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Isolation and identification of yeasts along wastewater treatment lines at Zagazig plant

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Abstract

Phenol is one of the most common pollutants in many kinds of industrial wastewater, some of which are in high heavy metals contents, resulting in more difficulties of biodegradation. This study aims to characterize the presence of yeasts along the processes in wastewater treatment plant (WTP) at Zagazig City. The most predominant species of yeast in wastewater were *Candida parasopilis* **(n=5),** *Candida krusei* **(n=8)** *Cryptococcus neoformans* **(n=3)**, *Geotrichum candidum.* **(n=9)** and *Saccharomyces cerevisiae* **(n=5)**. The distribution of these species in wastewater was 17, 27, 10, 30 and 17%, respectively. *Geotrichum candidum* and *Candida krusei* were the most common fungal species isolated and were present in a significant proportion in wastewaters. However, between the 30 yeast isolates, only 11 isolates (3: *C. parasopilis***,** 3: *C. krusei*, 3: *G. candidum* and 2: *S. cerevisiae***)** showed a high growth rate at phenol concentration reaching up to 20 mg/l. Also, all strains could grow at high concentration of zinc sulphate, reaching up to 12 mg/l. The untreated of *C. parasopilis* and treatment with 0.7 mg/l phenol concentration had a normal morphology with no cell morphological changes by scanning electron microscope. The results indicated that this strain is a good candidate to be used in further studies and different applications in the field of treated wastewater form phenol and heavy metal biosorption.

Keywords: Wastewater, Isolation, yeasts, *Candida*, Phenol, Zinc, Biodegradation

Introduction

Phenol is one of the most hazardous organic pollutants in wastewaters due to its toxicity, structural stability, and resistance to degradation even at low concentrations **(Chakinala et al., 2008).** Phenol is important in the field of environmental researches, because it has been frequently chosen as a hazardous pollutant. Hence, much data is available on its removal or destruction especially with respect to wastewater treatments. In the last decade, the treatment of aqueous effluents polluted with phenol and phenolic species has attracted much attention due to the toxicity and low biodegradability of these organic compounds. In the chemical industries, phenolic compounds are very difficult to process by conventional treatment methods such as activated sludge digestion, solvent extraction, chemical treatment, adsorption, etc. **(Ravanchi, et al., 2009; Zhang et al., 2012).**

The recent results recommended that biodegradation, chemical, electrochemical, and photocatalytic oxidation, solid phase extraction, ozonation, reverse osmosis/nanofiltration, and wet air oxidation are useful methods for removing low phenol concentrations, whereas liquid–liquid extraction, evaporation, membrane-based solvent extraction, adsorption, and distillation are suggested for high phenol concentrations removal (**Mohammadi, et al., 2015).** The wastes of more industrial units contain high concentrations of phenol, so the waste treatment by microorganisms is difficult. Phenol and their derivatives inhibit the growth of microorganisms in biological treatment processes because of their biotoxic and recalcitrant properties **(Chakinala et al., 2008).** Due to the toxicity of phenol for most micro-organisms at high concentrations, most of the biodegradation processes are carried out at low concentrations of phenol. Under aerobic or anaerobic conditions, phenol can be converted to harmless compounds by microorganisms. Some aerobic bacteria, *Candida* yeast and fungi use phenol as a source of carbon and energy, and degrade it **(Rappoport, 2003; Ahmed, et al., 2010, Mahgoub et al., 2015a).** Since heavy metals have a propensity to accumulate in selective body organs (such as brain and liver) their prescribed average safety levels in food or water are often misleadingly high. Use of biology based technologies, such as biosorption, is an attractive method for heavy metal removal from metal-laden effluents due to low cost and high efficiency of the process. Although biosorption is defined as the property of certain non-living biomaterials to bind and concentrate selected ions or other molecules from aqueous solutions (**Volesky, 2007; Jiang, et al., 2012)** it can occur in both living and dead microorganisms **(Tobin, et al., 1994; Fomina and Gadd, 2014).** The capability of degrading organic substances with high efficiency and tolerance of heavy metals as well as a wide range of environmental variations leads to the consideration of fungal strains to be applied in wastewater treatment **(Chen et al. 2002; Taştan et al. 2010).** Previous studies have reported the application of fungal isolates in biodegradation of kerosene, formaldehyde, and many other kinds of pollutants, which showed satisfactory performances **(Khan et al. 2015; Yu et al. 2014).** Although some genera of promising *Candida* capable of utilizing phenol as a

sole source of carbon and energy have been isolated from sludge and wastewater **(Mahgoub, et al., 2015a).** Additionally, it is of great importance to evaluate the tolerance of heavy metals by the isolated fungi since these metal ions always exist in some kind of phenol-containing wastewater and have inhibitory effect on cell growth. Biosorbents with different degrees of metal adsorption capacities, availability, and selectivity have been shown to be promising for metal removal from aqueous solutions in batch laboratory scale, but it is their applicability in continuous mode that makes this treatment method attractive for industrial application. In addition, a waste stream containing necessary food source and nutrients can be used to cultivate the biosorbents, thereby reducing the environmental footprint of the process by resource recovery and waste reduction. Based on our earlier work by **Mahgoub, et al., (2015a) and (2015b)** on the removal of Zn and biodegradation of phenol by *Candida* and bacteria in wastewater treatment system and *in vitro* using live cells as well as little information is available in literature on the use of unmodified live and resting cells for biosorption and biodegradation of phenol (**Gonen and Aksu, 2008; Mahgoub et al., 2015a).** Thus, the aims of this work were to isolate and characterize yeast capable of utilizing phenol as sole source of carbon and energy, establish optimal phenol removal conditions and investigate the effect of heavy metal (zinc sulphate) on isolated yeasts during along sewage water treatment lines.

Materials and Methods

Collection and analysis of sewage water samples

The samples of sewage water were collected from sewage water treatment plant located in Zagazig City, El-Sharkia Governorate, Egypt for characterization of heavy yeasts isolates. The samples were collected from different sites during conventional treatment process stages i.e. Untreated Wastewater (UW), Pre-primary Treatment (PPT), Primary Treatment (PT), Secondary Treatment (ST) and Treated Wastewater (TW). The wastewater samples were taken randomly to a depth of 5-10 cm in 300 ml sterilized brown glass bottles during April 2011 to January2012. The samples were placed in a container filled with ice, then transported to the microbiological laboratory, and stored at 4 ºC prior to analysis.

Yeast isolation and identification

Total yeasts were isolated on Rose Bengal Chloramphenicol Agar (RBCA, LAB) according to the method described by **Yarrow (1998)** and the plates were incubated at 30 °C for 24h. *Candida* spp. was isolated onto *Candida* Agar (Biolife, Milano, Italy)**.** In briefly the samples were serially diluted from 10^{-1} to 10^{-4} in the sterile buffered peptone water then 0.1 ml of each dilution samples were spread on *Candida* agar media plates and incubated at 35 ◦C for 48 h. Streaking was repeated on fresh agar to obtain pure cultures. The yeast cultures were kept at 4 ◦C for identification according to the guidelines of **Yarrow (1998) and Kurtzman *et al*. (2011).** All 30 isolates yeasts were identified based on API 20 C AUX system France according to the preparation of the manufacturing. The pure cultures were maintained at 4 °C in the Lab of Microbiology, Faculty of Agriculture, Zagazig University, Egypt for using in this research.

Effect of phenol on yeast strains growth

Phenol experiments were conducted to investigate the effect of phenol concentrations on 30 yeast strains activity. To evaluate the effect of phenol dosage, a contact time of 48 h was selected, and the phenol concentrations were ranged from 0.0 to 0.7, 10, 20, 30 and 40 mg/l. The phenol experiments were performed in 100 ml Erlenmeyer flasks. The flasks were inoculated with each yeast strains and incubated in a constant temperature incubator shaker at 30 ◦C at 150 rpm **(Mahgoub et al., 2015a).** Samples were taken after 48 h from each flask for culturing onto malt extract agar (Malt extract 15 g/l, peptone 5 g/l, agar 15 g/l, distilled water 1000ml/l pH5.4±0.2 at 25 °C). The results were expressed as plus or minus growth. All presented values are the averages of three replicates.

Scanning electron microscope (SEM)

Scanning electron microscopy (SEM) analysis was conducted for further exploration of the action of the phenol on *Candida parasopilis* cell morphology. An aliquot of 0.1 ml of yeast culture was inoculated into 10 ml malt broth and incubated at 30 ◦C with gentle agitation for 24 h. The cells were collected at 5000 rpm for 4 min at 4 ◦C. Cells were washed three times and re-suspended in phosphate buffer (pH 7.2) at the same volume. The phenol (0.0, 0.7 and 10 mg/l) was added to the cell suspension and incubated at 30 ◦C with gentle agitation overnight. The control sample was prepared similarly but without adding phenol. Yeast cells were recovered by centrifugation at 5000 rpm at 4 ◦C, washed with phosphate buffer (pH 7.2) and fixed in 2.5 % glutaraldehyde in phosphate buffer. The fixed yeast pellet was then dehydrated in graded alcohol series, dried and mounted onto stubs using double sided carbon tape, coated with thin layer of gold. All cell samples were examined in scanning electron microscope (JEOL-SEM, Japan) at Faculty of Agriculture, Research Park Cairo, Cairo University.

Effect of zinc on yeast strains growth

Zinc tolerance was conducted to investigate the effects of zinc sulphate concentrations on yeast strains activity. To evaluate the effect of zinc sulphate concentration a contact time of 48 h was selected and the zinc sulphate dosage was changed

from 0 to 5, 10, 20, 30 and 40mg/l. This experiment was performed in 100 ml Erlenmeyer flasks. The flasks were fixed in a constant temperature incubator shaker at 30 ◦C at 150 rpm **(Mahgoub et al., 2015a).** Samples were taken after 48 h for culturing onto malt agar (7456- India). The results were expressed as plus or minus growth. All presented values are the averages of three replicates.

Results and Discussion

Isolation and identification of yeasts from wastewater

In total, 30 yeast taxa were isolated from the wastewater samples during treatment process samples. Treated and untreated sewage water samples were collected during ten months from April 2011 and January 2012. The yeast numbers in every stage of treatment were determined by cultivation methods onto *Candida* Agar. Yeast isolates **(n=30)** initially identified as *Candida, Cryptococcus, Geotrichum* and *Saccharomyces* by morphological and biochemical analyses. The growth of these isolates on *Candida* medium and determination of their assimilation profiles with the API 20C AUX system identified five of the thirty isolates as *Candida parasopilis***,** eight as *Candida krusei*, three as *Cryptococcus neoformans*, nine as *Geotrichum candidum* and five as *Saccharomyces cerevisiae* **(Table, 1**).

Table 1. Biochemical characterization of the isolates yeasts based on carbohydrate interpretation using API 20 C AUX system France kit

Active ingredient	*Candida*	*Candida*	*Cryptococcus*	*Geotrichum*	*Saccharomyces*
D-Glucose	+	+	+	+	+
D-Galactose	+	-	+	+	+
Sucrose	+	-	+	-	+
Trehalose	-	-	-	-	+
Raffinose	-	-	-	-	-
β -Maltosidase	-	-	-	-	-
α-Amylase	-	-	-	-	-
	-	-	-	-	-
β-d-Glucuronidase	-	-	+	-	-
Urease	-		+	-	-

-, Negative reaction +, Positive reaction

The most predominant species of yeast in wastewater were *C. parasopilis* **(n=5),** *C. krusei* **(n=8)** *Cr. neoformans* **(n=3)**, *G. candidum.* **(n=9)** and *S. cerevisiae* **(n=5) (Table, 2).** The distribution of these species in wastewater was 17, 27, 10, 30 and 17%, respectively. However, *Geotrichum candidum* and *C. krusei* were the most common fungal species isolated and were present in a significant proportion in wastewaters. The total cultivable yeasts and *Candida* are in agreement with previously published studies on wastewater **(Mahgoub *et al*., 2015 a, b)**.

Table 2. The distribution of yeasts in wastewater samples collected during study

No	Strains	Percentage (%)
1	*Candida parasopilis*	(5) 17 %
2	*Candida krusei*	(8) 27 %
3	*Cryptococcus neoformans*	(3) 10%
4	*Geotrichum* spp.	(9) 30 %
5	*Candida krusei*	(5) 27 %
6	*Geotrichum* spp.	(9) 30 %
7	*Saccharomyces cerevisiae*	(5) 17 %
8	*Candida krusei*	(8) 27 %
9	*Geotrichum* spp.	(9) 30 %
10	*Geotrichum* spp.	(9) 30 %
11	*Candida krusei*	(8) 27 %
12	*Geotrichum* spp.	(9) 30 %
13	*Candida parasopilis*	(5) 17 %
14	*Geotrichum* spp.	(9) 30 %
15	*Saccharomyces cerevisiae*	(5) 17 %
16	*Candida krusei*	(8) 27 %
17	*Cryptococcus neoformans*	(3) 10%
18	*Candida parasopilis*	(5) 17 %

Table 2 Cont.		
19	*Geotrichum* spp.	(9) 30 %
20	*Candida krusei*	(8) 27 %
21	*Saccharomyces cerevisiae*	(5) 17 %
22	*Geotrichum* spp.	(9) 30 %
23	*Candida parasopilis*	(5) 17 %
24	*Saccharomyces cerevisiae*	(5) 17 %
25	*Candida krusei*	(8) 27 %
26	*Candida parasopilis*	(5) 17 %
27	*Geotrichum* spp.	(9) 30 %
28	*Cryptococcus neoformans*	(3) 10%
29	*Saccharomyces cerevisiae*	(5) 17 %
30	*Candida krusei*	(8) 27 %

Screening of *Candida* strains on different phenol concentrations

The growth patterns of 13 *Candida* strains and *Cr. neoformans* **(n=3)**, *G. candidum.* **(n=9)** and *S. cerevisiae* **(n=5)** on different concentrations of phenol is represented by the corresponding symbols in **Table 3**; the number of symbols corresponded to the extent of the growth pattern. According to the data given in **Table (3)**, all strains could grow even at high concentration, reaching up to 10 mg/l. However, between the 30 isolated yeast strains, only the 11 strains (3: *C. parasopilis***,** 3: *C. krusei*, 3: *G. candidum* and 2: *S. cerevisiae***)** showed a high growth rate reaching up to 20 mg/l. The application possibility of yeasts in wastewater treatment was early reported by some Japanese scientists **(Moriya, *et al*., 1990; Chigusa, *et al*., 1996).** Recent studies pointed that some yeasts could produce lipase or degrade phenol compounds and thus had the potentials in treating oil manufacturing wastewater **(Zheng, *et al*., 2001)** olive mill wastewater **(Goncalves, *et al*., 2009)** or reclaiming oil-contaminated sites **(Hesham, *et al*., 2006).**

Table 3. Growth of yeast strains in different concentrations of phenol

No	Strains	Control	0.7 mg/l	10 mg/l	20 mg/l	40 mg/l
1	*Candida parasopilis*	+++	+++	++	+	_
2	*Candida krusei*	+++	+++	+	+	_
3	*Cryptococcus neoformans*	+++	+++	++	-	_
4	*Geotrichum* spp.	+++	+++	+	-	_
5	*Candida krusei*	+++	+++	+	+	_
6	*Geotrichum* spp.	+++	+++	++	-	_
7	*Saccharomyces cerevisiae*	+++	+++	+	+	_
8	*Candida krusei*	+++	+++	+	-	_
9	*Geotrichum* spp.	+++	+++	+	-	_
10	*Geotrichum* spp.	+++	+++	+	-	_
11	*Candida krusei*	+++	+++	+	-	_
12	*Geotrichum* spp.	+++	+++	+	+	_
13	*Candida parasopilis*	+++	+++	+	+	_
14	*Geotrichum* spp.	+++	+++	+	-	_
15	*Saccharomyces cerevisiae*	+++	+++	+	-	_
16	*Candida krusei*	+++	+++	+	+	_
17	*Cryptococcus neoformans*	+++	+++	++	-	_
18	*Candida parasopilis*	+++	+++	+	-	_
19	*Geotrichum* spp.	+++	+++	+	+	_
20	*Candida krusei*	+++	+++	++	-	_
21	*Saccharomyces cerevisiae*	+++	+++	+	-	_
22	*Geotrichum* spp.	+++	+++	++	+	_
23	*Candida parasopilis*	+++	+++	+	+	_
24	*Saccharomyces cerevisiae*	+++	+++	+	+	_
25	*Candida krusei*	+++	+++	++	-	_
26	*Candida parasopilis*	+++	+++	++	-	_
27	*Geotrichum* spp.	+++	+++	++	-	_
28	*Cryptococcus neoformans*	+++	+++	++	-	_
29	*Saccharomyces cerevisiae*	+++	+++	++	-	_
30	*Candida krusei*	+++	+++	++	-	_

Our study also demonstrated that *Candida tropicales* could be advantageous in removal of phenol from wastewater **(Mahgoub *et al*., 2015 b).** Phenol can be converted to harmless compounds by microorganisms. Some aerobic bacteria and fungi use phenol as a source of carbon and energy, and degrade it **(Rappoport, 2003; Ahmed, et al., 2010).** The capability of degrading organic substances with high efficiency and tolerance of heavy metals as well as a wide range of environmental variations leads to the consideration of fungal strains to be applied in wastewater treatment **(Chen et al. 2002; Taştan et al. 2010).** Previous studies have reported the application of fungal isolates in biodegradation of kerosene, formaldehyde, and many other kinds of pollutants, which showed satisfactory performances **(Khan et al. 2015; Yu et al. 2014).**

Scanning electron microscope (SEM)

Different concentrations of phenol (0.0, 0.7 and 10.0 mg/l) were added to the cell suspension of *C. parasopilis* and incubated at 30°C with gentle agitation for 6 h. SEM at a magnification of 7500, 10000 or 20000× of *Candida* image are illustrated in Fig. (1). The image showed the untreated *C. parasopilis* and that treated with 0.7 mg/L phenol concentration had a normal morphology with no cell morphological changes. While *C. parasopilis* which treated with 10.0 mg/L phenol, the cells showed a shrunken surface (Fig .1). The cells in 10.0 mg/L were severely damaged shown as collapse and degradation of the cells. This strain is a good candidate to be used in further studies and different applications in the field of treated wastewater form phenol.

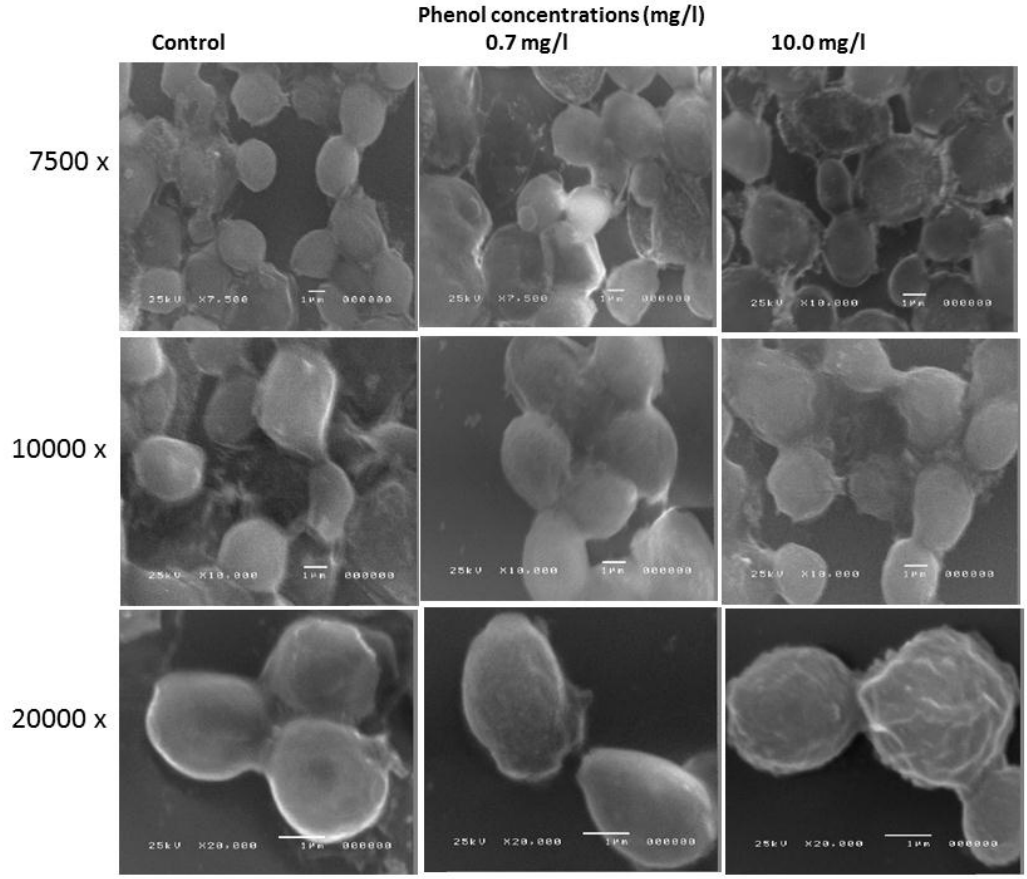

Fig. 1. Scanning electron microscope images of *Candida parasopilis* treated with different concentrations of phenol (0.0, 0.7 and 10.0 mg/l)

Screening of yeasts strains on diffrent zinc concentrations

The growth patterns of 13 *Candida* strains and *Cr. neoformans* **(n=3)**, *G. candidum.* **(n=9)** and *S. cerevisiae* **(n=5)** on different concentrations of zinc sulphate was represented by the corresponding symbols in Table (4), the number of symbols corresponded to the extent of the growth pattern. According to the data given in Table (4), all strains could grow at high concentration of zinc sulphate reaching up to 12 mg/l. However, between the thirty isolated yeast strains, only 11 strains (*C. parasopilis***,** *C. krusei*, *G. candidum* and *S. cerevisiae***)** showed a high growth rate reaching up to 24 mg/l. However, continuous operation system is the only viable way of treating large volume of wastewater in a reasonable time, and this is where most of the bench scale batch biosorption studies are limited in their scope. From the literatures, no studies have been reported on the use of yeast cells in a combined

continuous bioreactor–biosorption system for the removal of heavy metals from aqueous solutions. So, these results indicated that these strains could be used in a combined continuous bioreactor–biosorption system for the removal of heavy metals. Metal uptake is a combination of a metabolism independent physical process, followed by a metabolic step known as bioaccumulation (**Wehrheim and Wettern 1994).** Heavy metals which consist of Cd, Cr, Cu, Pb, Ni, Fe, Mn, Hg, Zn, Al, Se as well as metals of group III and IV, have toxic effects on microbial physiology **(WHO, 1999; Bruins *et al*., 2000).** However, biomaterials including fungal, biomass, marine algae, bacteria, yeasts, and waste sludge have been extensively investigated during the last decades for the removal of heavy metals and dyes because of their low cost, high efficiency, reduction in the amount of chemical and biological sludge, regeneration of biosorbent and the possibility of metal recovery **(Kratochvil and Volesky, 1998 and Mahgoub *et al*., 2015b).** Removal of heavy metals in continuous mode was earlier reported as a preferred choice in some metal adsorption studies. For instance, **Kapoor and Viraraghavan**, **(1998)** used immobilized cells of *Aspergillus niger* in a continuous operation for the removal of metal solutions containing cadmium, copper lead, and nickel. **Marques et al. (2007)** used a fixed-bed reactor for Cd removal using immobilized cells of an industrial strain of *Saccharomyces cerevisiae*.

Table 4. Growth of yeast strains in different concentrations of zinc sulphate

No	Strains	Control	1.0 mg/l	3.0 mg/l	6.0 mg/l	12.0mg/l	24.0mg/l	36.0 mg/l
1	*Candida parasopilis*	+++	+++	+++	++	++	+	_
2	*Candida krusei*	+++	+++	+++	++	+	+	_
3	*Cryptococcus*	+++	+++	+++	++	++	-	_
4	*Geotrichum* spp.	+++	+++	+++	++	+	-	_
5	*Candida krusei*	+++	+++	+++	++	+	+	_
6	*Geotrichum* spp.	+++	+++	+++	+++	++	-	_
7	*Saccharomyces*	+++	+++	+++	++	+	+	_
8	*Candida krusei*	+++	+++	+++	++	+	-	_
9	*Geotrichum* spp.	+++	+++	+++	++	+	-	_
10	*Geotrichum* spp.	+++	+++	+++	++	+	-	_
11	*Candida krusei*	+++	+++	+++	++	+	-	_
12	*Geotrichum* spp.	+++	+++	+++	+++	+	+	_
13	*Candida parasopilis*	+++	+++	+++	++	+	+	_
14	*Geotrichum* spp.	+++	+++	+++	++	+	-	_
15	*Saccharomyces*	+++	+++	+++	++	+	-	_
16	*Candida krusei*	+++	+++	+++	++	+	+	_
17	*Cryptococcus*	+++	+++	+++	++	++	-	_
18	*Candida parasopilis*	+++	+++	+++	++	+	-	_
19	*Geotrichum* spp.	+++	+++	+++	++	+	+	_
20	*Candida krusei*	+++	+++	+++	++	++	-	_
21	*Saccharomyces*	+++	+++	+++	++	++	-	_
22	*Geotrichum* spp.	+++	+++	+++	+++	++	+	_
23	*Candida parasopilis*	+++	+++	+++	++	+	+	_
24	*Saccharomyces*	+++	+++	+++	++	++	+	_
25	*Candida krusei*	+++	+++	+++	++	++	-	_
26	*Candida parasopilis*	+++	+++	+++	++	++	-	_
27	*Geotrichum* spp.	+++	+++	+++	++	++	-	_
28	*Cryptococcus*	+++	+++	+++	++	++	-	_
29	*Saccharomyces*	+++	+++	+++	++	++	-	_
30	*Candida krusei*	+++	+++	+++	++	++	-	_

Conclusion

In this work, the incidence of *C. parasopilis***,** *C. krusei, Cr. neoformans, G. candidum* and *S. cerevisiae* in wastewater treatment system was 17, 27, 10, 30 and 17%, respectively. The ability of these strains for biodegradation of phenol and biosorption of Zn ions was investigated. The yeast cells showed a higher growth rate reaching up to 20 mg/L and 12 mg/L phenol and Zn ions, respectively. Thus, live cells of yeast strains were used for phenol biodegradation and metal biosorption.

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عزل وتعريف الخمائر على طول خطوط معالجة مياة الصرف الصحي بمحطة الزقازيق

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يعتبر الفينول من اكثر الملوثات شيوعا في العديد من أنواع مياه الصرف الصناعي والتي يحتوي بعضها علي تركيزات مرتفعة من المعادن الثقيلة والتي تجعل عملية التحلل الحيوي أكثر صعوبة . وتهدف هذه الدراسة الي توصيف وجود الخمائر علي طول خطوط عملية معالجة مياه الصرف الصحي في محطة المعالجة بمدينة الزقازيق حيث كانت أكثر أنواع الخمائر تواجدا كل من : *Candida parasopilis* (عددها =3) و *C. krusei* (وعددها = 8) و*Cryptococcus neoformans* (وعددها = 3) و *Geotrichum* spp. (وعددها = 9) *Saccharomyces cerevisiae* (وعددها = 5) وكان نسب أنتشار هذه الأنواع 17 ، 27، 10، 30، 17% علي التوالي. وكانت أكثر تلك الأنواع الفطرية المعزولة كل من *C. krusei* و *Geotrichum* spp. حيث كان تواجدهم بصورة معنوية ملموسة. وعند قياس مدى تحمل العزلات للفينول لعدد 30 سلالة خميرة معزولة كان منهم فقط 11 سلالة وهم 2: *S.cerevisiae* و 3: *Geotrichum* spp. و 3: *C. krusei* و 3:*C. parasopilis* أكثر قدرة علي النمو في وجود تركيز عالي من الفينول حتي 20 ملليجرام / لتر . كما أظهرت معظم السلالات المتحصل عليها القدرة علي النمو علي تركيزات من كبريتات الزنك حتي 12 ملليجرام / لتر . وكان خميرة ال *C. parasopilis* غير المعاملة وكذلك المعاملة بتركيز فينول0.7 ملليجرام / لتر ذات شكل مورفولوجي للخلايا طبيعي مع عدم وجود تغيرات مورفولوجية بالخلية خلال الفحص بالميكروسكوب الإلكتروني النافذ. وتشير النتائج أن هذه السلالة من الخميرة سوف تكون نافعه في مجال البيئة والمعالجة البيولوجية لمياه المجاري لأزاله الفينول و امتصاص أيونات المعادن الثقيلة .