Inhibitory activity of *Saccharomyces cerevisiae*, *Rhodotorula glutinis* and *Lactobacillus* spp against *Escherichia coli* isolated from children diarrhea infection

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Abstract
The study was conducted to demonstrate the inhibitory activity of Yeast and *Lactobacillus* spp. *Escherichia coli* isolated from children's diarrhea Infection. Yeast isolates of *Saccharomyces cerevisiae* were isolated from dried date, *Rhodotorula glutinis* was isolated from apple fruit. Pure cultures were prepared on Sabouraud's dextrose agar (SDA). Lactobacillus spp. were obtained from Lacteol fort Sachets. A yeast and *Lactobacillus* spp isolates have been prepared and its antibacterial activity against *E. coli*. Our Results show variable effectiveness were observed, Lactobacillus spp had the highest inhibitory activity against *E. coli*, while Yeast interaction that the highest Inhibitory activity from yeast signal against *E. coli*. In conclusion: the results of *S. cerevisiae*, *Rh. glutinis* and *Lactobacillus* spp isolates showed inhibitory activity against *E. coli*. 

Keywords: Inhibitory activity, *Saccharomyces cerevisiae*, *Rhodotorula glutinis*, Children diarrhea

How to cite this article: Mulla Abid FN, Al-Dulaimi FKY, Al-Tarjuman JK (2020): Inhibitory activity of *Saccharomyces cerevisiae*, *Rhodotorula glutinis* and *Lactobacillus* spp against *Escherichia coli* isolated from children with diarrhea infection, Ann Trop & Public Health; 23 (7): 931-934. DOI: http://doi.org/10.36295/ASRO.2020.23714

Introduction
In recent years, using antibiotics as humans medicines showed a lot of increasing side effects such as the inhibition of intestinal flora[1], and the dependence on using probiotics (yeast, mold, and bacteria) as active antimicrobial agents are widely spread [2]. Probiotics are a group of organism those confer health benefit to consumers [3]. *S. cerevisiae* a unicellular yeast and one of the most explored organism in terms of medicine applications [4]. Several previous studies showed that *S. cerevisiae* can possess probiotic and antibacterial properties [2]. *S. cerevisiae* was effective in the treatment of acute diarrhea in children, also Probiotic Saccharomyces spp. may also help to re-establish a normal gut function, after long term antibiotic therapy [5, 6]. Previous reports involving both In vitro and in vivo studies have indicated that *Saccharomyces* sp. is able to prevent intestinal infection caused by *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus, Pseudomonas aeruginosa*, *Proteus vulgaris*, *Yersinia enterocolitica* and *Candida albicans*[7]. *Rhodotorula glutinis* is a pigmented yeast, part of the *Basidiomycota* phylum, particularly important for food industries because of its biotechnological potential and safety implications. Various strains of *Rhodotorula* represent important features such as the production of large amounts of carotenoids, single-cell proteins from ethanol, acetic acid and acetaldehyde. This review describes and discusses the biology and biotechnology of *Rhodotorula glutinis*, focusing on biochemical pathways, as well as carotenoids and pigment production. Carotenoids have been studied comprehensive and verified to show different beneficial effects on human health through serving as anti-inflammatory effect, antimicrobial and antioxidant activity [8, 9]. Most of *Lactobacillus* strains considered as probiotic bacteria, and play important role in improving human health as a result of its ability to produce many of the antimicrobial agents such as biosurfactants that play important therapeutic roles like much antibacterial activity, control diarrheal diseases, synthesis and enhancing the bioavailability of nutrients [10, 11]. This study aims to determine...
the Inhibitory activity of Saccharomyces cerevisiae, Rhodotorulaglutinis, Lactobacillus delbrukii and Lactobacillus fermentum against E.coli isolated from the children with diarrhea infection.

Materials and Methods
Microbial Isolation: Saccharomyces cerevisiae was isolated from dried date, Rhodotorula glutinis was isolated from apple fruit and identified by the vitek2 System (Germany). The yeast culture was maintained on PDA slants at 4°C and subcultured monthly. Lactobacillus delbrukii and Lactobacillus fermentum were obtained from Lacteol fort Sachets (Ramada, Egypt). This culture was maintained on MRS slants. Escherichia coli was isolated from children with diarrhea infection aged (1-5) years and identified by the vitek2 System (Germany). The bacteria culture was maintained at 4°C and subcultured monthly.

Preparation of Precipitate and Supernatants: S. cerevisiae, Rh. Glutinis Strains to be tested for antimicrobial activity were incubated in YPDB broth for 48 h at 35°C. The precipitate of yeast cells was obtained by centrifuging the culture at 5000 g for 20 min at 4°C. The supernatants were membrane filtered (Millipore, 0.22μm) and stored at 4°C. L.delbrukii and L. fermentum Strains to be tested for antimicrobial activity were incubated in MRS broth for 48 h at 37°C. The precipitate of Bacterial cells was obtained by centrifuging the culture at 5000 g for 20 min at 4°C. The supernatants were membrane filtered (Millipore, 0.22μm) and stored at 4°C.

Antimicrobial Assay: the activities of precipitate and supernatants produced from S. cerevisiae, Rh. glutinis, and Lactobacillus spp strains. The plates were poured with 20 ml Mueller Hinton Agar (HIMEDIA, INDIA). The pathogenic strain E.coli was spread on the surface of MHA by the loop. Wells of 5 mm in diameter were cut into these agar plates and 100 μl of the supernatants and precipitate were placed into each well. The culture plates were incubated at 37°C for 24 h and the zones of inhibition measured in diameter (mm) [13]. Antimicrobial tests were done in triplicate and the mean values were recorded. Statistical analyses were performed using SAS [14].

Results and Discussion
Table 1 Showed results that they Yeast and Lactobacillus spp strains have variable inhibitory activity against E. coli, where observed S. cerevisiae for inhibition zones precipitate and supernatant 17.2 and 14.4 mm, while Rh. glutinis observed 15.5 and 12.3 mm of inhibition zones precipitate and supernatant. L.delbrukii was observed the highest activity from yeast for inhibition zones precipitate and supernatant 18.1 and 20.6 mm, also L. fermentum was observed low activity from L.delbrukii for inhibition zones precipitate and supernatant 17.3 and 19.7 mm. Yeast interaction (S.cerevisiae + Rh. glutinis) were observed highest inhibitory activity from single yeast for inhibition zones precipitate and supernatant 19.6 and 16.3 mm, also (L.delbrukii +L. fermentum) interaction was observed highest activity from single bacteria for inhibition zones precipitate and supernatant 22.3 and 22.9 mm.

Table 1  Antimicrobial activity of Saccharomyces cerevisiae, Rhodotorulaglutinis and Lactobacillus spp against E.coli (Inhibition zones in millimeter).

<table>
<thead>
<tr>
<th>Strains</th>
<th>Zone diameters (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Precipitate</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>17.2</td>
</tr>
<tr>
<td>Rh. glutinis</td>
<td>15.5</td>
</tr>
<tr>
<td>L.delbrukii</td>
<td>18.1</td>
</tr>
<tr>
<td>L. fermentum</td>
<td>17.3</td>
</tr>
<tr>
<td>S.cerevisiae + Rh.glutinis</td>
<td>19.6</td>
</tr>
<tr>
<td>L.delbrukii +L.fermentum</td>
<td>22.3</td>
</tr>
</tbody>
</table>

These results were an agreement with previous studies in this field [15, 16, 17, and 18], several studies have also been reported with the use of S.cerevisiae as a potential biotherapeutic agent probiotic for the treatment of microbes associated diarrhea and colitis [19, 31]. The antibacterial capability of S. cerevisiae might be due to the production of extracellular protease [20]. Rh. glutinis contains carotenoids and material glycoprotein have been studied verified to antimicrobial and antioxidant activity [21]. The secretion of antimicrobial compounds from yeast interaction has led to the inhibition highest effects against E.coli. It was reported studies that the inhibition of yeast interaction was based on the protein excretion as toxins and glycoprotein [22, 30]. A study proved that S. cerevisiae has the ability to inhibit the diarrhea causative bacteria E.coli has shown that they strongly adhere to mannose on the surface of.
S. cerevisiae lectin receptors, once the invading microbe is bound to S. cerevisiae, it’s prevented from attaching to the intestinal cell wall [23]. Also, it was proved that yeast could be used for the treatment of diarrhea [24]. Another study showed the antagonistic activity of the yeast against Salmonella, Shigella and E. coli [5]. Several studies showed that the antibacterial mechanisms of Lactobacillus spp. may be due to a number of factors such as their symbiosis with potential pathogens and production of biosurfactants that inhibit pathogen adherence, decreased pH levels, competition for substrates, production of hydrogen peroxide (H2O2), lactic acid and bactericidal or bacteriostatic substances, involving diacetyl and small heat-stable inhibitory peptides (bacteriocins), this has led to the inhibition of E. coli [25, 26, 27, 28]. Table 2 showed the results that sensitivity to antibiotics was widely distributed E. coli isolates, and was found that the highest sensitivity was Gentamicin, Imipenem, and Cefixime (S), while shown Erythromycin, Ampicillin, Trimethoprim, and Amoxicillin-Clavulanic acid were resistant against E. coli. The resistance to antibiotics attributed as well as to acquired genes responsible for the resistance mounted on each of the bacterial chromosomes and the plasmid where the resistance gene on the plasmid has the ability to move from the bacterial cell to another through the processes of natural genetic exchange as conjugation, transduction, and transformation [29].

Table 2 Antimicrobial activity of antibiotics against E. coli (Inhibition zones in millimeter).

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Zone diameters (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>S</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>R</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>R</td>
</tr>
<tr>
<td>Imipenem</td>
<td>S</td>
</tr>
<tr>
<td>Cefixime</td>
<td>S</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>R</td>
</tr>
<tr>
<td>Amoxicillin-Clavulanic acid</td>
<td>R</td>
</tr>
</tbody>
</table>

R= Resistant. S= Sensitive.

Conclusion

The Saccharomyces cerevisiae and Rhodotorula glutinis singal isolates showed inhibitory activity against Escherichia compared with Yeast interaction that the highest inhibitory activity against Escherichia, Lactobacillus spp showed highest inhibitory activity against Escherichia, further research should be done to ensure safety and efficiency of the potential probiotic yeast and Lactobacillus spp.

References


