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Research paper

Analysis of Isolated Yeast Strains from Different Sources for Bio-ethanol Production

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ABSTRACT

The generation of bioethanol and alcoholic beverages uses yeast (*Saccharomyces cerevisiae*) on a worldwide scale; however, its activity is hampered by the buildup of intracellularly produced ethanol on cell viability. In the past, various yeast strains and their condition of culture is being employed to lessen the ethanol stress impact on the expression of the gene; however, these methods are affected by a range of factors. But there are similarities observed in the gene ontology because of the effects of ethanol, which indicates that the energy production constraints compromise *Saccharomyces cerevisiae's* ability to respond to stress from ethanol by increasing the expression of genes related to glycolysis and the mitochondrial TCA cycle while decreasing the expression of ATP-mediated growth-associated processes. *Saccharomyces cerevisiae* was used as the reference strain in the current study. Different strains of yeast were isolated from different materials, and the growth of the strains isolated was monitored.

Further, the ethanol measurement was carried out using the DNS method. The yeast strain with the best growth rate was used in the alcohol tolerance test to withstand ethanol stress. In the future, the molecular underpinnings of the yeast strain's tolerance to alcohol can help with genetic engineering to create methods for enhancing its function under ethanol stress.

1. Introduction

Owing to its large ethanol yield, high ethanol tolerance, and capability of fermenting a wide range of sugars, yeast, especially *S. cerevisiae*, is the prevalent microbe used in ethanol production when compared to other types of microorganisms. *Saccharomyces cerevisiae*, or sake yeasts, have about 20 percent of yield (v/v). Yeasts have faster rates of fermentation and are less tolerant of ethanol at high levels. Therefore, preventing sake quality decline brought on by the death of cells of yeast in the later stages of the fermentation faces a number of difficulties. Carbon dioxide (CO_2), the third largest component, makes up 0.03 percent of the atmosphere (Watanabe et al., 2011). This research is important because fossil fuel burning produces large amounts of CO_2 emissions, which contribute significantly to global

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Warming. As a result, we need alternative energy sources, especially those that are carbon neutral (Ramos et al., 2013). Bioethanol is an alternative to fossil fuels and can be used as an alternative to this; thus, microbial ethanol production is more significant (Demirbas et al., 2008). Corn is a substantial source of ethanol production in the US (Schmer et al., 2014), whereas sugarcane is one of Brazil's main ethanol sources. Brazil's bioethanol business has grown quickly in recent years as a result of the international crisis of oil, and genetic advances in cultivars have further increased sugarcane yield (Gnansounouet al., 2005). New variations of the sugarcane fermentation process are produced by technological and scientific innovation and have boosted the Brazilian distillery's productivity of bioethanol (Della et al., 2014).

High ethanol concentrations are produced during sake mash fermentation (Stanley et al., 2010); So many different strains of yeasts that are ethanol-tolerant are developed and employed in sake brewing. On the other side, sake, in particular, which has a gentle flavor and minimal acidity, bitterness, or astringency, is susceptible to this (Yamaoka et al., 2014). A possible strategy for increasing fuel generation on a wide scale would be the selection of novel yeast strains (Kumar et al., 2011). During industrial fermentation operations, yeast is subjected to a variety of stressful conditions that affect its activity and performance. The stress factors in live cells that have received the most research include variations in temperature, pH, and sugar content (Tenkolu et al., 2022). Alcoholic stress, however, also plays a crucial impact on the kinetics of yeast growth and its activity since ethanol is an internal substance, and its buildup causes yeast cells to die (Nagodawithana et al., 1976). The goal of the current research is to investigate the growth kinetics of strains estimation of ethanol from various samples.

2. Methodology

2.1 Yeast Strains

The strains were isolated from various sources, including grapes, oranges, sugarcane, baker's yeast, and TAPI. The isolation of yeast from the different sources was done by using a YPD media.

2.2 Media and cultural conditions

On a YPD medium, the yeast strains were cultivated. Media that had solidified contained 1.5% agar. In the prepared media (500ml) of YPD, 10^6 cells/ml from freshly prepared cultures were inoculated and left at 30°C, and the fermentation process was carried out. Samples are diluted serially. On YPD agar plates, cell growth was measured. Antibiotics like ampicillin were used to further purify the colonies that had formed and stop bacterial growth. After that, isolated pure colonies were preserved in a liquid YPD medium. For the metabolite analysis, cells from the pre-culture were employed.

2.3 Growth Kinetics

10^6 cells/ml isolated from each raw material were taken and added into the freshly prepared media (250 ml YPD) and cultured overnight on a shaker (orbital) at 160–170 rpm at 30°C to examine the growth of *S. cerevisiae. Saccharomyces cerevisiae* develops nicely in this environment. Afterward, the cultured cells were centrifuged for 5 minutes at 20,000g at 37°C, followed by washing and re-suspension in DNS. Cell growth at a predetermined time, i.e., at one h, two h, three h, and eight h) was monitored at 550 nm (Nordin et al., 2015).

2.4 Sugar estimation by DNS method

Each sample's culture supernatant was diluted to 0.5 ml, and then 0.5 ml of reagent (DNS) was mixed. Then the volume was then increased to 9 ml with the help of sterilized water (H_2O). This mixture was thoroughly agitated before being boiled to change the color and incubated overnight at 30°C. For the purpose of determining the amount of reducing sugar required to support the growth of yeast cells, the degree of change in color was measured at 600 nm at a predetermined time, i.e., at one h, two h, three h, and eight h).

2.5 Ethanol tolerance test

Testing for ethanol tolerance was done on the sugarcane yeast isolate. In broth (30 ml of YPD) with varying amounts of ethanol (control, 5 percent, 10 percent, and 15 percent w/v), the yeast strain was inoculated. Overnight, the tubes were incubated at 30 °C. By measuring optical density at 660 nm at

predetermined time gaps, the vitality of yeast cells was assessed (1, 2, 3, and 8 h).

3. Results

3.1 Growth Kinetics

After two hours, a considerable variation in the growth rates of the species isolated from various sources was seen, indicating the exponential stage of yeast strains. Table 1 displays the OD measured at various times at 550 nm.

3.2 Sugar estimation by DNS method

A change in hue is seen as a result of the DNS reagent's interaction with the sugar in the sample during the development of yeast cells. As demostrated in Table 2, the observed data represents the intensity of color change as OD, which is proportional (directly) to the reducing sugar concentration in the different sources.

Table 1. During the yeast growth from various samples, OD at 550 nm was observed

Samples	OD at a specific time								
	0	1	2	3	4	5	6	7	8
Grapes	0.317	0.329	0.348	0.387	0.523	0.8	1.121	1.221	1.234
Orange	0.071	0.2	0.284	0.249	0.293	0.321	1.393	0.385	1.101
Tadi	0.195	0.203	0.215	0.26	0.433	0.462	0.538	1.071	1.09
Baker's Yeast	0.21	0.249	0.292	0.282	0.489	0.618	1.127	1.132	1.181
Sugarcane	0.333	0.472	0.604	0.799	0.825	0.841	1.201	1.713	1.882

The data represents the mean values (n=3)

Table 2. To determine reducing sugar during in the yeast growth from various samples, at 600 nm OD was evaluated

Samples	OD at a specific time								
	0	1	2	3	4	5	6	7	8
Orange	0.527	0.476	0.467	0.329	0.302	0.296	0.079	0.067	0.056
Grapes	0.624	0.603	0.598	0.448	0.439	0.429	0.366	0.356	0.304
Tadi	2.06	0.648	0.567	0.492	0.457	0.440	0.306	0.296	0.289
Sugarcane	2.206	2.067	0.859	0.766	0.666	0.656	0.578	0.567	0.554
Baker's Yeast	0.852	0.803	0.798	0.685	0.667	0.595	0.397	0.378	0.367

The data represents the mean values (n=3)

3.3 Ethanol tolerance test

In 30 ml of broth (YPD) with varying amounts of ethanol (i.e., control, 5 percent, 10 percent, and 15 percent w/v), the yeast strain was inoculated. The tubes were kept at 30°C overnight for incubation. Figure 1 displays the results of noted OD at 660 nm at predetermined time gaps (one h, two h, three h, four h, five h, and six h) to determine the vitality of yeast cells.

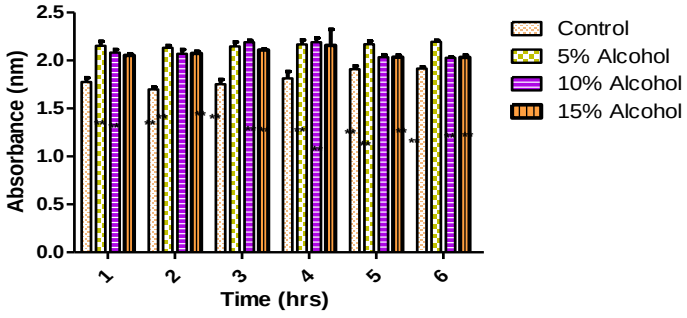

Figure 1 Results of tolerance test (alcohol) of *S. Cerevisiae* from the sources (sugarcane) at varying concentrations of ethanol and optical density measured at 660 nm. The data represents the mean values (n=3)

4. Discussion

Saccharomyces cerevisiae (yeast), the most researched eukaryote, is a helpful tool for the majority of basic eukaryotic organism studies (Sivasakthivelan et al., 2014). This is due to the fact that *S. cerevisiae* is unicellular, which often makes things easier and offers the combination of the fact that nearly all the biological processes seen in eukaryotes are also existing and highly maintained in *S. cerevisiae*. Furthermore, genetic manipulation is straightforward (Parapouli et al., 2020). Its biological characteristics make it very useful for biotechnology because of its capacity for fermentation, which produces alcohol and carbon dioxide, as well as its resilience to unfavorable low pH and osmolarity conditions. One of the most well-known applications for *S. cerevisiae* is the manufacture of food, and drinks, especially wine and biofuels (Parapouli et al., 2020).

4.1 Growth Kinetics

By assessing the specific growth rate, *S. cerevisiae* species were distinguished. In this stage, the starting cell concentration determines how many cells develop per unit of time (Nordin et al., 2015). Turbidity, a measure of yeast growth, is produced by the proliferation of yeasts. The spectrophotometer measures the percentage of transmission from 0.1 percent to 100 percent to determine how much light energy passes through the suspension. Optical density, is used to express the density of cell suspension. The findings demonstrate that the *S. cerevisiae* species isolated from sugarcane performs better than the other species (Cabañas et al., 2019).

4.2 Sugar estimation by DNS method

Using the DNS reagent, one may determine the amount of sugar present in a given sample. During fermentation, yeast transforms sugars and carbohydrates into carbon dioxide and alcohol. The findings imply that the *S. cerevisiae* species isolated from sugarcane is superior to other *S. cerevisiae* species (Tahir et al., 2010).

4.3 Ethanol tolerance test

The ability to tolerate ethanol was evaluated on the yeast isolates obtained from sugarcane, and the results indicate that the *S. cerevisiae* species isolated from the sugarcane can be used for the production of bioethanol.

5. Conclusion

One of the microorganisms that are always utilized to produce bioethanol is *S. cerevisiae*. Utilizing effective yeast strains with ethanol tolerance will increase ethanol yield during fermentation, lowering distillation costs and protecting the environment from dangerous pollutants. A variety of sources, including grapes, oranges, TADI, baker's yeast, and sugarcane, were used to extract the yeast. The sugarcane-derived yeast species had the best growth curve, in comparision to the other isolated samples.

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Declaration of Conflict

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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