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PHYSIOLOGICAL EFFECT OF VARIOUS ACOLHOLS AND DRUGS ON SACCHAROMYCES CEREVISIAE STRAINS OBTAINED FROM SUGAR-CANE JUICE

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ABSTRACT

The aim of the present study was to analyse the role of drugs and alcohols on growth of Saccharomyces cerevisiaeisolated from Sugar cane (Saccharumofficinarum) juice, which has been collected from different areas that have different atmosphere, soil composition, and temperature. The growth on Saccharomyces cerevisiae yeast is influenced by various alcohols (ethanol and methanol) and different drugs (Ampicillin and Cefotaxim). For characterization of physiological effects on Saccharomyces cerevisiae strains due to various Alcohols/drugs optical density wasmeasured by spectrophotometer.Experimental results indicate the inhibitory effect of alcohol and drug on growth on Saccharomycescerevisiaeunder aerobic conditions. Extensive research on factors effecting the growth of S. cerevisiae willhelp in improvising the conditions essential for fermentation.

Keywords: Saccharomyces Cerevisiae, Sugarcane, Alcohols and Drugs, Inhibitory Effect, Physiological Effects.

I. INTRODUCTION

Saccharomyces cerevisiae is a universal organism which traditionally used as in fermentation of food and wine industries; it belongs to kingdom of fungi and phylum of Ascomycota i.e. eukaryotic microorganism. *Saccharomyces cerevisiae* is yeast, may be useful or harmful in food. The yeast species useful in food are *Saccharomyces, Zygosacchoromyces* etc., the yeast species harmful are *Rhodotorula, Pichia, and Hansenula* etc. *Saccharomyces* are primitive and are morphologically very similar to ascomycetes, in which the mycelium is totally absent or poorly developed. *S. cerevisiae* is the best to be used in food industries, production of alcohol, glycerol, beer, yeast extract/vitamins, wine and distilled spiritsand are undesirable in causing spoilage of fruit juices, syrups, honey, meats etc.[1,3] Hence, they are known as "Bakers and Brewer's Yeast". It utilizes sucrose, glucose, fructose, maltose sensitive for NewFlophenotype [5]and maltotriose as carbon sources due to fermentation under anaerobic conditions. [3]The majority of brewery yeasts belong to NewFlo phenotype. [5]*Saccharomyces cerevisiae* is converts hexose sugars to ethanol, CO_2, acids that contribute to the sensory attributes of the food and beverage. **[21]**Yeast fermentative is the two types describe properly in the genus *Saccharomyces* what they are top and bottom fermentative yeast. Top yeast fermenters are very active and grow rapidly at 20°C. Bottom yeast fermentation is slowly grows at low temperature (10-15°C). [1]*Saccharomyces cerevisiae* is the cheapest strain used for sugar fermentation. [8,10]The sugarcane were used higher cultivation in

Brazil and second higher production of sugar cane in India in world, identify by Pandian(2014). [10,11]The yeast *Saccharomyces cerevisiae* as agent is uses more efficient conversion of sucrose into ethanol by new biotechnological process[12] in granular form or in pressed humid tablets, which is isolated from sugarcane juice. The genus *Saccharomyces* had highest incidence and it wasthe predominant yeast in industry.[9]Yeast growth is inhibited by low ethanol concentration, inhibiting cell division, decreasing cell-cell electrostatic repulsion [5] and specific growth rate, while high ethanol concentrations reduce cell vitality and increase cell death. The *Saccharomyces cerevisiae* is highly ethanol tolerant, relatively high ethanol concentrations inhibit cell growth viability and limiting fermentation productivity and ethanol yield.[2]However, the ethanol used competitive inhibition of phosphoglycerate kinase, phosphoglyceratemutase and pyruvate decarboxylase, that is non-competitive inhibition of the remaining nine enzymes clearly indicates a role ofglycolysis as an inhibited by alcohol.[13]

S. *cerevisiae* is generally highly tolerant against of various drugs. The high drug dosages are required for inhibition of yeast, those used for mammalian cells. The gene mutants have been responsible for drug resistance for cloned, which encodes an ABC transporter protein structurally and functionally homologous to the mammalian MDR (multiple drug resistance) proteins. The yeast cell surface has presumed to low permeability of drugs [4]. The some antifungal drugs are resistance, which increased *CDR1* expression is an adaptation response to the environment inside a human and other inimical mammalian host [6]. Antifungal drug therapy is no exception; resistance too many of the antifungal agents now in use has emerged. The fungi are eukaryotic organisms with a structure and metabolism that are similar to those of eukaryotic hosts [7].

The goal of the present study was to observe the physiologicaleffectsof alcoholic and drug onthe growth of S. *cerevisiae*yeast, isolated from sugarcane juice. Thestrains were characterized according to their abilities to ferment morphotype, biochemical.The growth of *Saccharomyces cerevisiae* cell has beeninhibitedand yeast metabolism by ethanol, methanol and Ampicillin and Cefotaxim drugs.The*S. cerevisiae* yeast isincreasing the levels of the desired flavoring compounds by fermentation process in the beverage.

II. MATERIALS AND METHODS

2.1 Yeast isolation

Samples of sugar cane juice were collected from different areas in previously sterilized 250-ml flasks. They were kept and transported in an ice-bag, stored at 0°C and processed as fast as possible. Triplicates of decimal dilutions in sterilized water were inoculated on YPDA medium containing potato infusion form (20% [wt/vol]), yeast extract (1% [wt/vol]), and agar (1.5% [wt/vol]) supplemented with dextrose (2% [wt/vol]) and chloramphenicol (0.01% [wt/vol]) or with ethanol (8% [wt/vol]). Plates were incubated at 30 - 35°C for 3-5 days.In order to maintain viability, the culture was stored at 4 °C and Isolated colonies were replicated in PDB medium containing potato infusion form, dextrose (20% [wt/vol]), ethanol (8% [wt/vol]), and chloramphenicol (0.01% [wt/vol]). Isolatedstrains were sub-cultured in PDB media, which incubatedat 28°C in orbital shaker at 200 rev/ min for 48 hours.*Saccharomyces* yeast strains were normally subjected to similar conditions during the fermentation process. These isolates were first classified on the basis of their morphological characteristics, biochemical test and then tested for their ability to grow at higher temperatures (25-28°C) in the presence of 8% (wt/vol) ethanol, which is to the stressing environmental conditions that occur during sugar cane fermentation.

2.2 Fermentation Conditions

Yeast strain were grown in 100 ml culture bottle containing 50 ml of PDB medium andalso used the same composition of those different isolates. The cultured bottle were incubated overnight at 25°C in an orbital shaker at 200 rev/ min. Fermentation was carried out in 25 ml test tube with 10 ml volume, fitted with a fermentation lock and a septum seal for sample, and maintained,incubated overnight at 25°C in an orbital shaker at 200 rev/ min. Yeast growth was periodical measurement of absorbance at 600nm for optical density (OD_{600nm}) by spectrophotometer (PC based Instrument).

2.3 Preparation of Concentration

The *S. cerevisiae* was fermentation monitored by periodical determination consumption of drugs and increase of death cells). The physiological effects were observed of different concentration (control, 05%, 10%, 20%, 30% and 50%) broad range of ethanol, methanol on potato dextrose broth media (ϒTM-Media), and the yeast strain was founded maximum growth of inhibition by 10% of alcohol. The narrow range like as (control 1.0%, 2.0%, 4.0%, 6.0%, 8.0% and 10%)were used.Ampicillin injection (Ampicillin) (control, 50mg, 100mg, 150mg,) and taxim injection (Cefotaxime) (control, 50mg, 100mg, 150mg, 200mg,) drugs, were used in narrow range as Ampicillin (25 mg, 50 mg, 75mg and 100mg) and Cefotaxime used as (10 mg, 25 mg, 50 mg, 75 mg, 100 mg). The effects of growth on *Saccharomyces cerevisiae* by different concentration of drugs have been measured by spectrophotometer, Model-UV-VIS Spectrophotometer 119, Systronic (PC based Instrument), using an absorbance at 600 for optical density (OD_{600nm}). Quantitative measurements were performed by spectrophotometer at $OD_{600nm.}$

2.4 Reproducibility of the Results

All experiments were run at least three times and all reported data are mean values.

III. RESULT

3.1 Obtained Strain

*Saccharomyces cerevisiae*has obtained from sugar cane juice for different sub-region as Agra, Faridabad, Greater Noida, Noida and Mainpuri, and stored at 4°C. The*S. cerevisiae* culture was further used at overnight to each isolates.

3.2 Physiological effects on Saccharomyces Cerevisiae

First up all used of broad test different concentration of alcohol (ethanol & methanol) as control, 5%, 10%, 20%, 40% and 50% that *Saccharomyces cerevisiae*strain has inhibited growth maximum effect on 10% alcohols. The physiology effects have higher concentration alcohols of *Saccharomyces cerevisiae*on PDB media, than used of suitable concentration of alcohols as control, 1%, 2%, 4%, 6%, 8% and 10% (table no. 1), and same way used of methanol concentration, seen table no. (2).*Saccharomyces cerevisiae*has decreased growth rate by different concentration of ethanol (Graph no. 1) and also effects of methanol different concentration (Graph 2). Now,three rounds of experiments were conducted by mean. In the experiment, a broad range of six twofold serial dilutions were tested based on the Minimum Inhibitory Concentration (MIC). Thedifferent concentrations of drugs were used of narrow range as follow as first, second and third rounds.A narrow range of ampicillin drug has used as (0 mg/ml, 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml); this is effects on different concentration (table no. 3). A narrow range of ampicillin drug concentration has inhibited the growth rate of

*Saccharomyces cerevisiae*at (Graph no. 3). Second drug of Cefotaxime is used a narrow range as (0 mg/ml, 10 mg/ml, 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml), It is effects of cell mutation by different concentration (table no. 4). *Saccharomyces cerevisiae* has inhibited the Cells growth Cefotaxime (Graph no, 4). The growth of *Saccharomyces* stains has checked on the basis of Optical density by spectrophotometer. The physiology effects wereobserved by suitably concentration alcohols and drugs on the growth of *Saccharomyces cerevisiae*.

3.3 Determination of Cell growth

Saccharomyces cerevisiae strain have been measured by spectrophotometer, Model-UV-VIS Spectrophotometer 119, Systronic (PC based Instrument), used an absorbance at 600 for optical density (OD_{600nm}). Quantitative measurements were performed by spectrophotometer at $OD_{600nm.}$ The approximate number of cells in a culture can be determined with a spectrophotometer by measuring the optical density (OD) at 600 nm. Cultures should be diluted such that the observed reading (OD_{600}) is <1. That ranges were optical density between an OD_{600} = 1.0 is approximately equal to 10^7or 3 x 10^7 cells/ml.[14,23] That is maximum optical density 2.5 equal to 7.5 x10^7 cells/ml and minimum optical density 0.028 equal to 0.084 x10^7 cells/ml.

Table 1: Different concentration of ethanol, inhibited the growth of*Saccharomyces cerevisiae* strain from different area

	Optical density (OD_{600nm})						
Ethanol concentration	0%	1%	2%	4%	6%	8%	10%
Agra	2.5	1.473	1.076	1.024	0.926	0.23	0.131
Faridabad	2.5	1.62	1.256	1.194	1.097	0.43	0.011
Greater Noida	2.5	2.044	1.852	1.28	0.359	0.125	0.041
Noida	2.5	1.626	1.597	0.683	0.076	0.0495	0.028
Mainpuri	2.5	1.885	1.852	1.597	0.256	0.041	0.026

Table 2: Different concentration of methanol, inhibited the growth of *Saccharomyces cerevisiae* strain from different area

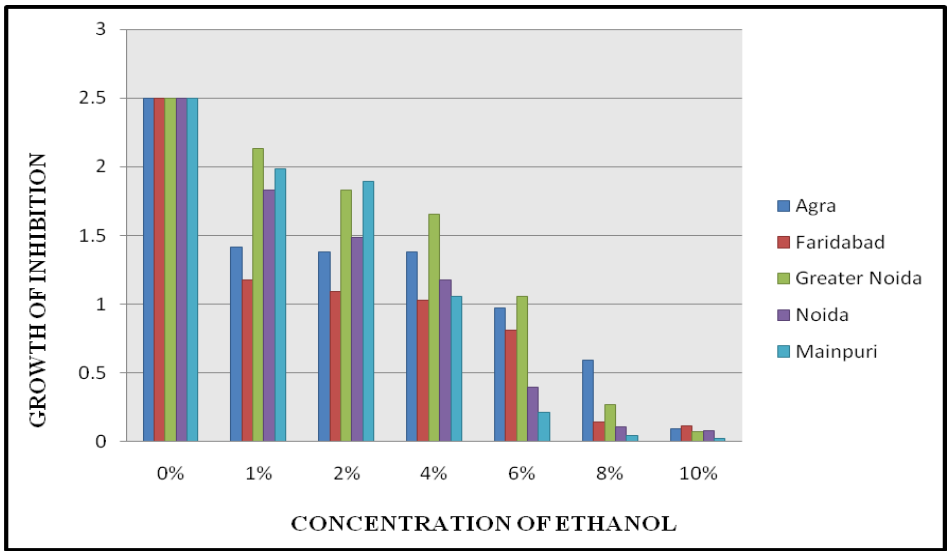
	Optical density (OD_{600nm})						
Methanol concentration	0%	1%	2%	4%	6%	8%	10%
Agra	2.5	1.417	1.378	1.377	0.971	0.591	0.091
Faridabad	2.5	1.179	1.091	1.028	0.809	0.139	0.116
Greater Noida	2.5	2.134	1.833	1.656	1.054	0.27	0.069
Noida	2.5	1.833	1.483	1.179	0.393	0.104	0.08
Mainpuri	2.5	1.983	1.894	1.054	0.212	0.04	0.023

Table 3: Different concentration of ampicillin drug, inhibited the growth of *Saccharomyces cerevisiae* strain from different area

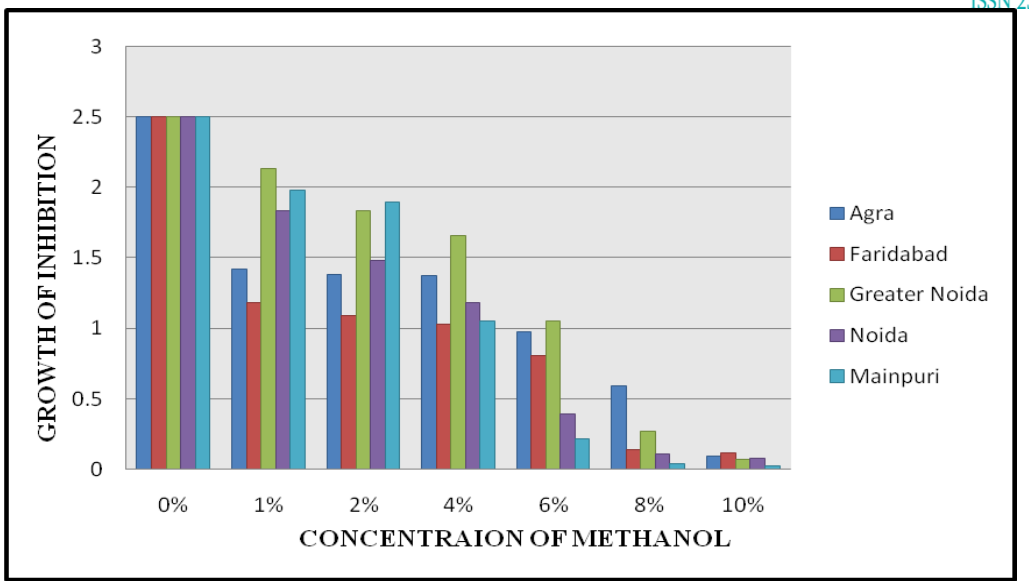
	Optical density (OD_{600nm})				
Ampicillin concentration	0 mg	25 mg	50 mg	75 mg	100 mg
Agra	2.5	2.5	1.25	0.551	0.259
Faridabad	2.5	2.5	1.355	1.109	0.456
Greater Noida	2.5	2.5	1.803	0.937	0.35
Noida	2.5	2.303	1.5	1.109	0.676
Mainpuri	2.5	1.584	1.454	0.885	0.47

Table 4: Different concentration of Cefotaxim drug, inhibited the growth of *Saccharomyces cerevisiae* strain from different area

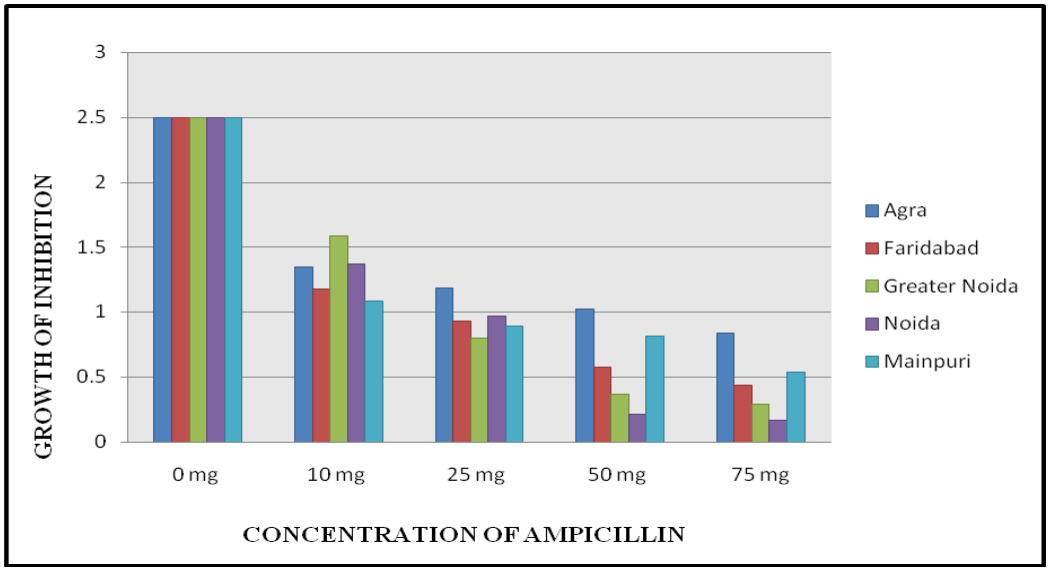
	Optical density (OD_{600nm})					
Cefotaxim concentration	0 mg	10 mg	25 mg	50 mg	75 mg	100 mg
Agra	2.5	1.352	1.184	1.025	0.84	0.163
Faridabad	2.5	1.18	0.937	0.578	0.439	0.081
Greater Noida	2.5	1.59	0.803	0.369	0.295	0.057
Noida	2.5	1.371	0.969	0.219	0.171	0.046
Mainpuri	2.5	1.089	0.895	0.821	0.541	0.06



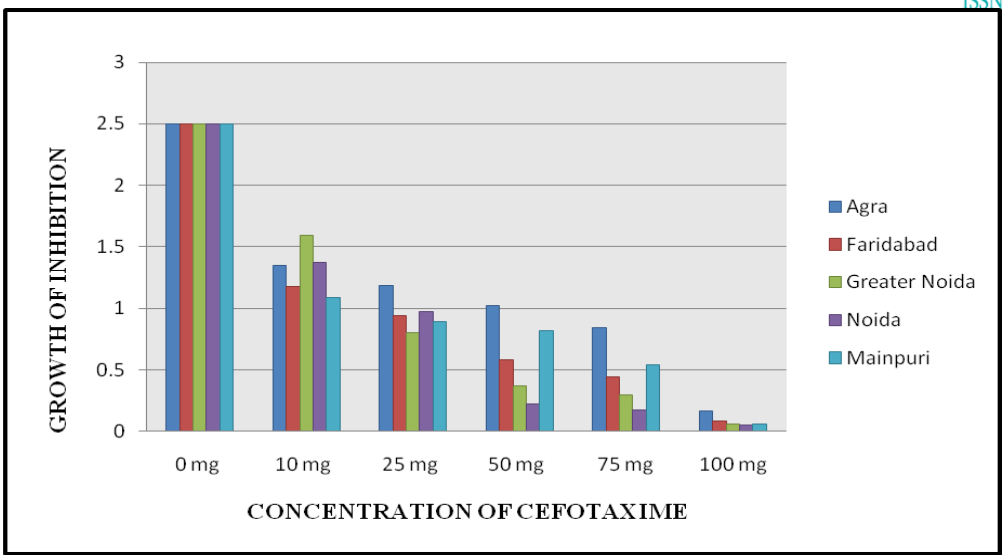
Graph 1: Effects of growth rate, different concentration of ethanol against *Saccharomyces cerevisiae* strain from different areas.



Graph 2: Effects of growth rate, different concentration of methanol against *Saccharomyces cerevisiae* strainfrom different area



Graph 3: Effects of growth rate, different concentration of Ampicillin drug against *Saccharomyces cerevisiae* strain from different area



Graph 4: Effects of growth rate, different concentration of Cefotaximedrug against *Saccharomyces cerevisiae* strain from different area

IV. DISCUSSION

The application of biotechnology is used scientific and engineering principles to the processing of materials by biological agents provide good services. The biotechnology is used to improvement of the product produced by microbes. Those are used in the synthesis of products like organic acids, acetone, enzyme, and many drugs**.** **[14]***S. cerevisiae* has exhibited a reduce energy requirement at low specific growth rates.**[15]**The fermentation temperature has been usedfor optimum at 30°C. The growth of yeast cell has been observed inhibition during fermentation at higher temperatures, has been found in previous study. [8]The fermentation was similar results reported by Walker (2006), that all the isolate ferment at least one type of sugar. However, *S. cerevisiae* isolates which ferment glucose, galactose, maltose, sucrose and raffinose. *S. cerevisiae* yeast is the most commonly used of fermentation in food and beverage industries. The ethanol effects on yeast cell utilised measurements of glucose uptake, the growth was inhibited by 12% ethanol and the losses in viability were observed 18% ethanol.[13]The effect of ethanol concentration on the cell growth and viability of *Saccharomyces cerevisiae* observed. The main target of ethanol is considered to be the cytoplasmatic membrane of yeast cells by Thomas (1978).Alcoholstress have toxic effect on lipids, and protein of plasma membrane and organelles (mitochondria) of *S. cerevisiae and* decrease their structural integrity.[5]Comparative to previous study, *S.Cerevisiae*yeast has observed fermentation properly optimum at 30°C, which has not foundphysiological effect of growth during fermentation at higher temperature.*S.Cerevisiae* has inhibited growth by different concentration as 0%, 1%, 2%, 4%, 6%, 8% and 10% of ethanol and methanol (table 1, 2).The maximum growth of*S.Cerevisiae* has been inhibited by 10% of alcohol. They reported that *S. cerevisiae* is an acidophilic organism and grow better under acidic conditions. [19]

Saccharomyces is ubiquitous Ascomycota yeast used in the food industry in the production of foodstuffs, wines, and beers. [11] The several species were includes *Saccharomyces* genus as *S. cerevisiae. S. boulardii,* which is the treatmentor prevention of antibiotic-associated diarrhea (AAD) for approved in many countries. *S. cerevisiae* is apart of the normal flora of the gastrointestinal tract, the respiratorytract, and the vagina.[16,18]The isolates of

antimicrobial agents susceptibility test were performed against different agents including: Ampicillin (AMP), Clindamycin (CA), Erythromycin (E), Cefotaxime (CTX) etc. and the ampicillin and Clindamycin were selected as a more antibiotics resistance by bacterial isolates.[20]In the present studies, AMP and CTX were inhibited the growth of *S.Cerevisiae* yeast, which used of different concentration of drugs as 0mg/ml, 10mg/ml, 25mg/ml, 50mg/ml, 75mg/ml and 100mg/ml (see table 3,4).The maximum growth of *Saccharomyces* strain has been inhibited by 100mg/ml concentration of drugs. The physiological effects have been found on the growth of *S.Cerevisiae* yeast by different concentration of alcohol and drugs.Comparative to previous study like Amphotericin B is involved in the cell wall stress response pathway and the silver nanoparticles (Ag-Nps) exhibited a potent antifungal activity against *C. albicans*and *Saccharomyces cerevisiae* strains observed.[15,16] This study is similar to the antifungal activity in the MIC values of Amphotericin Band Fluconazole, whichused as a positive control and the inhibition of bud growth correlates with membrane damage. [7]

V. CONCLUSION

The study is very important enhancement of fermentation efficacy.*Saccharomyces cerevisiae* strains obtained for sugar cane juice from different sub-region as Agra, Faridabad, Greater Noida, Noida and Mainpuri District. The physiological effects of *Saccharomyces cerevisiae* has been observed on PDB media.The effect of alcohol on the growth rate of *Saccharomyces cerevisiae* which used of different concentrations of ethanol and methanol has 0%, 1%, 2%, 4%, 6%, 8% and 10% that is found maximum growth inhibited by 10% alcohols. The growth of Saccharomyces *cerevisiae*has been decreased by different concentration of ampicillin and Cefotaxim drugs, that have observed maximum growth cell inhibition by used of 100 mg/ml drugs. The different concentrations have been used of drugs as 0 mg/ml, 10 mg/ml, 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml.

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