may not represent the total ethanol produced for both (natural and commercial S.Cerevisiae) due to potential losses during the process. Therefore, the reported efficiency likely underestimates the actual fermentation performance of the yeast.

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Conflict of Interest: The authors have no conflict of interest to declare.

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