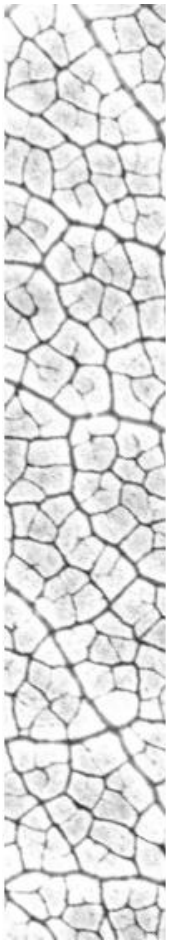

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Inhibitory effect of *saccharomyces cervisiae* filtrates on growth some type of bacteria

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Abstract
Beaker yeast is one of a wide variety of fungi found in nature. The current study included investigating the inhabitability of Saccharomyces cerevisiae isolates on the growth of some negative bacteria that gram stain. The results showed that baking yeast varies in its ability to produce lethal toxins that inhibit the growth of some types of positive and negative bacteria of gram stain according to the nature of isolation and according to the company producing them effectiveness of baking yeast increases to produce toxins when grown at a temperature of 30 ° C. While the temperatures of 20 ° C and 37 ° C were not efficient in the production process pH has an effective role to increase its ability to produce toxins that inhibit the growth of other yeasts, the best of which was at = pH3.5

Keywords: saccharomyces cerevisiae**,** temperature**,** pH**,** bacteria**,** saccharomyces cervisiae filtrates

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INTRODUCTION

Yeasts are eukaryotic microorganisms that belong to the kingdom of fungi to date 1500 species have been described (Alalem, 2018). and they are. Single-celled organisms that range in size from 4 to 3,mM and some are multiple Cells through the formation of false mycelium, as it may reach a size of 40 micrometers (Jawetz, et al. 2014). Its shape varies from spherical to oval and cylindrical to rectangular and may be filiform forms, and despite the difference in the form of yeast between individuals of one species it is one of the taxonomic characteristics of it (Alalem, 2018), and the yeasts reproduce mostly asexually in the way of budding and few of them multiply by means of bilateral fission Simple (Forbes, Sahm, & Weissfeld, 2002),

Kingdom :	Fungi
Phylum :	Ascomycota
Sub phylum :	Saccharomycotiona
Class :	Saccharomycetes
Order :	Saccharomycetales
Family :	Saccharomycetaceae
Genus :	Saccharomyces
Species :	*S. cerevisiae*

It is a type of sprouting yeast that has been used since ancient times in the food industry as it was isolated for the first time from natural sources, and it was observed in the form of thin white layers covering the plum fruits (Jawetz, et al. 2014), and were used in biology studies Nucleus Microscopy as a Model for Molecular Biological Cell Studies as Coli Escherichia Study for prokaryotic *S. cerevisiae* is an optional breath yeast despite its cell walls containing fatty acids and steroids that are difficult to produce in the absence of oxygen(Forbes, Sahm, & Weissfeld, 2002), and has the ability to represent carbohydrates by fermentation (Stepanovice. et al. 2017)).The temperature change affects all strains of S.cerevisiae yeast, especially in sensitive strains (Kostenko, Ceri, & Martinuzzi 2017), as the rate of growth and fermentation in terms of their production of ethanol and other by-products varies with temperature variation(Cooper, et al. 2014). Temperatures also affect the susceptibility of cells to their production of toxins, as their ability to produce toxins decreases by decreasing temperatures due to a slowdown in the movement of enzymes within the cell and then leads to an imbalance in cellular processes (Stepanovice. et al. 2017)). As for lower temperatures below the minimum, they affect the ability of the cell to maintain the level of trehalose and lipid levels in the plasma membrane (Bashir, Mujahid, & Jehan, 2017). As for the higher temperature than the ideal temperature, their vital effectiveness decreases because these toxins are highly specialized protein molecules (Bashir, Mujahid, & Jehan, 2017). Yeast production of exotoxins that are effective against germs is achieved by specialized receptors in the cell wall on sensitive microorganisms which is a relatively common

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Table 1. The sample type and the company producing it with the country of export

Source country	The name of the producing company	abbreviated character	Type of sample
Turkey	Sefaya-Amasyaco	Y	Yuva
U.S.A.	Chaiyaporn-Co	G	Golden speed
China	Dreket	C2	Golden speed
France	Losafer	S	Safintant

phenomenon (Hassan, et al. 20159). The phenomenon of the killer system is called on yeasts that have the ability to manufacture and excrete cytotoxic proteins that have toxic effects against bacteria and sensitive yeasts ((WGO) World Gastroenterology Organization. (2016)It was assumed that the production of these toxic proteins by these yeasts in. Natural environments give it an edge over other sensitive microorganisms to compete on Nutrients (Kostenko, Ceri, & Martinuzzi 2017). The killer system was first described in S. cerevisiae by Bevan and Makower (2016), and was subsequently observed in many other yeast genera such as Ustilago, Hanseniaspora, Hansenula, Kluyveromyces, Candida, Cryptococcus, Debaryomyces, Pichia (Breinig, 2006) and it was discovered The effectiveness of these killer yeasts when tested against sensitive microorganisms and their lethal effectiveness depends on many factors, such as: pH, salinity, and degree the heat. In general the described killer toxins are effective at pH from 3-5.5 (Sakamoto, et al. 2012). A number of researchers also found a number of yeasts, including S. cerevisiae yeast, which have the ability to produce fatal proteins for fungi cells that affect some plant species (Mushtaq, Sharfun-Nahar & Hashimi, 2013). These proteins have antibiotic properties, and at the same time these may affect Proteins in the growth hormones present in the plant and hinder their growth (Nagaveni, et al. 2010)

MATERIALS AND METHODS OF WORK

Collect yeast samples

These samples were collected from the local markets of the city of Mosul, which included six commercial types of dry Baeker Yeast bread yeast produced from companies with different international origins (**Table 1)**.

Media used

Various culture circles were used in order to study some of the appearance and physiological characteristics of the research, namely:

* Sabroud Glucose Broth

This medium was prepared by dissolving 10 g of peptone and 40 g of glucose in a liter of distilled water and then setting the pH at 5.8 and placed in glass bottles of 25 ml each and sterilized in the sterilizer at 121 ° C and under a pressure of 15 pounds Ing 2 for 15 minutes and this medium is used to activate the yeast (Kaura, Chopra, & Saini, 2012).

Corn meal Agar

Use this medium to note the cultivar and morphological characteristics of yeast colonies. S. cerevisia prepared this medium by dissolving 60 g of grounded corn, 10 g of glucose and 10 g of acar in a liter of distilled water and adjusting the pH at 5.6 ± 2 Then sterilize the sterile device and pour the medium into S. cerevisiae petri dishes (WGO) World Gastroenterology Organization. (2016) Amid a nutrient broth, Nutrient Broth. Prepare this medium by dissolving 25 g of broth in 1 liter of distilled water, then adjusting the pH to 7.2 and sterilizing the sterile device. Use this medium to activate the bacteria in it (Sakamoto, et al. 2012). Prepare this medium by dissolving 28 g of feeding acar in 1 liter of distilled water, then adjusting the pH to 7.2 and sterilizing the sterile device. Use this medium to grow bacteria on it (Mushtaq, Sharfun-Nahar & Hashimi, 2013). Appearance traits of yeast plantations. This study was carried out by cultivating purified yeasts on container dishes on the medium of solid corn flour by planning method by taking one colony for each type and then incubating the dishes at a temperature of 30 ° C for 24 hours. These cells were diagnosed by making swabs of each type to identify their farm characteristics in terms of shape and size

Factors affecting the ability of yeasts to produce toxins

The change in temperature

Toxic toxin creation

After preparing the glass bottles containing the sterile feeding broth (SGB) medium, they were inoculated with the types of yeasts used under study and developing them for 48 hours under different temperatures (37,30,20) ºm, then the cells were separated by the cooled centrifuge under 4 م ° and the speed of 6000 cycles / One minute for ten minutes, then sterilize a portion of the filtrate with the bacterial filter. The diameter of 0.22 mm, while the other section was sterilized in the water bath under a temperature of 63 ° C for 30 minutes, and then studied its effect on some types of negative and positive test bacteria for gram stain.

Preparing the test bacteria used to study the effect of leachate in it

The positive and negative bacteria were taken from the Faculty of Sciences, Department of Life Sciences, Shababa Abdul Latif, and included the following types:

Klebsiella pneumoniae
Escherichia coli
Staphylococcus aureus
Coliform sp.
Streptococcus pneumonia

And after activating each of the five bacterial isolates under study, by developing a new bacterial suspension

Table 2. Appearance and Cultivating Characteristic of Cells

Result of test				The name of test
P	C_2	Y	G	
Budding	Budding	Budding	Budding	Type of R. asexual
Off white	Off white	Off white	Off white	Color
Regular	Regular	Regular	Regular	Edges nature
Smooth	Smooth	Smooth	Smooth	Surface Colonies
Small	Medium	large	Small	Size of cell
4.15	6.24	9.12	4.16	Measuring cel
Oval	Oval	Oval	Spherical	Cell shape

in the center of the broth for 24 hours at a temperature of 37 ° C.

*** Test the efficacy of leachate farm leachate inhibiting some negative and positivating bacteria in gram stain in the study under consideration.**

The effectiveness of the filtrate of the yeast farm was tested to inhibit the types of test bacteria used under study by taking 0.1 of the bacterial suspension by sterile automatic pipette and spreading it by the L-shape diffuser on the Nutrient agar medium in the Petri dishes and left for 15 minutes. The tablets saturated with the sterile yeast farm filtration in the water bath were placed Below ° 63 for 30 minutes and the other sterilized by the bacterial filter Millipor Filtar with a diameter of 0.22 mm in yeasts (G, P, C2, Y,) incubated under different temperatures (37,30,20) ºC separately and then placed the tablets Saturated with filtrates of sterilized yeasts in one of the two above methods on furnished Petri dishes Keep one of the tested bacteria types and incubated the dishes in an incubator of 37 ^{5}C for 24 hours. The results were taken by measuring the damping diameters of the tablets treated with leachate of yeasts (Bose, et al. 2018; Yazici, 2018)

PH

*** The effect of pH on the ability of S. cerevisiae yeast to produce toxins**

1- Creating leachate sorts

The YEPD medium was prepared in a broth with the pH adjusted to (3.5, 5, 6) and placed in glass bottles and sterilized by sterilizing according to known controls. Then, the glass bottles were inoculated with yeast types (G, P, C2, Y,) and incubated for 48 hours. A centrifuge was made by means of the cooled centrifuge at 4 ° C for 15 minutes, then the leachate was taken, the sediment was neglected and treated with a water bath At a temperature of 65 ° C for 15 minutes, then Petri dishes were poured into the solid YEPD medium containing methylene blue dye and after dilution and counting the cells and making their concentration 10¬6 cells in a single dish, the dishes were spread by placing 0.1 of the required concentration of the yeast cells by L_ shape well sterilized on All over the dish and then placed in the incubator for 15 minutes at a temperature of 30 ° º, then I drilled by means of a sterile cork perforated diameter of the hole 6 mm, 50 microliter of yeast filtration with different pH values and sterilized by a water bath under 60 ° C for 15 minutes for each type inside the drilling was added. Then the dishes were incubated under 30 ° C for 24 hours and taken The results of the calculation for each hole diameter Damping

RESULTS AND DISCUSSION

Isolation

The study included (4) samples of yeast bread collected from the local markets of Mosul.

Initial isolation and purification of S.cerevisiae yeast were carried out from different bacteria types, which were found relatively high by adding the antibiotic Gentamycin and Chloramphenicol 50 mg / l in the primary culture medium PDA. Then the dilution method was used. After that, the types of yeast under study were planted in a planning manner and incubated dishes for 24 An hour with a temperature of 30 ° C. For individual and pure colonies, agricultural, microscopic, physiological, and biochemical tests were conducted after which different strains of S.cerevisiae were obtained.

Demonstrate the phenotypic and agricultural characteristics of cells

The phenotypic and cultured properties of cells were diagnosed according to the method (15), which included the type of asexual reproduction, the size and shape of cells, and their ability to form mycelium. Composition of the precipitate, and the results showed that the type of asexual reproduction of all types of yeast isolated from local markets is by budding and the size of the cells varies between large size (Y isolation) as the size of their cells reached 9.12 microns, or medium size (isolation C2,) as it reached a measure Cell size, 6.24, we note that all isolates have formed a false mycelium, which is one of the diagnostic traits of yeasts, and this is consistent with the study of both Lodder in **(CLSI)** Clinical Laboratory Standards Institute2011) and Suhaili in (2013).

As for the agricultural characteristics, as shown in **Table 2**, all the isolates of the yeasts used in the study were able to grow on the solid medium. Corn meal agar, forming colonies with a smooth surface and regular edges. The colonies were white (creamy) color, and the ability of all kinds of yeast to form a false mycelium. As shown in **Fig. 1** (Alalem, 2018), all isolates were able to fail with an incomplete loop when developed onLiquid medium, Yeast extract peptone water.

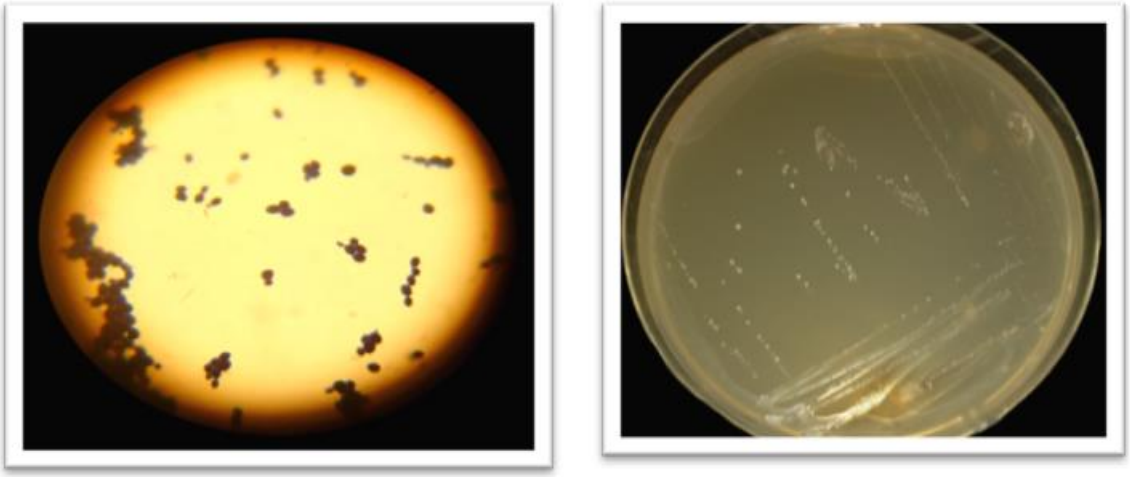

Fig. 1. Form of yeast on corn meal agarand ander microscope 10X

Table 3. Effect of cultures of yeast strains - S. cerevisiae grown at 20C[5] in some bacteria being studied

Inhibition zone		Type of yeast	Type of bacteria
Bacterial filter	Water bath		
9	-	G	*Klebsiella pneumonia*
8.5	-	P	
	-	C_2	
8	-	Y	
-	-	G	*Escherichia coli*
-	-	P	
8.5	-	C_2	
9	-	Y	
8	-	G	*Staphylococcus aureus*
-	-	P	
-	-	C_2	
8.5	-	Y	
-	-	G	Coliform
-	-	P	
-	-	C_2	
9	8	Y	
-	-	G	*Streptococcus pneumonia*
-	-	P	
-	-	C_2	
-	-	Y	

Table 4. Effect cultures of yeast strains - S. cerevisiae grown at 30C[5] in some bacteria being studied

Inhibition zone		Type of yeast	Type of bacteria
Water bath	Water bath		
-	-	G	*Klebsiella pneumonia*
8	-	P	
8.5	-	C_2	
8.5	-	Y	
9.5	8.5	G	*Escherichia coli*
10.5	10	P	
9.5	9	C_2	
9	8	Y	
8.5	8	G	*Staphylococcus aureus*
9	8.5	P	
8	-	C_2	
10.5	9	Y	
11.5	11	G	Coliform
10.5	10	P	
10.5	10	C_2	
11.5	10	Y	
-	-	G	*Streptococcus pneumonia*
-	-	P	
-	-	C_2	
-	-	Y	

The effect filtrates *S. cerevisiae* yeast strains grown at different temperatures on inhibition growth of some bacteria under study

Temperature affects the growth of baking yeast - *S. cerevisiae* and its ability to produce toxins that inhibit the growth of some negative and positive bacteria gram stain. The effect of *S. cerevisiae* yeast cultures in different temperatures has varied in some types of bacteria under study, as the results of their development at 20 ° C have shown that the *S. cerevisiae* leachate filtrate has a slight inhibition ability in the growth of some types of bacteria under study as isolation has shown Y inhibition capacity for each growth of *K.pneumoniae* and *S. aureus* and *E. coli* and *Coliform* and very simple compared to the rest of the isolates and isolate C_2 showed its ability to simple inhibition of two types of bacteria *E. coli* and *K.pneumoniae*. Compared to the isolation P, which showed an inhibitory effect in only one type of bacteria, *K.pneumoniae*, as shown in **Table 4**. And by observing **Table 3**, we find that when using leachate semen. S. cerevisiae grown at a temperature of 30 ° C. The yeast farm activity seemed to increase and was better as the inhibitory effect of S.cerevisiae yeast isolates was greater in diameter except for *K.pneumoniae* was better than 30 ° C. In particular, isolation G of the filtrate isolates grown at temperature 20 m It was distinguished by the presence of a clear and differential effect on the variation of the bacteria under study and by using sterile filtrates by water bath or isolated using the bacterial filter milli por, as an inhibitory effect was observed for all isolates of *S. cerevisiae*. (G, P, C2, Y) in all types of negative bacteria that are positive for the of gram stain under study with the exception of *S. pneumoniae* and the largest inhibition diameter for isolation (G, Y) was 11.5 mm for sterile filter with bacterial filter milli por On Coliform bacteria, then *E. coli* bacteria followed by *S. aureus* and then *K.pneumoniae.* The results of the use of fermented leachate farm filtrates at a degree of (37) C. showed a weak effect in inhibiting bacterial growth in

Table 5. Effect cultures of yeast strains - S. cerevisiae grown at 30C[5] in some bacteria being studied

Inhibition zone		Type of yeast	Type of bacteria
Water bath	Water bath		
9.5	9	G	*Klebsiella pneumonia*
9.5	9	P	
8.5	-	C_2	
8	-	Y	
-	-	G	*Escherichia coli*
-	-	P	
-	-	C_2	
-	-	Y	
-	-	G	*Staphylococcus aureus*
-	-	P	
-	-	C_2	
-	-	Y	
-	-	G	Coliform
-	-	P	
-	-	C_2	
-	-	Y	
-	-	G	*Streptococcus pneumonia*
-	-	P	
-	-	C_2	
-	-	Y	

K.pneumoniae, as they were close by using sterile filtration (P, G) filters. Whereas, the inhibitory diameter (8, 8.5 mm) of the filtrate isolates (C2, Y) was only using the bacterial filter as shown in **Table 4**. Through the results of the previous three tables, we note the variation of the effect of the filtration of S.cerevisiae yeast cultures taken from different companies on the growth of some types of negative bacteria and positive for the dye of cram, we find that the best temperatures that stimulate the ability of yeasts to produce lethal toxins inhibiting the growth of bacteria is a temperature of 30 ° C compared to By developing it at temperatures of 37 ° C and 20 ° C It agrees with Polonelli and Morace (16) who assert that temperature has an effect on the ability of yeasts to produce toxins, that bacterial filter sterilization was better than water bath because toxic toxins when treated with a water bath may lose their toxic capacity.

The weak inhibitory effect in microorganisms may be due to the type of strain Yeast, its ability to produce toxins, and its incubated temperature according to it.

The process of purification of yeasts to obtain the yeast of S. cerevisiae in a pure form free from bacteria that may reach 41% may lead to a weakening of its ability to produce toxins.

The absence of inhibitory secretions in the isolates of S and F isolates resulted in their neglect in subsequent studies.

The effect of pH on the ability of yeast S.cerevisiae to produce toxins:

In this study, it was found that there is a relationship between sensitive yeasts and the ideal pH of the deadly efficacy. Some S. cerevisiae isolates used in the study showed the highest efficacy at pH = 3.5 values. These fatal strains were diagnosed with the presence of death halos (deposition of the blue double dye pigmentation) for sensitive cells as shown In Table, we notice that the highest inhibitory diameter was for the nominal leaky of isolate G as it reached (12.5.13.5) mm on Y and C2 respectively

Whereas, this filtrate did not affect the rest of the other strains used under study that were immune to the toxins exposed to it. explained that Pretoxin gene under appropriate conditions produces toxins of twice the magnitude of two and a half times compared to non-stimulating conditions.

The toxic leaky effect of Y isolation also affected C2 isolation, as the inhibition diameter reached (11.5) mm. From these results, we notice a difference in the isolates used in the current study between sensitive cells and the killer cells of the yeast S.cerevisiae, while no toxic leakage affected all the yeasts prepared under the pH (5,6) In any type of yeast under study, this is consistent with what was found by Schmitt and Schernikau (2019), as it confirmed that lethal toxins produced by fermented yeasts from fruits affected the inhibition of the growth of sensitive yeasts when the pH decreased. To 3.5 Similar results were obtained by Herero and his group in 2014, Schmitt and Schernikau in 2017 in insulated wines from wine.

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