

The By-product Metabolic Effect of *Saccharomyces Ellipsoideus* against Some Medically Important Bacterial Species from Conventional And Modified Growth Media

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Abstract

Saccharomyces ellipsoideus is a yeast species with metabolites that enhance resistance to acidity, act as an anti-inflammatory agent, and stimulate the immune system. Additionally, *S. ellipsoideus* exhibits antimicrobial effects against intestinal bacterial pathogens. *S. ellipsoideus* yeast was sourced from local markets in Ramadi City to be tested against the growth of bacterial species, including *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Escherichia coli*, *Serratia marcescens*, *Pseudomonas aeruginosa*. Bacterial susceptibility to *S. ellipsoideus* metabolites was tested using the agar diffusion method. The study findings indicate that *S. ellipsoideus* cultivated in modified media demonstrated increased antimicrobial activity against the five bacterial species over time. The most significant antimicrobial effects were observed against *E. coli*, *P. aeruginosa*, and *S. marcescens*, particularly after an incubation period of 168 hours, where inhibition zones of 19 mm and 18 mm were recorded, respectively. *S. ellipsoideus* effectively inhibits the growth of five bacterial species. Moreover, the antimicrobial activity of *S. ellipsoideus* was significantly enhanced when grown in modified media compared to nutrient media.

Introduction:

Saccharomyces spp. are of the most extensively studied probiotic strains. These microorganisms are widely used as both a preventive and therapeutic agent in many countries [1,2]. Yeasts and their metabolic products play a crucial role in food processing and preservation, particularly in fermentation and bread production. Additionally, yeast-derived metabolites have significant applications in various industries, including pharmaceuticals, food, beverages, and industrial enzyme production [3,4].

In societies that prioritize a healthy lifestyle, probiotics such as *S. boulardii* are commonly incorporated into daily diets. This yeast is considered safe for use in both the food and pharmaceutical industries and has demonstrated its ability to support digestive health and protect against inflammatory conditions [5,6]. Furthermore, *S. boulardii* can regulate antioxidant activity and exhibit antimicrobial effects against intestinal pathogens [5,7,8].

Probiotics are defined as live microorganisms that provide health benefits to the host when consumed in adequate amounts [9]. Strains such as *Saccharomyces spp.* are particularly notable for their resistance to stomach acidity and their ability to survive harsh conditions. Moreover, they are not inhibited by bacterial antibiotics and can integrate harmoniously with the nutrient gut microbiota [9,10]. Due to these properties, *Saccharomyces spp.* has been widely used as an oral probiotic to treat various gastrointestinal conditions, including bacterial diarrhea, colitis, and cholera, among other diseases caused by intestinal pathogens [9,4,11].

The unique properties of *Saccharomyces spp.* have been the subject of numerous studies, which highlight its ability to produce metabolites that enhance its resistance to acidic environments, reduce inflammation, stimulate the immune system, and combat intestinal bacterial pathogens [9,12]. Recent research has also shown that *Saccharomyces spp.* produces biologically active secondary metabolites with antimicrobial properties. These metabolites affect the biofilms of antibiotic-resistant bacterial species such as *Escherichia coli*, *Serratia marcescens*, and *Pseudomonas aeruginosa* [13, 6].

The antibacterial properties of *S. ellipsoideus* secondary metabolites are of significant interest for the development of novel antibacterial treatments, particularly in addressing multi-drug-resistant pathogens. This study aims to evaluate the antibacterial effects of *S. ellipsoideus*'s secondary metabolic products in different nutrient media and compare their efficacy. Given the scarcity of studies focusing on this subject, this research could provide valuable insights into the potential applications of *S. ellipsoideus*-derived metabolites in antimicrobial therapies. This study aims to evaluate the impact of secondary metabolic products derived from nutrient and modified media of *S. ellipsoideus* on various bacterial species.

Materials and Methods

Sample Collection

Fresh fruit samples, including grapes, figs, and lychees, were collected from markets in Anbar City, Iraq, to isolate *Saccharomyces ellipsoideus*. These samples were placed in sterile polyethylene bags and transported to the laboratory for yeast isolation. Proper preservation measures were taken before and after analysis to ensure sample integrity.

Isolation of *S. ellipsoideus* yeast

A one-gram sample was isolated from the collected and prepared fruit and mixed with 9 mL of sterile peptone water (Oxoid, England). A serial dilution of this mixture was then performed up to 10^{-5} . Subsequently, 1 mL of the diluted mixture was cultured using the pour plate technique on Sabouraud dextrose agar medium (Oxoid, England) (pH 5.5) [14]. The inoculated Petri plates were incubated at 37°C for 3–7 days. Finally, the morphological characteristics of *Saccharomyces ellipsoideus* colonies were examined microscopically.

Isolation and cultivation of bacterial strains

The bacterial strains listed in Table 3 were obtained from the microbiology laboratory at the Burn Center of Ramadi Hospital. These strains were isolated from various clinical samples.

To cultivate the isolated bacterial strains, a nutrient agar culture medium (Mast diagnostic, USA) was prepared and autoclaved at 121°C and 15 psi for 15 minutes. The sterilized medium was then poured into Petri dishes and allowed to solidify. Subsequently, 0.5 mL of each bacterial sample was evenly distributed over the surface of the medium. The stability of the medium was verified before incubation. The inoculated plates were then incubated at 37°C for 24 hours to allow bacterial growth.

Identification of yeast *S. ellipsoideus*

To identify *S. ellipsoideus* yeast, biochemical tests were applied, which included utilization of a nitrogen source, utilization of a carbon source, or fermentation of a carbon source, in addition to tests for acid and ester production, urea hydrolysis a gelatin liquefaction test, and an H₂S test, as mentioned by [15-17].

Preparing the modifying nutrient medium for yeast *S. ellipsoideus*

The nutrient medium was prepared according to the instructions reported by AL Zubaidy and Khidhr, (2013), by dissolving 28 g of the medium in 1000 mL of sterile distilled water [16]. The mixture was then heated and continuously stirred for 10 minutes to ensure complete dissolution. Afterward, the nutrient medium (Mast diagnostic, USA) was sterilized at 121°C and 15 bar pressure for 30 minutes. The sterilized medium was subsequently maintained in a water bath at 37°C.

Once the temperature of the nutrient medium was verified, *Saccharomyces ellipsoideus* was added after they were mixed with 5 mL of 100% alcohol for each capsule. The mixture was incubated at 37°C for 72 hours. After this incubation period, 30 g of sugar was added per 250 mL of the nutrient medium containing *S. ellipsoideus*. The solution was then incubated for 168 hours under continuous monitoring and shaking.

Following incubation, the yeast filtrate was separated using No. 1 filter paper. To obtain *S. ellipsoideus* extract and its secondary metabolites from the modified nutrient medium, the filtrate was subjected to ultrasonic treatment and then centrifuged at 5000 rpm for 10 minutes. The final extract, containing *S. ellipsoideus* and its secondary metabolites, was collected. A 0.22 mL aliquot of this extract was used to assess its antibacterial activity against bacterial strains isolated from burn injuries [16].

Antibacterial activity of *S. ellipsoideus* in the nutrient medium and modified medium against bacterial species

Bacterial species *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus epidermidis*, *Serratia marcescens*, and *Pseudomonas aeruginosa* were cultured on nutrient agar medium (Mast diagnostic, USA) for 24 hours. Using a sterile borer, three wells (5 mm in diameter) were drilled in each plate pre-inoculated with the bacteria. Subsequently, 0.22 mL of *S. ellipsoideus* yeast suspension, obtained after centrifugation, was added to each well.

The antibacterial activity of *S. ellipsoideus* was tested using the agar diffusion method [18]. The inoculated plates were then incubated at 37°C for 24 hours to allow propagation. After incubation, the inhibition zones were measured as outlined by Drew *et al.* (1972) [19].

The effectiveness of *S. ellipsoideus* in both nutrient and modified media was determined based on the bacterial isolates exhibiting inhibition zones larger than 6 mm, indicating that *S. ellipsoideus* had antibacterial activity against these bacterial species.

Statistical Analysis

In order to investigate the significance of our results T-TEST was performed using Microsoft Excel 2011 program.

Results and Discussion

Identification of *S. ellipsoideus* by biochemical characteristics

Saccharomyces ellipsoideus was identified using biochemical testing, and the results, as shown in Table 1, indicated that this yeast species utilizes carbon and nitrogen sources for production. However, it does not utilize galactose as a carbon source. Furthermore, the yeast isolation results were negative for the use of lactose, galactose, starch, and nitrates, as well as for urea hydrolysis and gelatin liquefaction. On the other hand, the isolate showed positive results for all other biochemical tests, confirming that this yeast isolate is *S. ellipsoideus*. These findings are in agreement with studies conducted by AL Zubaidy and Khidhr in Iraq and Hossain *et al.* in Bangladesh [16, 17].

Table 1: Biochemical tests for identification of *S. ellipsoideus* yeast.

Tests		Result
Carbon utilization	Fructose	+
	Glucose	+
	Sucrose	+
	Lactose	-
	Galactose	-
	Starch	-
	Raffinose	+
Nitrogen utilization	Peptone	+
	Nitrate	-
	Asparagine	+
	Ammonium sulfate	+
Ester production	+	
Acid production	+	
Cyclohexamide Resistance	+	
Urea hydrolysis	-	
Gelatin Liquefaction Test	-	

Morphological and biochemical examinations of bacterial isolates

Bacterial isolates *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus epidermidis*, *Serratia marcescens*, and *Pseudomonas aeruginosa* were identified through Morphological and biochemical examinations, as shown in Table 2.

The phenotypic characterization of these five bacterial species involved several steps. First, Gram staining was performed to observe the specific staining and morphology of each species. This was followed by examining cell shape and appearance, growth on MacConkey agar (Oxoid , England), mannitol salt (Mast group/ UK) and blood agar media (Oxoid , England) , and growth at temperatures of 4°C and 42°C. All bacterial isolates grew on blood agar, and all except *S. epidermidis* grew on MacConkey medium. At 42°C, all isolates exhibited growth, but none grew at 4°C, except for *S. marcescens*. *S. epidermidis* grew on mannitol salt agar plates. These phenotypic characteristics align with results reported in previous studies [20-23].

Biochemical tests were conducted to further identify the bacterial species. These included tests to produce enzymes such as catalase, oxidase, and urease, as well as tests for indole, methyl red, Voges-Proskauer, and citrate utilization. All bacterial species produced catalase and oxidase enzymes but did not produce urease. The results for *E. coli* indicated positive results for methyl red, indole, and negative citrate utilization. Furthermore, the *S. marcescens*, *S. epidermidis*, and *P. aeruginosa* isolates were positive when they tested for citrate utilization, while *E. faecalis* showed negative outcome for the same test. The biochemical results of this study are consistent with findings from several other studies [20-24]

Table 2: Phenotypic and biochemical examinations of bacterial isolates.

Test type	<i>E. faecalis</i>	<i>E. coli</i>	<i>S. epidermidis</i>	<i>S. marcescens</i>	<i>P. aeruginosa</i>
Gram stain	+	-	+	-	-
Cell shape and appearance	Cocci	Rod-shape cells, Pink colonies, smooth	Cocci, spherical cells	Rod-shaped	Rod-shaped
Growing on MacConkey medium	+	+	-	+	+
Growth on blood culture medium	+	+	+	+	+
Growth at 4 °C	-	-	-	+	-
Growth at 42 °C	+	+	+	+	+
Oxidase enzyme	-	-	-	-	+

Catalase enzyme	-	+	+	+	+
Urase enzyme	-	-	+	-	-
Indol	-	+	-	-	-
Methyl red	-	+	-	-	-
Voges proskauer	+	-	+	+	-
Citrate utilization	-	-	-	+	+

Thirty-four bacterial isolates, including *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Escherichia coli*, *Serratia marcescens*, and *Pseudomonas aeruginosa*, were obtained from the Burn Center at Ramadi Hospital in Anbar City, Iraq. Details of these isolates are presented in Table 3.

Table 3: Shows the bacteria strains used in this study.

Bacteria strains	Number	Source
<i>Enterococcus faecalis</i>	3	Baghdad Hospital Burn Center
<i>Staphylococcus epidermis</i>	8	
<i>Escherichia coli</i>	11	
<i>Serratia marcescens</i>	11	
<i>Pseudomonas aeruginosa</i>	6	
Total		39

Comparison of antibacterial activity of secondary metabolic products of *S. ellipsoideus* in nutrient media and modified media against bacterial species

The effect of the filtered *S. ellipsoideus* in nutrient media and modified media against five types of bacteria was determined by conducting an antimicrobial activity test of *S. ellipsoideus* against bacterial pathogens. The results showed that the filtrated *S. ellipsoideus* had antibacterial activity when grown in both media. Still, the inhibition zone of the growth of the bacterial species also depended on the incubation time. The results showed the effect of the filtered *S. ellipsoideus* in the nutrient and modified media through the inhibition zones for the growth of *E. faecalis*, *E. coli*, *S. epidermidis*, *S. marcescens*, and *P. aeruginosaa* as shown in Fig 1- 5.

The results showed that the filtered *S. ellipsoideus* had a clear effect on the growth of *E. faecalis*, as the inhibition zone increased with an increasing incubation period. On the other hand, it was observed that the filtrated *S. ellipsoideus* effect on the growth of *E. faecalis* was significantly positive in the modified media as shown in Fig. 1. Where the inhibition zone was 16 mm after 168 hours of incubation. The effect of this filtration is explained by the fact that *S. ellipsoideus* cells contain α -d-glucan and β -d-glucan sugars that interact directly with the cell walls of this type of bacteria. Consequently, this interaction causes *E. faecalis* cells to adhere to the outer walls, causing damage and preventing their growth. These results are consistent with the results of a study

conducted by Miller *et al.* when they indicated the possibility of producing quantities of various sugars that have a clear effect on the bacterial species that cause many diseases such as *E. faecalis* [25].

Also, Rajkowska *et al.* reported that the closely species *S. boulardii* exhibits antagonistic activity against pathogenic bacteria such as *E. coli*, *E. faecalis*, and *P. aeruginosa* [26].

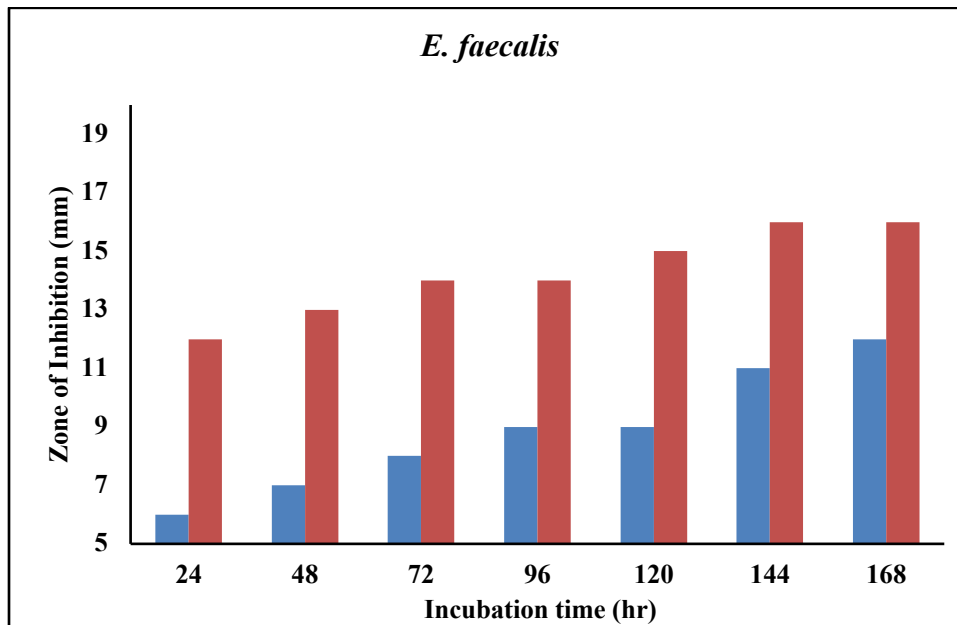


Fig. 1 The effect of secondary metabolic products of *S. ellipsoideus* cultivating in nutrient and modified media against *E. faecalis* (P value < 0.01).

The results also showed that the effect of secondary metabolic products of *S. ellipsoideus* had a clear effect on the growth of intestinal bacteria *E. coli*, and the effect in nutrient and modified incubated media was high. The inhibition zone for the effect of yeast filtrate after 168 hours of incubation reached 19 mm. The results proved that the effect of *S. ellipsoideus* in the modified media is greater than in the nutrient media (Fig. 2). This explains that the effect of the yeast filtrate on the growth of *E. coli* is at its highest levels, as the media contains sugars and alcoholic substances. This is because the presence of *S. ellipsoideus* in such a medium makes it able to produce additional substances with a high effect against pathogenic bacteria. In addition, the reason for this may be the production of substances such as mannose by this type of yeast, which has a great ability to adhere to the surfaces of bacterial cells, including *E. coli* bacteria. Thus, its growth is greatly inhibited. These results are consistent with the findings of Jalal and Aziz, they confirmed in their study that the membrane of the closely species *S. boulardii* is rich in mannose and that pathogenic bacteria have a high ability to bind to the mannose present in that yeast. This may prevent bacteria from sticking to the surfaces that feed them, which weakens their ability to grow and reproduce [27].

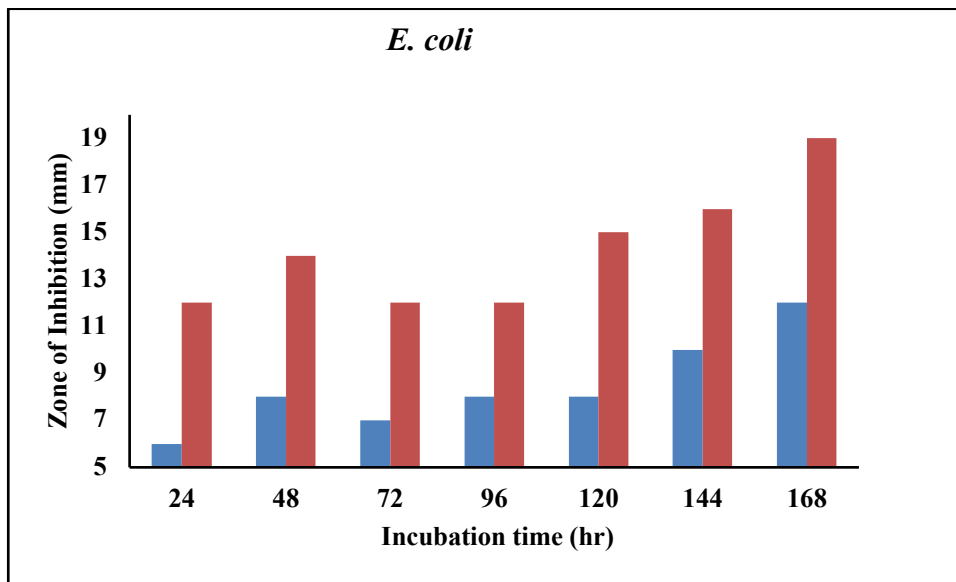


Fig. 2 The effect of secondary metabolic products of *S. ellipsoideus* cultivating in nutrient and modified media against *E. coli* (P value < 0.01).

Also concerning the effect of filtrated *S. ellipsoideus* in nutrient media on the growth of *S. epidermidis*, it was a clear effect and the highest effect of the filtrate was 12 mm for the zone of inhibition when using the isolated filtrate after 168 hours, but the effect was higher in the modified media where the zone of inhibition reached 16 mm at the same incubation time, with a clear significant difference as shown in Fig. 3. This indicates that yeast uses sugars and alcohols as reinforcing materials to produce substances with a microbial effect against isolated bacterial species, including *S. epidermidis*. These results are consistent with the findings of Ali et al. who reported that the closely species *S. boulardii* possesses antibacterial activity due to the secretion of capric acid, which contributes to inhibiting the formation of bacterial cell membranes. Thus, preventing the adhesion of bacterial cells to the food medium by destroying their biofilms, which gave results with a higher effect on the bacteria under study [28].

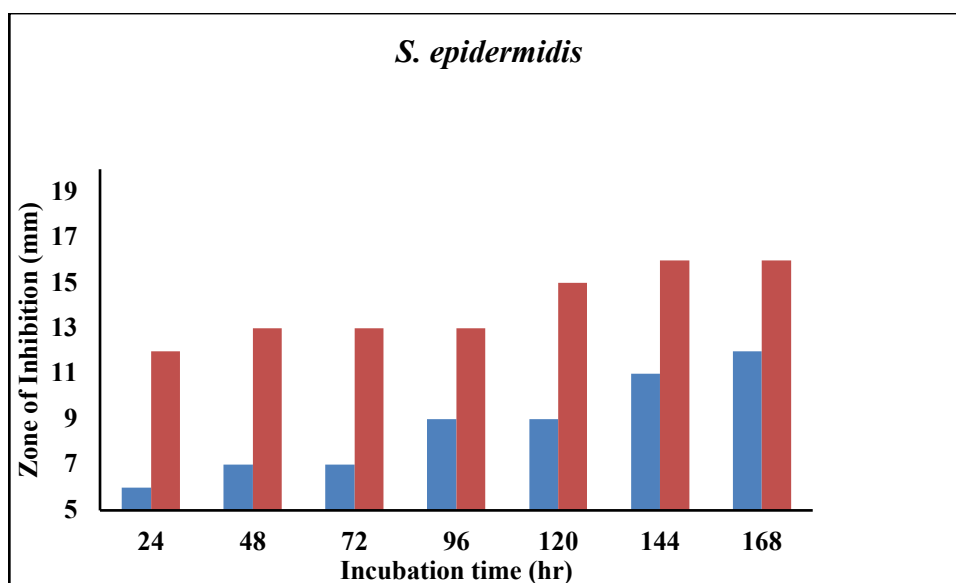


Fig. 3 The effect of secondary metabolic products of *S. ellipsoideus* cultivating in nutrient and modified media against *S. epidermidis* (P value < 0.01).

Also, the filtrated yeast in both media gave a high killing effect against bacterial cells of *S. marcescens* (Fig. 4), as seen with *E. coli* cells. The reason for this may be as pointed out by Canani et al. in their study, where they stated that the closely yeast *S. boulardii* produces small peptides, including serine protease, which are capable of inhibiting the toxin activities of bacterial cells and leading to the degradation of toxin receptors present on the surface of the bacterial cell, thus inhibiting their activity and growth [29].

The results showed that the *P. aeruginosa* bacteria is one of the species most affected by filtrated *S. ellipsoideus* due to the modified media, especially at the incubation period of 168 hours, where the zone of inhibition reached 19 mm. This confirms a difference in the effect of *S. ellipsoideus* in nutrient media and modified media on *P. aeruginosa*, as shown in Fig. 5, including all bacterial species under this study. This result is consistent with a study conducted by Venkateswarulu *et al.* It was concluded that the closely species *S. boulardii* has a pesticide activity against *P. aeruginosa* bacteria. This is because this yeast secretes substances such as peptides that work to separate bacterial toxins or reduce the sugar levels of bacterial cells and thus prevent their growth [30].

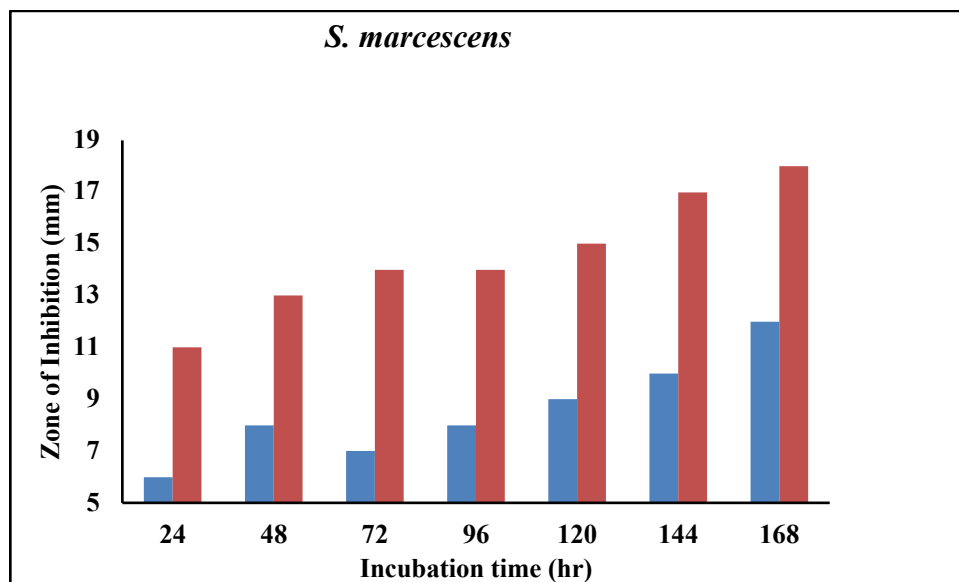


Fig. 4 The effect of secondary metabolic products of *S. ellipsoideus* cultivating in nutrient and modified media against *S. marcescens* (P value < 0.01).

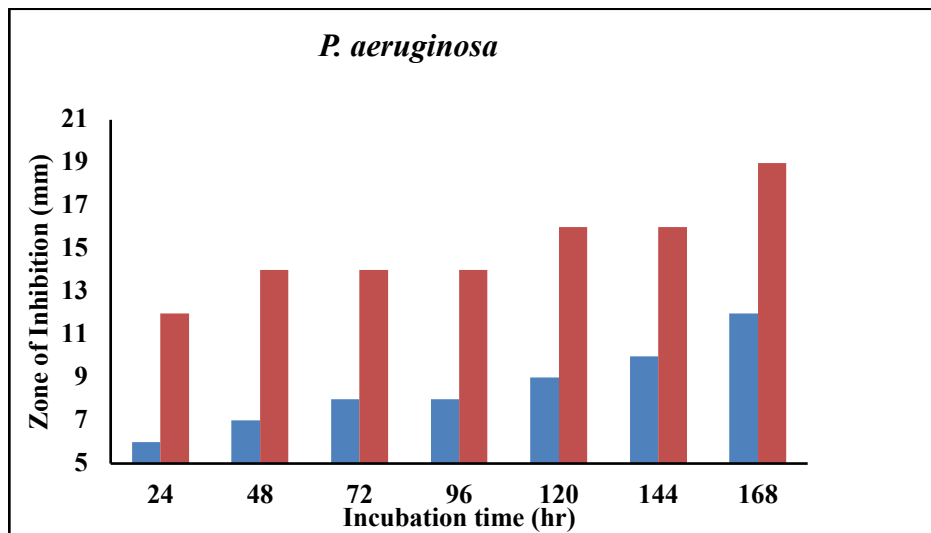


Fig. 5 The effect of secondary metabolic products of *S. ellipsoideus* cultivating in nutrient and modified media against *P. aeruginosa* (P value < 0.01).

Moreover, the results indicate that of *S. ellipsoideus* in the modified media as the incubation period increases, the active substances that have positive antimicrobial activity against the five bacterial species increase. However, the *S. ellipsoideus* had high antimicrobial activity against *E. coli*, *P. aeruginosa*, and *S. marcescens*, respectively as shown in Fig. 6. This may be because yeast consumes nutrients extensively and its cells produce high levels of inhibitory substances that have a high effect against bacterial species such as sugar, enzymes, inhibitory proteins, polysaccharides, and a group of metabolites that have biological activity (gamma aminobutyric acid, 2-hydroxyisocaproic acid, P-aminobenzoic acid, polylactic acid, shikimic acid, tyrosol) [5,6, 31].

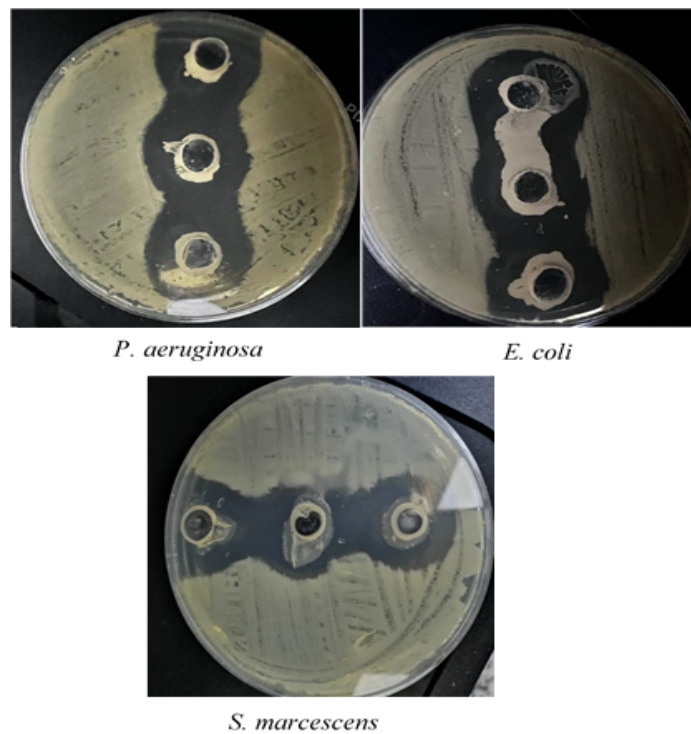


Fig. 6 Shows the effect of modified media for *S. ellipsoideus* on isolated bacteria *E. coli*, *P. aeruginosa*, and *S. marcescens*.

Conclusion

The current study concluded that *S. ellipsoideus* belongs to probiotics and plays an important role in antimicrobial activity. Also, this yeast proved effective in inhibiting five types of bacteria: *E. faecalis*, *S. epidermis*, *E. coli*, *S. marcescens*, and *P. aeruginosa*. In addition, the microbial activity of *S. ellipsoideus* yeast against the five types of bacteria was clear and high when grown in the modified media compared to its growth in the nutrient media.

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التأثير الأيضي للمنتج الثانوي لـ *Saccharomyces ellipsoideus* ضد بعض الأنواع البكتيرية المهمة طبيياً من وسائط النمو التقليدية والمعدلة

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نلاصة:

Saccharomyces ellipsoideus هو نوع من الخميرة مع نواتج أيضية تعزز مقاومة الحموضة وتعمل كعامل مضاد للالتهابات وتحفز الجهاز المناعي. بالإضافة إلى ذلك، يُظهر *S. ellipsoideus* تأثيرات مضادة للميكروبات ضد مسببات الأمراض البكتيرية المعوية. تهدف هذه الدراسة إلى تقييم تأثير المنتجات الأيضية الثانوية المشتقة من المغذيات والوسائط المعدلة لـ *S. ellipsoideus* على أنواع بكتيرية مختلفة. الطرق: تم الحصول على خميرة *S. ellipsoideus* من الأسواق المحلية في مدينة الرمادي، لاختبارها ضد نمو الأنواع البكتيرية بما في ذلك *Escherichia coli* و *Serratia marcescens* و *Pseudomonas aeruginosa*. تم اختبار حساسية البكتيريا لنواتج أيض *S. ellipsoideus* باستخدام طريقة انتشار الأجار. النتائج: تشير نتائج الدراسة إلى أن *S. ellipsoideus* المزروعة في بيئات معدلة أظهرت نشاطاً مضاداً للميكروبات متزايداً ضد الأنواع البكتيرية الخمسة بمرور الوقت. لوحظت التأثيرات المضادة للميكروبات الأكثر أهمية ضد *E. coli* و *P. aeruginosa* و *S. marcescens*، وخاصة بعد فترة حضانة مدتها 168 ساعة، حيث تم تسجيل مناطق تثبيط 19 مم و 18 مم على التوالي. الاستنتاج: خلصت الدراسة إلى أن *S. ellipsoideus* يثبط بشكل فعال نمو خمسة أنواع من البكتيريا. علاوة على ذلك، تم تعزيز النشاط المضاد للميكروبات لـ *S. ellipsoideus* بشكل ملحوظ عند نموه في بيئات معدلة مقارنة بالوسائط المغذية.

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