SCREENING OF SOME YEAST ISOLATES FOR THEIR ANTIBACTERIAL ACTIVITY

Hosny M.Gamal-Eldin[1], Osama A. Seoudi[1], Baraka, A. Abd El-Salam[2] and Hemat A. Mahmoud[2]

[1] Agricultural Microbiology Department, Faculty of Agriculture, Fayoum University
[2] Dairy Research Department, Food Technology Research Institute, Agricultural Research Center

ABSTRACT

Sixty-nine yeast isolates were collected from different sources. The isolates were tested using agar well diffusion and disc diffusion assays against eight medically important indicators. Results indicated that 48 (69.6%) of isolates exhibited antibacterial activity against one or more of the eight indicator bacteria. When pH was adjusted to 6.5, only 26 isolates were effective. The stability of yeast isolates supernatants at pH 6.5 against proteolytic enzymes was also tested. Only sixteen isolates were effective against test microorganisms. When the supernatants were treated with catalase and trypsin, only thirteen isolates showed antibacterial activity. The isolate Eg-Y2 was very effective against *Cl. tyrobutyricum* which is considered as one of the most dangerous anaerobic pathogens. . The 13 isolates were identified as *C. pelliculosa ,C. guillermondii, C. glabrata, C. famata, Cryptococcus neoformans, Rodo muciluginosa* using the 20 CUX API system.

KEY WORDS: screening, yeast, pathogens, supernatants, antibacterial activity.

INTRODUCTION

Yeasts are eukaryotic microorganisms classified in the kingdom Fungi; yeast cells are typically single, small and oval. The yeast constitutes a large and heterogeneous group of microorganisms that are currently attracting the increased attention from scientists and industry. Other advantages of yeast when used for biological studies are well defined genetic system and highly versatile DNA transformation system **(Guthrie and Fink, 1991).**

Numerous and diverse biological activities make yeast promising candidates for a wide range of applications not limited to the food sector. In addition to their major contribution to flavor development in fermented foods, their antagonistic activities towards undesirable bacteria, and fungi are now widely known. These activities are associated with their competitiveness for nutrients, acidification of their growth medium, their tolerance of high concentrations of ethanol, and release of antimicrobial compounds such as antifungal killer toxins or "mycocins" and antibacterial compounds. While the design of foods containing probiotics (microorganisms that confer health benefits) has focused primarily on *Lactobacillus* and *Bifidobacterium*, the yeast *Saccharomyces cerevisiae var. boulardii* has long been known effective for treating gastroenteritis. **(Hatoum *et al*, 2012)**. The antimicrobial effects of yeasts

are present in fermented foods and beverages, **(Lowes *et al* (2000) and Viljoen, (2006)** reported the actions of organic acids, antibiotic factors, volatile acids, hydrogen peroxide, and various other substrates excreted by yeasts in the product.

Yeasts are very common in the environment, and are often isolated from sugar-rich materials. Examples include naturally occurring yeasts on the skins of fruits and berries (such as grapes, apples, or peaches), and exudates from plants (such as plant saps or cacti). Some yeasts are found in association with soil and insects **(Suh *et al,* 2005 and Slavikova& Vadkertiova, 2003)**.The ecological function and biodiversity of yeasts are relatively unknown compared to those of other microorganisms **(Herrera and Pozo, 2010)**.Yeasts, including *Candida albicans, Rhodotorula rubra, Torulopsis* and *Trichosporon cutaneum,* have been found living in between people's toes as part of their skin flora **(Oyeka and Ugwu, 2002)**. Yeasts are also present in the gut flora of mammals and some insects (**Martini, 1992)** and even deep-sea environments host an array of yeasts **(Bass *et al,* 2007 and Kutty &Philip, 2008)).**

Biological control of post-harvest diseases of fruits and vegetables by microbial antagonists is well documented, but the majority of the information is related to bacterial and fungal antagonists. In the preceding decade the interest in yeast antagonists has been increasing with the aim to isolate such yeast strains.
The aim of this work has been designed to isolate and screen yeasts from different sources and studying their ability as antibacterial agents.

Materials and Methods

Food Samples

Samples of Raw milk, Yoghurt, Rayeb milk , Butter milk, Kariesh cheese, Mesh cheese, Butter, Ras cheese, Grape, Grape leaves, Guava, Sugar cane, Cane juice, Mussels ajwa, Pear, Black honey and Dates were collected from the local market and factories.

Indicator microorganisms

Six Gram - negative bacteria (*Escherichia coli* ATCC 0157, *E. coli* ATCC 25922, *Salmonella typhi* ATCC 13076, *Pseudomonas aeruginos* ATCC 9027 and *Ps. aeruginos* ATCC 27853, and three Gram positive bacteria (*Listeria monocytogenes* ATCC 15313, *Bacillus cereus* ATCC 13753, *Staphylococcus aureus* ATCC 8095 and two spore forming anaerobic bacteria (*Clostridium butyricum* ATCC 8260 *and Clostridium tyrobutyricum* ATCC 25755) were used as indicator microorganisms for the detection of antimicrobial activity.

All aforementioned strains were obtained from the Culture Collection of Agricultural Microbiology Department, Faculty of Agriculture, Fayoum University. Except *Cl. butyricum* ATCC 8260 and *Cl. tyrobutyricum* ATCC 25755 and *Ps. aeruginosa* ATCC 27853 were obtained from Egyptian Microbial Culture Collection (EMCC) at Cairo Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams Univ.

Media used

-Potato dextrose agar medium (PDA) (**Atlas and Parks 1997**) was used for growing fungi and *yeast*.

-Luria-Bertani agar medium (LB) (gL^{-1}): (**Atlas and Parks 1997**) was used for all indicator bacteria.

- Sabouraud dextrose agar media (**Oxoid, 2006**) was used for yeast.

-Thioglycollate Agar media was used for *Clostridium butyricum* and *Cl. tyrobutryricum.*

-Lugol's Iodine Solution (Soriful *et al.*, 2013), KI (10%) and I_2 (5%) was also used.

Methods

Isolation of yeasts:

Isolation and screening of microorganisms from naturally occurring processes have always been the most powerful means of obtaining useful cultures for scientific and commercial purposes. This is certainly true for yeast which play an important role in a large number of various traditional food fermentations. In order to achieve the objectives of the present study, sixty-nine yeast were isolated from 35 samples obtained from 17 different sources.

A sample of 1g or 10 ml was added to sterile saline solution (100 ml) and shaken for 30 minutes. Ten – fold and 100 fold serial dilutions were prepared and 0.1ml plated onto acidified dextrose agar plates then incubated at (30˚C, 72 hours). The purified 69 isolates are designated Eg-Y (Egyptian yeast) and their sources are listed in **Table (1)**.

Table (1): Sources of different yeast isolates

	Isolate	Source	NO.	Isolate	Source	NO.	Isolate	Source
1	Eg-Y1	Grape leaves	24	Eg-Y24	Cane juice	47	Eg-Y47	Ras cheese
2	Eg-Y2	Grape leaves	25	Eg-Y25	Ras cheese	48	Eg-Y48	Ras cheese
3	Eg-Y3	Grape leaves	26	Eg-Y26	Ras cheese	49	Eg-Y49	Carrot
4	Eg-Y4	Grape leaves	27	Eg-Y27	Ras cheese	50	Eg-Y50	Carrot
5	Eg-Y5	Guava	28	EG-Y28	Ras cheese	51	Eg-Y51	Mesh cheese
6	Eg-Y6	Guava	29	Eg-Y29	Ras cheese	52	Eg-Y52	Mesh cheese
7	Eg-Y7	Rayeb milk	30	Eg-Y30	Dates	53	Eg-Y53	Mesh cheese
8	Eg-Y8	Rayeb milk	31	Eg-Y31	Dates	54	Eg-Y54	Mesh cheese
9	Eg-Y9	Mussels ajwa	32	Eg-Y32	Dates	55	Eg-Y55	Mesh cheese
10	Eg-Y10	Mussels ajwa	33	Eg-Y33	Dates	56	Eg-Y56	Kariesh cheese
11	Eg-Y11	Sucker cane	34	Eg-Y34	Yoghurt	57	Eg-Y57	Kariesh cheese
12	Eg-Y12	Sucker cane	35	Eg-Y35	Yoghurt	58	Eg-Y58	Kariesh cheese
13	Eg-Y13	Sucker cane	36	Eg-Y36	Yoghurt	59	Eg-Y59	Kariesh cheese
14	Eg-Y14	Pear	37	Eg-Y37	Grape	60	Eg-Y60	Kariesh cheese
15	Eg-Y15	Pear	38	Eg-Y38	Grape	61	Eg-Y61	Apple
16	Eg-Y16	Black honey	39	Eg-Y39	Grape	62	Eg-Y62	Apple
17	Eg-Y17	Butter	40	Eg-Y40	Grape	63	Eg-Y63	Apple
18	Eg-Y18	Butter	41	Eg-Y41	Yoghurt	64	Eg-Y64	Apple
19	Eg-Y19	Butter	42	Eg-Y42	Rayeb milk	65	Eg-Y65	Labenah
20	Eg-Y20	Butter milk	43	Eg-Y43	Rayeb milk	66	Eg-Y66	Labenah
21	Eg-Y21	Butter milk	44	Eg-Y44	Rayeb milk	67	Eg-Y67	Labenah
22	Eg-Y22	Butter milk	45	Eg-Y45	Ras cheese	68	Eg-Y68	Labenah
23	Eg-Y23	Cane juice	46	Eg-Y46	Ras cheese	69	Eg-Y69	Labenah

Preparation of cell – free yeasts culture supernatants.

Yeast isolates were grown in potato dextrose broth at 30˚C for 72h. The cultures were centrifuged at 10000xg for 15 min at 4˚C and the resulted supernatant was designated crude cell – free culture supernatant (CCFCS). To eliminate growth inhibition caused by organic acids and hydrogen peroxide, the pH of the CCFCSs was

adjusted to 6.5 and of catalase (1mg mL-1) was added. These supernatants were used immediately or stored at -20˚C until needed.

Antimicrobial activity assay

Agar well diffusions method

Agar well diffusion method was used as described by **Wolf and Gibbons (1996).** Briefly, 20 ml of Luria bertani agar medium **(Atlas and Parks 1997),** inoculated with 1% tested organisms suspension were cooled at 45˚C and poured into sterile Petri dish and allowed to solidify at room temperature. Wells of 6 mm diameter were cut in the solidified agar using a sterile metal Cork borer and filled with 0.1ml of yeast supernatant. The plates were left at 4-5˚C for 2hrs to allow diffusion of the substances and then incubated aerobically for 24h at optimum temperature for each of the tested organisms. Absence or presence of inhibition zones as well as their diameters were recorded.

Biological activities of secondary metabolites produced by Egyptian yeast isolates

In this study, 4 *in vitro* screenings were performed. The first one was devoted to general screenings of the 69 isolates for antimicrobial activities, while the second 3 were devoted to specific screenings using 13 selected isolates. Selection of these 13 isolates was based on the results of the first screening.

Screening of yeast isolates for antibacterial activity

The search for new antibiotics and other bioactive compounds has been intensified due to the development of multiple resistances in pathogenic bacteria and lack of effective apices against various infectious- diseases. Each of the 69 isolates was *in-vitro* screened for antibacterial activity using a panel of medically important 8 indicator bacteria, (*E. coli* ATCC 25922, *E. coli* ATCC 0157 *Sal. typhi, L. monocytogenes, Staph. aureus, Bacillus cereus, Ps. aeruginosa* ATCC 9027 and *Ps. aeruginosa* ATCC 27853).

Results in **Table (2)** indicated that 48 (69.6%) of isolates exhibited antibacterial activity against one or more of the 8 indicator bacteria. Out of 69 isolates, 17 were active against all the 8 indicator bacteria

Regarding the sensitivity of the 8 indicator bacteria to the 69 yeasts isolates, **Figure (1)**, show that 37 isolates were active against *E.coli* and 37 were active against *Sal. typhi*. Contrary to this result, screening of actinomycetes and fungi by **(Lazzarini *et al*., 2000; and Suay *et al*., 2000)** for antibacterial activity, showed that activity against Gram-negative bacteria were generally far less common than against Gram-positives. It is worth noting that there is an unmet medical need for antibiotics acting on Gram-negative pathogens. Furthermore, Eg-Y12, 13 and 14 were active in their effect against *Sal. typhi* while the strain showed resistance to Eg-Y15. On the other hand, *Ps. aerginosa* was highly resistant to Eg-Y8, 10 and 11 while Eg-Y9 was very effective against the tested microbe **photo (1)**.

Table (2): Antibacterial activity of crude cell - free culture supernatants (CCFCS) of Yeast isolates against indicator bacteria using agar well diffusion assay.

NO.	Isolates	Inhibition zone diameter (mm) against indicator bacteria							
		E.coli 0157	**E.coli 25922**	***L. monocytogenes***	***Sa. Typhi***	***Ps. aeruginos* 27853**	***Ps. aeruginos* 9027**	***B.cereus***	***Staph. aureus***
1	Eg-Y1	-	-	-	-	15	-	-	-
2	Eg-Y2	23	20	27	25	28	27	30	25
3	Eg-Y3	-	-	-	-	-	23	-	-
4	Eg-Y4	28.5	25.5	23	27	28	22	21	27
5	Eg-Y5	12	20	-	18.5	19	-	15	-
6	Eg-Y6	-	-	-	-	-	-	-	-
7	Eg-Y7	22	20	-	16	24	16	16.5	31.5
8	Eg-Y8	-	-	-	-	-	-	-	-
9	Eg-Y9	26	20.5	29	27	29	23	24	29
10	Eg-Y10	12	16	-	13	19	-	-	-
11	Eg-Y11	-	-	-	-	-	-	-	-
12	Eg-Y12	19	27	26.5	24.5	31	23	26.5	20
13	Eg-Y13	26	26.5	24	15	25	24	21	27
14	Eg-Y14	15	28	-	16	24	-	20	-
15	Eg-Y15	-	-	-	-	-	-	-	-
16	Eg-Y16	-	15	-	-	-	-	-	-
17	EG-Y17	17.5	12	-	-	-	-	-	-
18	Eg-Y18	-	15	-	-	-	-	-	-
19	Eg-Y19	-	-	-	-	-	-	-	-
20	Eg-Y20	20	20	22	21	24	25	28	24
21	Eg-Y21	19	18	-	17	24	-	12	26
22	Eg-Y22	-	-	-	-	-	-	-	-
23	Eg-Y23	20.5	19	-	21	21	13	15.5	36
24	Eg-Y24	-	-	-	-	-	-	-	-
25	Eg-Y25	12	-	-	-	-	-	-	-
26	Eg-Y26	19	30	28	28	28	16	24	39
27	Eg-Y27	-	15.5	-	-	-	-	14	33
28	Eg-Y28	-	-	-	-	-	-	-	-
29	Eg-Y29	-	-	-	-	-	-	-	-
30	Eg-Y30	-	-	-	-	-	-	-	-
31	Eg-Y31	-	-	-	-	-	-	-	-
32	Eg-Y32	-	-	-	-	-	16	-	-
33	Eg-Y33	-	-	-	-	-	-	-	-
34	Eg-Y34	20.5	19.5	30	25	24	26	24.5	28
35	Eg-Y35	11.5	13	11.5	12	-	-	11	20.5
36	Eg-Y36	21	24	25	28	26	-	13	27.5
37	Eg-Y37	-	-	21.5	23	-	-	17	-
38	Eg-Y38	-	-	22.5	25	-	-	23	-
39	Eg-Y39	-	-	19	18.5	-	-	15	-
40	Eg-Y40	-	-	21	24.5	-	-	21	-
41	Eg-Y41	25	22	26	24	35	24	30	29
42	Eg-Y42	-	-	-	-	-	-	-	31.5
43	Eg-Y43	18	13	14	14	-	-	15	-
44	Eg-Y44	25	-	16	25.5	-	-	20	19.5
45	Eg-Y45	-	-	-	-	-	-	-	-
46	Eg-Y46	-	16	20	21	-	-	-	-
47	Eg-Y47	25	19	23.5	24	24.5	24	25	26
48	Eg-Y48	28	16	18	26	-	-	15.5	30
49	Eg-Y49	24	23	26	22	25.5	16	25	-
50	Eg-Y50	-	-	-	-	-	-	-	-
51	Eg-Y51	-	22	25	22	20	15	15	-
52	Eg-Y52	-	-	-	-	-	-	-	-
53	Eg-Y53	29	28	20	25	29	28	24	36
54	Eg-Y54	15	30	26	24	26	-	23	-
55	Eg-Y55	17	25	24	21	26.5	23	-	32
56	Eg-Y56	27	28	29	23	28	17	24	29
57	Eg-Y57	-	-	-	-	-	-	28	-
58	Eg-Y58	-	18.5	15	17	-	-	16	-
59	Eg-Y59	-	-	-	-	-	-	-	-
60	Eg-Y60	-	-	-	-	-	-	-	-
61	Eg-Y61	-	-	-	-	-	-	-	-
62	Eg-Y62	19	18	22	17	24.5	18	19.5	35.5
63	Eg-Y63	27	24	25	23.5	27	27	27	20
64	Eg-Y64	-	-	-	-	-	-	-	-

Table (2):Continued									
65	Eg-Y65	-	-	-	-	-	-	-	-
66	Eg-Y66	25	24.5	23	22	26	24	23	22
67	Eg-Y67	-	-	-	-	-	-	-	-
68	Eg-Y68	-	-	-	-	-	-	-	-
69	Eg-Y69	19	22	15	17	20	15	15	20

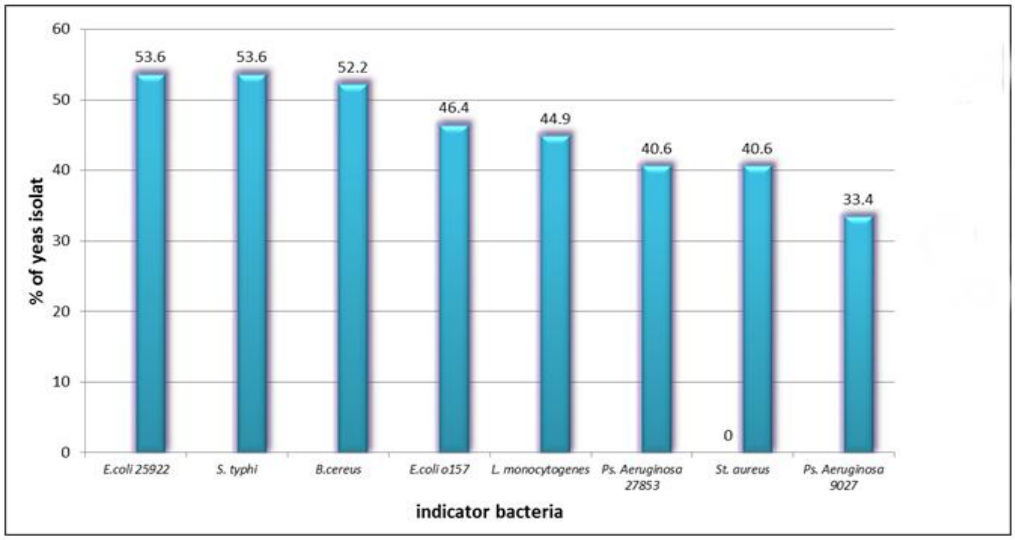

Fig (1): Percentage of yeast isolates inhibited growth of each indicator bacteria.

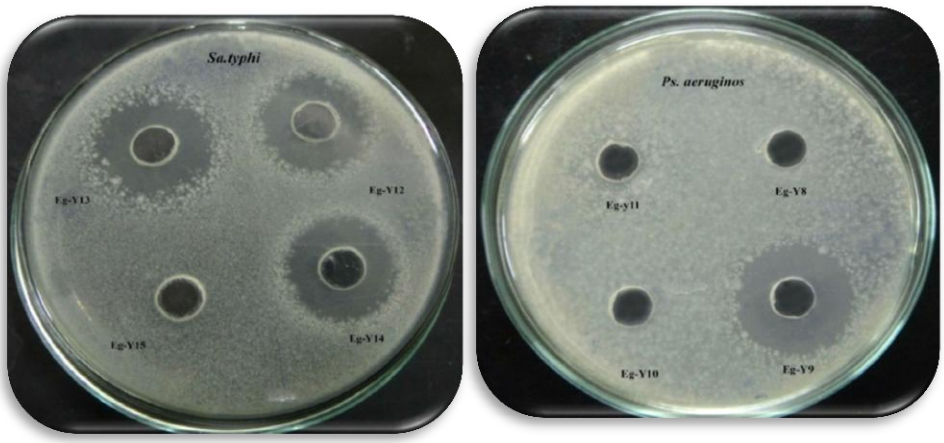

Photo (1): Antibacterial activity of cell - free supernatants of yeast isolates against 2 indicator bacteria using agar well diffusion assay

Antibacterial activity of Yeast isolates supernatants after pH adjustment to 6.5.

It was obvious from the data obtained in **Table (3)** that the most tested pathogenic strains were sensitive to the most studied of adjusted crude cells - free Yeast isolate supernatants (CCFYIs) to pH 6.5. Also, it could be noticed that, no antibacterial activity of 7 adjusted (CCFYIs) to pH 6.5 was recorded

toward all tested indicator bacteria. Generally, most result of (CCFYIs) to pH 6.5 had the lowest antibacterial activity against *E. coli* 0157 and *E. coli* ATCC 25922. The highest inhibition zones of all tested indicator bacteria were observed with Eg-Y9, Eg-Y12 and Eg-Y41.

Table(3): Antibacterial activity of adjusted crude cells - free supernatants of Yeast isolates to pH 6.5 against indicator bacteria using agar well diffusion assay.

NO.	Isolates	Inhibition zone diameter (mm)								
		Indicator bacteria								
		E.coli 0157	*E.coli* 25922	*L monocytogenes*	*Sa. typhi*	*Ps. aeruginosa* 27853	*Ps. aeruginosa* 9027	*B. cereus*		*Staph. aureus*
								Cells	spores	
1	Eg-Y2	22.5	21.5	25	23	28	26	29.5	27	22
2	Eg-Y4	24	19.5	21.5	20	25	20	20	19.5	25
3	Eg-Y7	-	-	-	-	-	-	-	-	-
4	Eg-Y9	24	17.5	28.5	24	27	23	23.5	21	29
5	Eg-Y12	17.5	26	26	24	23	21	24	24	29.5
6	Eg-Y13	24	24	21	14	23.5	22.5	24	24.5	26.5
7	Eg-Y14	-	-	-	-	-	-	-		-
8	Eg-Y20	19	18.5	21	19	23	24	27	25	22
9	Eg-Y21	-	-	-	-	-	-	-		-
10	Eg-Y23	-	-	-	-	-	-	-		-
11	Eg-Y26	18	23	26	24	26	14	21	20	28
12	Eg-Y34	19	17	25	24	22	24	24	24	28
13	Eg-Y36	20	22.5	24	27	24	23.5	25	25	27.5
14	Eg-Y41	22	20	25	23	26	22	30.5	29.5	28
15	Eg-Y44	12	-	14	22	-	-	12	-	20
16	Eg-Y47	23.5	19	23	24	22	19	23	24	25
17	Eg-Y48	-	-	-	23.5	-	-	-	-	28
18	Eg-Y49	20	19.5	16	20	20.5	15	20	19	-
19	Eg-Y51	-	-	-	-	-	-	-	-	-
20	Eg-Y53	26	24	19	21	25	21.5	24	21	31.5
21	Eg-Y54	17	20	25	20.5	25	20.5	25.5	24	-
22	Eg-Y55	-	-	-	-	-	-	-	-	
23	Eg-Y62	14	15	16	14	19	18	19	17	-
24	Eg-Y63	-	-	-	-	-	-	-	-	
25	Eg-Y66	23.5	23	21	20.5	24	22	20.5	20	21.5
26	Eg-Y69	18	19	14.5	16	19.5	14	14.5	14	18

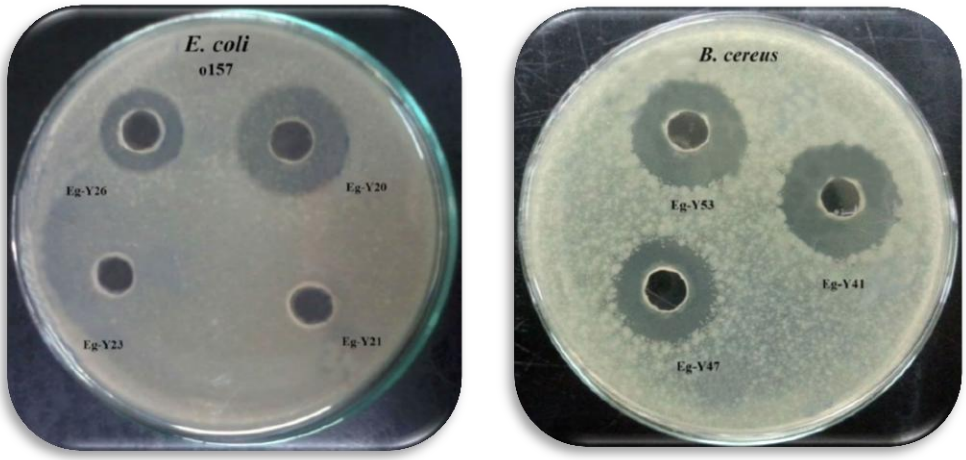

Photo (2): Antibacterial activity of cell - free supernatants of yeast isolates after adjustment pH to 6.5 against 2 indicator bacteria using agar well diffusion assay.

From **photo (2),** the test against both *E. coli* and *B. cereus*, resulted in a high effect of Eg-Y20 against *E. coli*, moderate effect of Eg-Y26 and no effect of Eg-Y21& 23. All tested isolates; Eg-Y41, 47 & 53 against *B. cereus* were very effective.

Table (4): Antibacterial activity of adjusted crude cells - free yeast isolate supernatants to pH 6.5 and treated with catalase against indicator bacteria using agar well diffusion assay.

NO.	Isolates	Inhibition zone diameter (mm)							
		Indicator bacteria							
		E.coli 0157	**E.coli 25922**	***L.monocytogenes***	***Sa.typhi***	***Ps.aeruginos* 27853**	***Ps. aeruginos* 9027**	***B.cereus***	***Sapht. Aureus***
1	EG-Y2	15	14	16	16.5	25	-	22.5	21.5
2	EG-Y4	13	-	12	13.5	23	-	14	23
3	EG-Y9	20.5	15.5	20.5	17.5	26	-	22.5	29
4	EG-Y12	14	15.5	19.5	15.5	19	-	20.5	28.5
5	EG-Y13	20	14	19	19	24.5	-	20	21
6	EG-Y20	15	13.5	17	16	21.5	-	22.5	18
7	EG-Y26	-	-	-	-	-	-	12	17.5
8	EG-Y34	16.5	12	18	15.5	24.5	-	23	25.5
9	EG-Y36	-	-	-	-	18.5	-	-	-
10	EG-Y41	19	15.5	17.5	16.5	18	-	20.5	26
11	EG-Y47	13	15	17	18	17.5	-	16	22
12	EG-Y49	19	16	15	19	19.5	-	18	-
13	EG-Y53	21	20.5	17	19.5	21	-	20	25
14	EG-Y54	-	-	-	-	-	-	-	-
15	EG-Y66	21	20.5	19	19	21	-	17.5	20
16	EG-Y69	17	15	13.5	14.5	19	-	14	18

Antibacterial activity of Yeast isolates supernatants after pH adjustment to 6.5 treated with catalase.

It was obvious from the obtained data in **Table (4)** that the tested indicator bacteria revealed different response to the examined adjusted (CCFYIs) to pH 6.5 and treated with catalase. Among these examined (CCFYIs) of Eg-Y9, Eg-Y12, Eg-Y41 and Eg-Y53 showed strongly inhibited activity against most studied indicator bacteria. It was of interest to notice that, no inhibitory effect of Eg-Y36 and Eg-Y54 against all tested indicator bacteria. Moreover, *Pseudomonas aeruginosa* ATCC27853 strain was more resistant toward all examined (CCFYIs).

Stability of yeast isolates supernatants (after adjustment pH to 6.5 and treated with catalase) against proteolytic enzymes.

The inhibitory effect of adjusted crude cells - free Yeast isolate supernatants to pH 6.5 and treated with catalase and trypsin against indicator bacteria using agar well diffusion assay is given in **Table (5**). It could be seen that, the trypsin treatment of adjusted (CCFYIs) to pH 6.5 and treated with catalase was not affected their antibacterial activity that appeared in adjusted (CCFYIs) to pH 6.5 and treated with catalase. This result suggests that the inhibitory substances of Yeast isolate supernatants are not proteinaceous nature.

Table (5): Antibacterial activity of adjusted crude cell - free supernatants of yeast isolates to pH 6.5 and treated with catalase and trypsin against indicator bacteria.

NO.	Isolates	Inhibition zone diameter (mm)						
		Indicator bacteria						
		E.coli 0157	*E.coli* 25922	*L.monocytogenes*	*Sal. typhi*	*Ps.aeruginos* 27853	*B.cereus*	*Staph. aureus*
1	Eg-Y2	14	13	14.5	16	23.5	21	20
2	Eg-Y4	12.5	-	12	13	22	14	21.5
3	Eg-Y9	19	15	20	17	24	22	25
4	Eg-Y12	14	15	18	14	17	19	26.5
5	Eg-Y13	19	13.5	19	18	22	20	21
6	Eg-Y20	15	12.5	16	15,5	20	20,5	17.5
7	Eg-Y34	16	12	17	15	21	21	22
8	Eg-Y41	18	15	16.5	16	17	19	21.5
9	Eg-Y47	13	14.5	17	18	16.5	16	21
10	Eg-Y49	18	15	15	17	18	17	-
11	Eg-Y53	19.5	17	16	19	20	19.5	24
12	Eg-Y66	19	18	19	18	20.5	17	19
13	Eg-Y69	17	14.5	13	14	19	14	18

It's obvious from the **Table (5)** that Eg-Y9 gave the best antibacterial results against all tested bacteria especially *L. monocytogenes* and *Ps. aeroginosa* followed by Eg-Y1, Eg-Y66 and Eg-Y47. When pH was adjusted to 6.5 all isolates showed no activity against *Ps. aeroginosa* 9027 which showed high resistance. Similar results were reported by **Goerges *et al* (2006)** and **Goerges *et al* (2011).**

Screening of yeast isolates against spore forming anaerobic bacteria.

Table (6) presents the antibacterial activity of yeast isolate supernatants against spore forming anaerobic bacteria**.** It is obvious that the (CCFYIs) had the highest inhibitory effect against *Cl. butyricum* ATCC 8260 *and Cl. tyrobutyricum* ATCC 25755 compared with those of (CCFYIs) after adjustment to pH 6.5 and elimination of hydrogen peroxide. The obtained data revealed that the *Cl. butyricum* ATCC 8260 *and Cl. tyrobutyricum* ATCC 25755 were more sensitive to the supernatant of Eg-Y2 and Eg-Y20. Also, it could be noticed that the supernatant of Eg-Y12 did not affect the growth of *Cl. butyricum* ATCC 8260 and *Cl. tyrobutyricum* ATCC 25755.

Table(6): Antibacterial activity of Yeast isolates supernatant against spore forming anaerobic bacteria.

NO.	Isolates	Inhibition zone diameter (mm) against indicator bacteria*					
		Clostridium butyricum			*Clostridium tyrobutyricum*		
		Cr	Ph	Cata	Cr	Ph	Cata
1	Eg-Y2	18.5	17	16	18	17	16.5
2	Eg-Y4	4.5	4	4	5	4.5	4
3	Eg-Y9	2	0	0	2	0	0
4	Eg-Y12	0	0	0	0	0	0
5	Eg-Y13	3	2	0	4	2	0
6	Eg-Y20	16	15	14	16.5	15.5	14
7	Eg-Y34	3	2	2	3.5	2.5	2
8	Eg-Y41	3.5	2	2	3	2	2
9	Eg-Y47	3	0	0	2.5	0	0
10	Eg-Y49	4	2	0	3	0	0
11	Eg-Y53	5	3	3	4.5	3	3
12	Eg-Y66	3	1	1	3	1	0
13	Eg-Y69	3	0	0	2	0	0

*****Cr = crude cell-free culture supernatants (CCFCS)& Ph = (CCFCS) after adjustment to pH 6.55
Cata = (CCFCS) after adjustment to pH 6.55and elimination of hydrogen peroxide

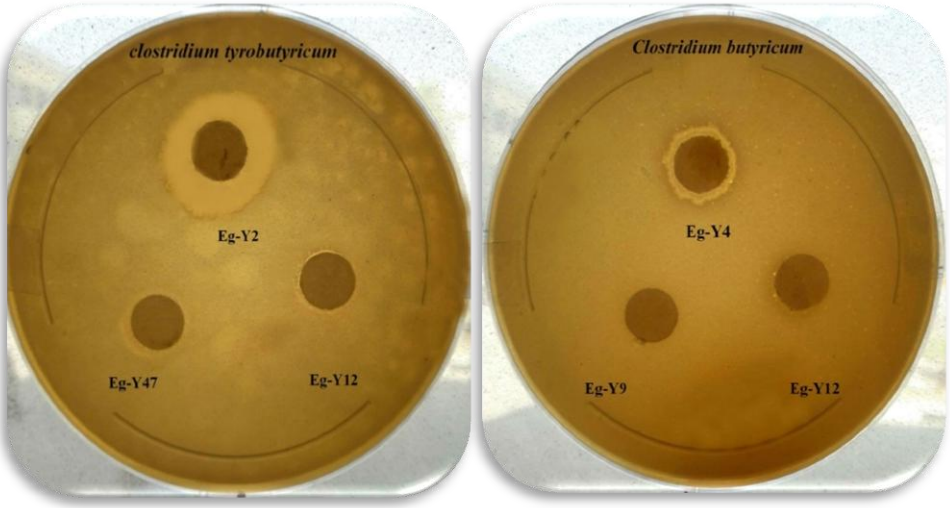

Photo (3): Antibacterial activity of yeasts isolates against spore forming anaerobic bacteria using agar disc diffusion assay.

It could be noticed from **Photo (3)** that the isolate Eg-Y2 was the best in its antibacterial effect against *Cl. tyrobutyricum* while Eg-Y47 and Eg-y12 had no effect. On the other hand, Eg-Y4 showed a slight effect while Eg-Y9 and Eg-Y12 exhibited no effect at all. **Fatichenti, *et al.* (1983)** reported that the yeast *Debaryomyces hansenii* showed and antagonistic activity against both strains.

Identification of yeast isolates

Identification of the selected yeast isolates to species level was carried out on the basis of their biochemical profiles of carbohydrate fermentation patterns obtained by API 20 CUX kits in **Table (7).** According to the API database correlation, the identification of yeast isolates showed that both Eg-Y2 and Eg-Y20 isolates were identified as *Candida pelliculosa*. Isolate Eg-Y4 was identified as *Cryptococcus neoformans.* Both Eg-Y49 and Eg-Y69 isolates were identified as *Candida guillermondii.* The Eg-Y9, Eg-Y13, Eg-Y34, Eg-Y41, isolates were identified as *Candida glabrata*; isolate Eg-Y53 as *Candida famata* and isolate Eg-Y66 as *Rodo. muciluginosa.*

Table (7): Carbohydrate fermentation patterns of selected yeast isolates by API 20 CUX system.

Characteristics*	Yeast- isolates												
	2	4	9	12	13	20	34	41	47	49	53	66	69
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycerol	+	-	+	-	+	+	+	-	+	+	+	+	+
Calcium 2-Keto-Gluconate	-	+	-	+	-	-	-	-	+	+	+	-	+
L- Arabinose	-	+	-	+	-	-	-	-	+	+	-	+	-
D-Xylose	+	+	-	+	-	-	-	-	+	+	+	+	+
Adonitol	-	+	-	-	-	-	-	-	+	+	+	+	+
Xylitol	-	+	-	+	-	-	-	-	+	+	+	+	+
D-Galactose	+	+	-	+	-	-	-	-	+	+	+	+	+
Inositol	-	+	-	+	-	-	-	-	-	-	-	-	-
D- Sorbitol	+	+	-	+	-	+	-	-	+	+	+	+	+
Methyl – α D-Glucopyranoside	+	+	-	+	-	+	-	-	+	+	+	-	+
N-Acetyl-Glucosamine	-	+	-	-	+	-	-	-	+	+	+	-	+
D-Cellobiose	+	+	-	+	-	+	-	-	+	+	+	+	+
D-Lactose	-	-	+	-	-	-	-	-	-	-	+	-	-
D-Maltose	+	+	+	+	-	+	-	-	+	+	+	+	+
D-Saccharose	+	+	+	+	-	+	-	-	+	+	+	+	+
D-Trehalose	+	+	-	+	-	+	-	-	+	+	+	+	+
D-Melezitose	+	+	-	+	-	+	-	-	+	+	+	-	+
D-Raffinose	+	+	-	+	-	-	-	-	+	+	-	+	-

*** The Score of the result tests: -, negative test; +, positive test**

Conclusion:

It could be concluded that the obtained yeast isolates had good antibacterial effect especially against G- tested bacteria and Clostridium which give them the privilege for future work to investigate the possibility of using them in food processing.

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فحص بعض عزلات الخميرة لأنشطتها المضادة للبكتيريا

حسني محمد جمال الدين[١], اسامة عبد التواب سعودي[١], بركة ابو اليزيد عبد السلام[٢], همت عبد القادر محمود على[٢]

[١] قسم الميكروبيولوجيا الزراعية - كلية الزراعة - جامعة الفيوم.

[٢] قسم بحوث الالبان - معهد بحوث تكنولوجيا الاغذية– مركز البحوث الزراعية - جيزة.

تم عزل69 عزلة خميره من مصادر مختلفه وتم إختبار قدرة كل منها على إنتاج مواد أيضيه ثانوية ذات نشاطات بيولوجية. ودراسة النشاط المضاد للبكتيريا باستخدام ٨ سلالات بكتيريا مرضية مختلفة بطريقة الديسكات ونتائج هذه الدراسه أظهرت أن من بين العزلات الـ٦٩أظهرت٤٨منها (٦٩,٦%) نشاطا مضادا لنمو واحدة أو أكثر من الدلائل البكتيرية.

- عند ضبط الحموضة لراشح سلالات الخمائرعند ٦,٥ لوحظ ان ٢٦ عزلة لها نشاط مضاد مضاد للبكتريا لـ٧ عزلات خمائر تجاه كل الدلائل البكتيرية.

- وبدراسة التأثير المثبط بعد اضافة انزيم الـ catalase لراشح سلالات الخمائر المضبوط حموضتة اوضحت النتائج ان ١٦ عزلة خمائرلها تأثير مثبط. وأيضاً عند معاملة الراشح بكل من انزيم الـ trypsin & catalase

أظهرت ١٣ عزلة فقط تأثيرها كمضاد للبكتريا المختبرة.

- وقد اختبرت الـ ١٣عزلة لقدرتها على إنتاج مواد أيضية ثانوية ذات تأثير مضاد للبكتريا المتجرثمة اللاهوائية.

(*Clostridium butyricum* ATCC 8260 and *Clostridium tyrobutyricum* ATCC 25755)

ووجد ان من بين العزلات الـ١٣ لوحظ ان السلاتين كانت اكثر حساسية للعزلة Eg-Y2.

- ثم تم تصنيف الـ١٣ عزلة خمائر باستخدام نظام API 20 CUX واوضحت نتيجة هذا الاختبار ان هذه العزلات هى *Candida pelliculosa ,Cryptococcus neoformans, Candida guillermondii, Candida glabrata, Candida famata, Rodo muciluginosa.*