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Article

Effect of Baker Yeast crude Killer Toxin on some Pathogenic Microorganisms

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Abstract
Many microorganisms, including yeasts, produce protein substances that are toxic to other microorganisms in the competitive communities in which they live. Bread yeast is one of these unicellular microorganisms that produce these inhibitory substances. This study looks at the effects of killer toxins produced by bread yeast on test bacteria and fungi. According to the findings, the inhibitory activity increased with the supernatant concentration. It differed in the extent of its effect according to the type of bacteria, and E. coli isolate was the most sensitive to the yeast killer toxin. The ideal conditions for producing killer toxins were yeast cultivation in GYEPB, PH at 5.5, incubation at 30 c for 24 hours, and ventilation.

Keywords: Baker yeast, Killer toxin, Antimicrobial activity, Saccharomyces cerevisiae, Optimum condition.

Introduction

Yeasts are non-filamentous, unicellular fungi that reproduce through budding or fission [1] discovered killer toxins in Saccharomyces cerevisiae, and they have since been discovered in a variety of additional yeast genera [2] Exotoxins, or mycotoxins, are toxic proteins produced by a variety of yeast species [3] Torulaspora delbrueckii (a yeast species belonging to the Saccharomycetaceae family) and Wickerhamomyces anomalous (a yeast species belonging to the Wickerhamomyceteae family) both have been shown to produce powerful glycoproteins or active toxic proteins as killer toxins [4]

Materials and Methods

Collection of samples: Baker's yeast is available commercially (Saf-instant, France).
Pathogenic strains: Six isolates were acquired from the biological laboratory of the College of Science at Al-Mustansiryah University in Iraq: "E.coli, S.aureus, P.mirabilis, K.pneumoniae, P.aeruginosa, Candida albicans." As a stock cell culture, all isolates were stored in nutrient agar slants at 4°C for the duration of the study.
Yeast activation: Activation of yeast using the Barnett [12] technique.
Identification of S. cerevisiae isolates: Isolates were identified depending on the cultural characteristics, microscopic characteristics, and biochemical tests. Primary diagnostic of isolates based on morphological characteristics of the colonies, including colony shape, height, color, and odor, was studied depending on the

growth of yeasts on Sabouraud agar [13] Before being viewed under a microscope, a smear of the chosen colonies was prepared and stained with lacto phenol cotton blue. The biochemical tests included glucose, raffinose, lactose, fructose, sucrose fermentation, nitrate reduction, and urease synthesis.

Killer toxin Extraction: The killer toxin was extracted from yeast using the Aziz [14] technique.

100 ml of YEGPB broth was added to a 250 ml conical flask, inoculated with 3 ml of activated yeast, and the pH was adjusted to 5.5 before incubation at 30°C and 125 rpm/min for 24 hours in a shaking incubator. The yeast cells were separated using a cooling centrifuge at 4°C for 20 minutes at 5000 rpm/min, and the supernatant was used as a crude extract for yeast killer toxin. The supernatant was sterilized with Millipore microfilters with a diameter of 22.0 m, and a sample of the filtrate was cultured on a solid Sabouraud medium to check that it was free of organisms. A dialysis bag mediated by sucrose concentrated the filtrate once and twice.

Antimicrobial activity: As indicated by Gupta [15], Antimicrobial activity was established using the Well diffusion method.

Transfer 100 µl of the prepared bacterial suspension to the surface of the Mueller Hinton solid medium by the diffusion method with a glass spreader. Leave the dishes to dry at room temperature for 15 minutes. A cork Borer was used to make holes of 5 mm diameter, and then the holes were filled with 100 µl of S.cerevisiae filtrate (raw, concentrated once, and concentrated twice). The dishes were kept at room temperature for an hour, then incubated for 24 hours at 37°C. After the incubation period, the diameters of the clear zone around the holes were estimated in mm.

Effects of certain circumstances on the generation of killer toxins: Effects of medium, pH, temperature, period of incubation, and ventilation on killer toxin formation:

1. The effect of the culture medium:

Yeast was grown in different culture media that included yeast extract glucose peptone broth(YEGPB), Sabouraud dextrose broth(SDB), Potato dextrose broth(PDB), Date extract broth(DEB), and wheat bran extract broth(WBEB).

2. The effect of temperature: Yeast was grown in liquid YEPDB medium and incubated in a shaker incubator at different temperatures (25, 30, 35, 40) C for 24 hours.

3. The effect of pH: Yeast was grown in a liquid YEPDB medium at different pH levels (4, 5, 5.5, 6, and 7) and incubated at 30°C for 24 hours.

4. The effect of incubation period: Yeast was grown in a liquid YEPDB medium and incubated at 30°C for 24 hours in a shaker incubator at a speed of 120 rpm at different times (18, 24, 48, and 72) hours.

5. Ventilation effect: Two cultures of yeast were prepared. The first culture was cultured for 24 hours at 30° C in a stable incubator, while the other culture was incubated in a shaking incubator at 120 rpm.

Results

Cultural characteristics of the yeast isolate: The colonies that grew on the solid medium were round, white to cream in color, with smooth edges, a convex shape with a sticky texture, and a diameter of 1 to 2 mm. The vegetal cells of the yeast were oval to spherical, arranged in a bee-like structure, with a sizeable apparent nucleus and a considerable gap that encompassed most of the cell, as well as the presence of buds in multiple locations and the absence of fungal mycelium.

Antimicrobial activity: The killer toxins showed varying degrees of results when the sensitive bacterial strains were tested. Table 1 shows the killer toxin effect by a distinct halo of growth inhibition around a killer strain's colonies.

(Table, 1) shows the antagonistic activity of S.cerevisiae Killer toxin (concentrated once and twice) grown in GYEPB medium against test bacteria and fungi has different effects on the growth of pathogenic microorganisms with inhibitory diameters ranging from 11 to 23 mm. The results of the statistical analysis indicated that there was a significant difference between the effects of the two concentrations of the supernatant on one isolate under study, except for Candida yeast, which did not show any significant difference, and there is a substantial difference in the total effect of the two concentrations on all isolates. The diameters of the inhibition zone of the onetime concentrate in isolates of "E.coli, P.mirabilis, K.pneumoniae, p.aeruginosa, S.aureus, C.albicans" are 15,14,13,11,11,17 mm respectively, As the diameters of the inhibition zones of tow time concentrate supernatant were 23,20,19,16,17,19.5 mm, respectively.

Conditions affecting toxin production:

The optimum medium for the formation of killer toxin: There is a higher production of toxin against pathogenic microorganisms in the GYEPB medium than PDB, WBEB, and DAB, as demonstrated in Table 1. In SDB, no killer toxin production was seen. Culture conditions may significantly impact the development of killer toxins, and ideal conditions may need to be discovered empirically. The statistical analysis results indicated significant differences in the medium's effect on the toxin's productivity on the bacteria and fungi, except for the PDB medium, the toxin production in it was not significant between isolates and others. Previous research has demonstrated that adding yeast extract and organic nitrogen compounds in the growing medium enhances the production of killer toxins [23] In grape must, YEPDB, and a variety of other culture media, yeasts produce glycoprotein toxin that kills sensitive strains [24]. However, changes in killer toxin generation in different culture media utilized in this study could be attributable to variances in carbon and nitrogen supplies and other components.

Medium	**Diameter of inhibition zone / pathogenic microorganisms**					
	E.coli	P.mirabilis	K.pneumoniae	p.aeruginosa	S.aureus	C.albicans
YEPDB	20	14.5	17	17	15	12.5
SDB	0	15	0	12	11	10
PDB	12	12	11	13	10	10.5
DEB	15	15	14	13.5	10.5	13
WBEB	12	10.5	10	14	12	11
LSD	5.62*	4.41 *	5.77 *	4.52 *	4.63 *	3.06 NS

Table 1: Antimicrobial activity of killer toxin of S.cervisiae cultivated in different media against pathogenic microorganisms.

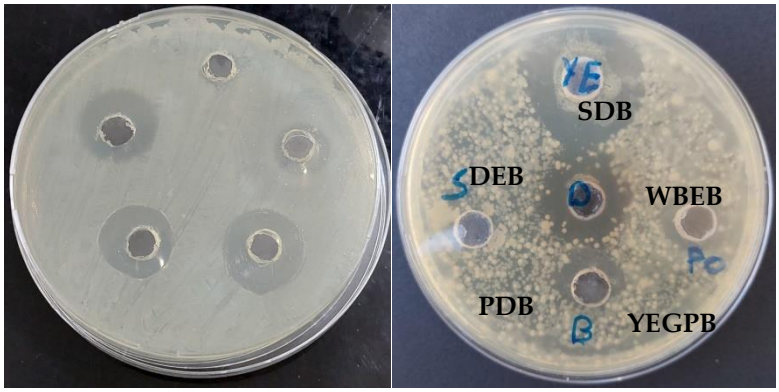

Figure 1: Antimicrobial activity against pathogenic microorganisms.

The optimum PH for the formation of killer toxin: Table 2 shows that the ideal pH for toxin production was 5.5. There was very little output at pH values 4, 5, 6, and 7. The ideal pH for creating and stabilizing various killer toxins has been changed. For example, the optimum pH for the development and stability of K1 killer toxin ranges from 4.6 to 4.8. In contrast, the optimum pH for K2 killer toxin ranges from 2.9 to 4.9 [25] The results of the statistical analysis showed that there were significant differences at the level ($P \leq 0.05$) in the diameters of the inhibition zones for all pathological isolates, except for the PH value = 4, which did not show any significant difference between one isolate and another. S.cerevisiae is an acidophilic organism, which means it thrives in acidic environments.

PH	Diameter of inhibition zone / pathogenic microorganisms						LSD value
	E.coli	P.mirabilis	K.pneumoniae	p.aeruginosa	S. aureus	C.albicans	
4	15	12	13	11	14	12	3.35 NS
5	19	14.5	12.5	17	15	13	4.51 *
5.5	23	18	18.5	17	16	15.5	4.77 *
6	20	17	16	12.5	17	14	4.69 *
7	15	10.5	13	0	12	12.5	5.03 *
LSD	4.22 *	4.61 *	4.37 *	3.95 *	3.87 *	3.65 NS	---
*** (P≤0.05), NS: Non-Significant.**							

Table 2: Antimicrobial activity of killer toxin of S.cervisiae cultivated in different PH against pathogenic microorganisms.

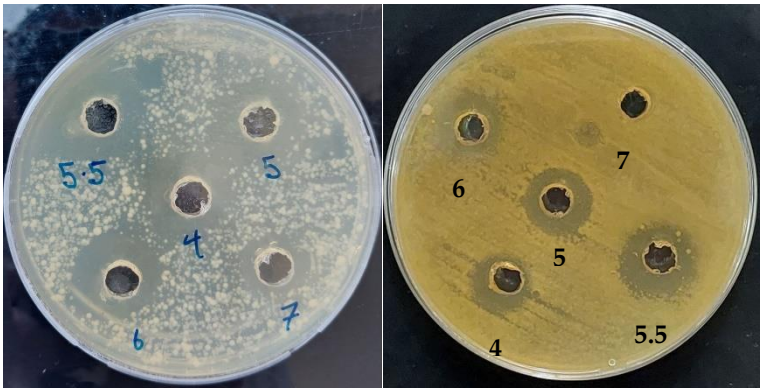

Figure 2: Antimicrobial activity against pathogenic microorganisms.

The optimum temperature for the formation of killer toxin: At an incubation temperature of 30°C, the most significant formation occurred, while at 40°C the formation was reduced. The little formation was observed at 35,25°C (table 3). The same results were seen for other toxic materials [28] The majority of the yeast colonies formed at 37 or 40 Co, there was no sign of killing, and all strains displayed an increase in the frequency of non-killer colonies with rising temperature [19] The results of the statistical analysis indicated significant differences at the level (P<0.05) between diameters of the inhibition zones for all isolates except for the temperature of 25, which did not show any significant differences in their effect on isolates.

Temperature/C	Diameter of inhibition zone / pathogenic microorganisms						LSD value
	E.coli	P.mirabilis	K.pneumoniae	p.aeruginosa	S. aureus	C.albicans	
25/C	15	14	15.5	13	14.5	15	3.02NS
30/c	18	17	16	14	16	15	3.91 *
35/c	13	14	14	12	16.5	11	4.17 *
40/c	0	0	0	0	0	0	0.00 NS
LSD value	4.37 *	4.55 *	3.97 *	3.83 *	4.69 *	4.03 *	---
*** (P≤0.05), NS: Non-Significant.**							

Table 3: Antimicrobial activity of killer toxin of S.cervisiae cultivated in different temperatures against some pathogenic microorganisms.

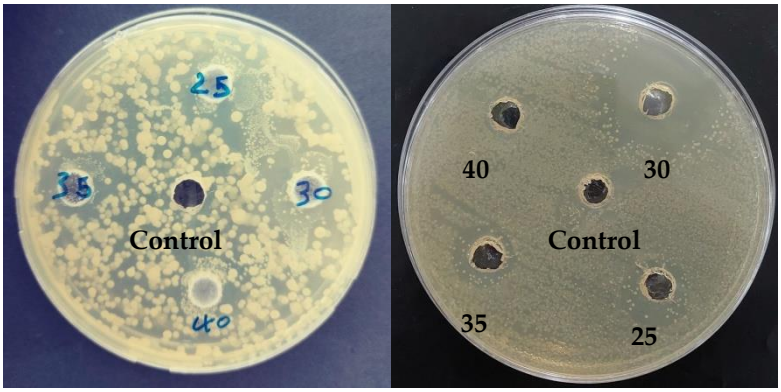

Figure 3: Antimicrobial activity against pathogenic microorganisms.

The optimum incubation period for the formation of killer toxin: When S.cerevisiae was grown on a GYEPB medium at 30°C, minimal formation of the killer toxin was noticed at 18,48 h, the maximum formation was noticed at 24 h, and no production was observed at 72 h, as indicated in table 4. The results of the statistical analysis showed that there were significant differences at the level (P≤0.05) in the diameters of the inhibition zones for all pathological isolates, except for the incubation period of 18 hours, which did not show a significant difference in its effect between one isolate and another.

Period/ hour	Diameter of inhibition zone / pathogenic microorganisms						LSD value
	E.coli	P.mirabilis	K.pneumoniae	p.aeruginosa	S. aureus	C.albicans	
18	15	14	15.5	14.5	15	15	2.86S
24	19	17	18	15	18	16	3.81 *
48	13	14	18	14	18	15	4.07 *
72	0	0	0	0	0	0	0.0NS
LSD	3.98 *	3.75 *	4.05 *	3.79 *	4.27 *	4.63 *	---
*** (P≤0.05), NS: Non-Significant.**							

Table 4: Antimicrobial activity of killer toxin of S.cervisiae cultivated in different incubation periods against pathogenic microorganisms.

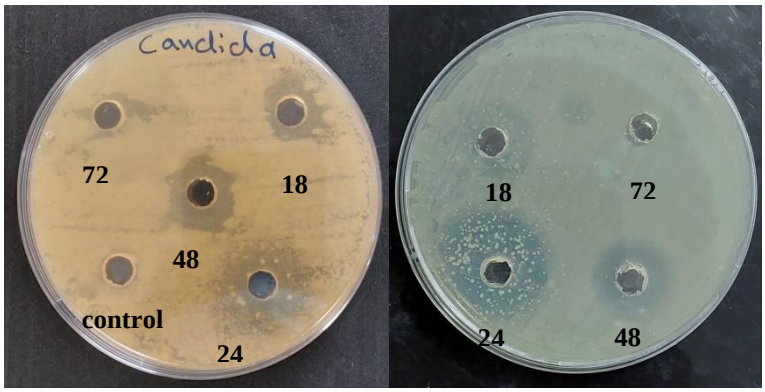

Figure 4: Antimicrobial activity against pathogenic microorganisms.

Incubator	Diameter of inhibition zone / pathogenic microorganisms						LSD value
	E.coli	P.mirabilis	K.pneumoniae	p.aeruginosa	S. aureus	C.albicans	
Shaker	20	19	18	16	15	14	4.36 *
Static	15	15	15	14	13	13.5	2.65 NS
LSD	3.81 *	3.78 *	3.02 NS	2.76 NS	3.15 NS	2.09 NS	---
* (P≤0.05), NS: Non-Significant.							

Table 5: Antimicrobial activity of killer toxin of S.cervisiae cultivated in different incubators against pathogenic microorganisms.

Discussion

Biochemical test: Shows the ability of the selected isolates to ferment the carbon sources of sugars represented by glucose, fructose, sucrose, maltose, and raffinose, while they were unable to consume lactose as a carbon source, and the production of urease enzyme, indicating that it belongs to the yeast S. cerevisiae [12].

It is suggested that the receptor type and the yeast supernatant concentration play a role in the variation in the degree to which these substances affect bacteria [17]. The crude supernatant did not give any inhibitory action on all the test isolates, and this agrees with the researcher [18]

It is clear from the above-increased effect of killer toxin with increased concentration that the yeast S.cerevisiae has a broad antimicrobial activity that has an inhibitory effect against Gram-negative, positive bacteria and fungi. This may be due to its ability to produce acidic inhibitory substances, a lethal protein nature similar to the bacteriocins secreted by bacteria with a specialized action in destroying the plasma membranes of sensitive cells, causing the loss of their cellular contents as well as inhibiting the transport of amino acids [19] Shareef and Al-Dabbagh [20] showed that the inhibitory protein substances produced by S.cerevisiae differ in their inhibitory activity against bacterial isolates depending on the receptors on the surface of the bacterial cells as well as the mechanism of action of the inhibitory proteins produced by them that have a role in affecting the cell wall, according to Table 1, that the diameters of the inhibitory zones increase with increasing protein concentration, which is consistent with what was stated by [21]. Despite the discovery of the phenomenon of killing among yeasts for a long time and the succession of studies on the mechanism of the effect of toxins on microorganisms, reports, research and studies on the effect of yeast toxins on bacteria or the mechanism of inhibiting the growth of pathogenic bacteria remained unknown or studied in an integrated manner [22].

The optimal pH range for yeast growth can range from 4 to 6 depending on temperature, oxygen availability, and yeast strain [26]. The pH influences toxin production because of its role in the solubility of medium substrates and its

impact on the substrate's ionization. It also has an impact on enzyme stability and productivity [27].

When tested at 18 to 30 °C, Candida nodaensis killer toxin shows killer action but loses its murderous phenotype at 40 °C. This is comparable to what has been indicated for other killer toxins [29] Surprisingly, killer activity was observed under a wide range of PH, temperature, and oxygen concentrations [2] Similar studies on enzyme invertase produced from S.cerevisiae found the optimum temperature for enzyme production is 30c. The production of enzymes decreases at low and high temperatures because of effects on microorganisms' growth and enzymatic reactions inside the cell [30].

The measured killing activity raised with cell titer during the exponential growth phase and leveled off as cells reached their stationary phase [31]. A strategy is also needed for other killer toxins whose formation and secretion occur during the producer yeast's exponential growth, rising to the beginning of the stationary phase [29]. The synthesis of toxins decreased due to the medium's nutritional depletion and repression of an enzyme [32].

Effect ventilation for toxin production: As shown in Table 5, the best route for toxin production is incubation with shaking rather than static incubation. This is in agreement with [33]. It is reported that an increase in yeast cell biomass and mannan content occurs under aerobic conditions. 34. Shaking homogenizes the components of the culture medium with each other, with the air and the organism. Thus, the organism, the latter, is stuck in the culture medium throughout the growth period, which helps to achieve its metabolic activities correctly. The shaking increases the dissolution of oxygen in the culture medium [35]. According to the statistical analysis findings, there were significant differences at the level ($P \leq 0.05$) in the diameters of the inhibition zones for all pathological isolates in the shaking incubator. In contrast, no significant difference appeared between the isolates in the stable incubator [36] They studied the inhibitory activity of yeast grown under aerobic conditions, which has a substantial impact on its ability to produce inhibitory substances that are believed to have a protein nature and whose synthesis activated under aerobic conditions in microorganisms. As a result of the negative correlation between biomass production and alcohol produced in culture media, biomass production increases [34]. Therefore, it is appropriate to use a shaking incubator because low ventilation leads to anaerobic conditions that inhibit the enzyme systems necessary for forming carbohydrates in cells [37,38,39].

Conclusion

Based on the findings, it can be concluded that (GYEPB) medium, PH 5.5, 30 C for 24 hours, and incubated in a shaker incubator, the yeast S. cerevisiae could yield the most killer toxin.

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