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Yeast Microflora of Some Aquatic Habitats in El-Minia Governorate, Egypt

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ABSTRACT: 269 yeast strains were isolated from water samples collected from different sites in Minia governorate. These included 126 strains from fresh water, 108 strains from sewage and 35 strains from wastewater from sugar-cane factory. On the basis of 23 different physiological and morphological merkmals, the isolated strains were assigned to 16 species belonging to 11 genera. Total yeast cell counts as well as spectra of yeast species were highly variable in tested water. Total yeast cell counts ranged between $3.0\times10^3/l$ and $1.8\times10^6/l$ for fresh water, $3.0\times10^4/l$ and $3.0\times10^7/l$ for sewage and $1.5\times10^6/l$ and $2.6\times10^7/l$ for wastewater from sugarcane factory. *Debaryomyces hansenii, Rhodotorula mucilaginosa* and *Torulaspora delbrueckii* were the dominant species in fresh water, whereas *Debaryomyces hansenii, Thrichosporon beigelii, Rhodotorula mucilaginosa* and *Kluyveromyces marxianus* were the dominant species in sewage and *Saccharomyces cerevisiae, Kluyveromyces marxianus* and *Trichosporon beigelii* were the dominant species in wastewater from sugar-cane factory. Yeast human pathogens, Trichosporon beigelii, Rhodotorula mucilaginosa and *Candida albicans* were encountered in water samples indicating that water in El-Minia governorate is also polluted by some pathogenic yeasts.

KEYWORDS: Yeast, aquatic habitats, Egypt.

The occurrence of yeasts in aquatic habitats was reported by several investigators(Kriss *et al.*, 1952, 1967; Spencer *et al.*, 1969, 1970, 1974a; Simard and Blackwood, 1971a, b; Viviani and Tortorano, 1976; Hinzelin and Lectard, 1978 and Hagler and Mendoca-Hagler, 1981). Data of these investigations support the idea that most yeasts of aquatic habitats have terrestrial origin and some species, such as *Debaryomyces hansenii* and *Rodotorula spp.*, are physiologically adapted for survival and increase in population in water environment with increased nutrients. The correlation between yeast counts and pollution of water has been previously observed by Wollett *et al.*(1970), Viviana and Tortorano(1976), Vaatanen(1980a, b) and Hagler *et al.*, (1981). This pollution is reflected on the chemical, physical and microbiological characteristics of water. This correlation is indicated from the observation that yeast cell densities in polluted water and sewage exceed that of nonenriched water. Also, yeast counts in sewage were reported to be higher than those recorde for fresh water(Cooke *et al.*, 1960; Cooke & Matsuura, 1963, 1969; Cooke, 1965, 1970; Meyers *et al.*, 1967; Crow *et al.*, 1976 and Hinzelin and Lectard, 1979). Along the same line, pollution of fresh water streams receiving sugar-cane wastes from sugar-cane factories by yeasts was reported by Ahearn *et al.*(1968). Different yeast species including *Saccharomyces exiguus* and its anamorph *Candida holmii* as well as other *Saccharomyces spp.* have been reported to prevail in fresh water receiving sugar-cane waters(Ahearn *et al.*, 1968).

Due to health hazard which might arise as a result of pollution of water with pathogenic yeasts, it was of interest to study yeast flora of water samples from different habitats in El-Minia governorate as a part of an extensive survey aiming to study yeast flora in different habitats in this governorate in order to evaluate its role in pollu-

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ting the environment.

Materials and Methods

Collection of water samples: 112 fresh water samples were collected from water streams of Ibrahimea canal and its irrigation branches at different localities in El-Minia governorate. These sites were located in Maghagha, Beni-Mazar, Matay, Samalot, El-Minia, Abo-Korkas, Malawy and Dermowas. 27 sewage samples were gathered from 2 different sewage drainage stations(A, B). Station A is found outside El-Minia City(at El-Minia University) where a limited number of population work, whereas sewage of station B(at Ard-Soltan) comes from different areas with a high number of population and receives wastewaters of different industries. 12 wastewater samples from sugar-cane factory(at Abo-Korkas) were collected from a canal receiving this wastewater. 25 m*l* proteins of each water samples were collected in sterile conical flasks and transported directly to the laboratory. Sampling was performed monthly for one year period.

Isolation and identification of yeast strains: 0.5 m*l* portions from the prepared decimal dilutions of water samples were spread on plates containing YM-agar medium(Lodder, 1970) adjusted to pH 3.5 and the plates were incubated at 28℃ for 2-3 days. In case of sewage samples, an additional set of plates was incubated for the same period at 37℃. Developing yeast colonies were counted and after microscopic examination, yeast strains were isolated, purified, preserved on YM-agar slants and stored at 4℃. Yeast identification was performed on the basis of the various physiological and morphological characteristics as indicated by Lodder(1970), Barnett *et al.*(1983) and Kreger van Rij(1984).

Results and Discussion

Fresh water: Results of table 1 showed that, yeasts were detected in 59(ca. 53%) out of 112 fresh water samples collected from 8 different localities in El-Minia governorate and that, total yeast counts in the investigated fresh water samples ranged between $3.0\times10^3/l$ and $1.8\times10^6/l$. Results also showed that,f relatively high yeast total counts as well as more yeast species were recorded in water samples collected from Maghagha, Dermowas, Abo-Korkas and El-Minia, whereas low yeast counts and relatively few yeast species were found in fresh water from Beni-Mazar, Matay, Samalot and Malawy(Table 1). Other investigators reported that, clean fresh as well as marine waters usually contain below 100 yeasts/*l*(Van Uden & Ahearn, 1963; Ahearn *et al.*, 1968, 1969; Cooke, 1970; Meyers *et al.*, 1970; Ahearn, 1973; Spencer *et al.*, 1974a; Colwell *et al.*, 1976 and Hagler & Mendoca-Hagler, 1981), whereas yeast cell densities in polluted water frequently exceed $10^3/l$(Van Uden & Fell, 1968 and Ahearn, 1973). Moreover, a correlation was found between yeast counts and chemical, physical and microbiological parameters at various sites with different levels of pollution (Wollett *et al.*, 1970; Simard & Blackwood, 1971b; Noel & Simard, 1973; Viviana & Tortorano, 1976; Vaatanen, 1980a,b and Hagler *et al.*, 1981). In the light of the results presented in Table 1, it could be concluded that fresh water in El-Minia governorate is polluted by yeasts and the level of pollution varies among the different localities. The highest level of water pollution was observed in water samples collected from Maghagha, whereas the lowest was recorded in Samalot.

On the basis of morphological and physiological merkmals, 126 yeast strains isolated from fresh water samples were assigned to 12 species belonging to 9 genera(Table 1). It is clear that, spectra of yeast species as well as the dominant ones varied in fresh water samples collected from different localities. *Debaryomyces hansenii, Rhodotorula mucilaginosa* and *Torulaspora delbrueckii* occurred in high numbers in 6 different localities but were missed in two localities. It is possible that these species are physiologically adapted for survival in aquatic habitats than the others. This observation was previously reported for *Debaryomyces hansenii* and *Rhodotorula spp.* by other investigators(Cooke *et al.*, 1960; Capriotti, 1962a,b; Fell, 1967, 1974; Hoppe, 1972, Ahearn, 1973 and Hagler & Ahearn,

Table 1. Yeast species isolated from aquatic habitats.

	Number of isolated strains (269)	Fresh water from locality								Sewage		Waste-water from sugar-cane factory
		Kaghagha	Beni-Mazar	Katay	Samalot	El-Minia	Abo-Korkas	Kalawy	Dermowas	A	B	
Number of samples tested	14	14	14	14	14	14	14	14	14	12	15	12
Number of samples containing yeasts	10	6	5	5	8	10	5	10	12	15	12	
Percentage of yeast occurrence	71	43	36	36	57	71	36	71	100	100	100	
Total count of yeast cells/*l*		1.4×10^{-4} to 1.8×10^{6}	1×10^{-4} to 5.4×10^{5}	1.2×10^{-4} to 1.3×10^{5}	3×10^{-3} to 4.2×10^{4}	1.8×10^{-4} to 4.8×10^{4}	3.6×10^{-4} to 3.8×10^{5}	1.2×10^{-4} to 1.3×10^{5}	1.2×10^{-4} to 2.0×10^{4}	3×10^{-4} to 1.8×10^{6}	1.9×10^{-5} to 1.6×10^{7}	1.5×10^{-6} to 2.6×10^{7}
Species												
Debaryomyces hansenii	48	8(4)**	4(4)	6(4)	--	4(2)	2(1)	--	4(4)	6(5)	14(6)	--
*Rhodotorula mucilaginosa**	42	2(1)	---	---	4(2)	4(1)	4	2	2(1)	10(2)	14(1)	--
*Trichosporon beigelii**	38	8(4)	--	---	--	---	---	2(1)	2(2)	4(2)	14(2)	8(2)
Kluyveromyces marxianus	34	4	---	---	--	---	2(2)	--	---	6(3)	10(2)	8(3)
Torulaspora delbrueckii	24	2(1)	---	---	--	4(2)	2(2)	--	---	---	8(1)	--
Saccharomyces cerevisiae	20	---	2(2)	---	2(2)	2(1)	4(2)	4(2)	---	---	8(3)	12(5)
Candida blankii	18	4	---	---	--	---	---	---	---	---	4	4(1)
Hansenula polymorpha	12	---	---	---	--	4(1)	---	2	---	---	6	2(1)
Cryptococcus albidus	10	---	---	---	--	2	2	--	---	---	---	1
Clavispora lusitaniae	8	---	---	---	--	4	2(1)	2(2)	---	1	---	---
Rhodotorula aurantiaca	6	---	---	2(1)	--	4(1)	2(2)	--	---	---	---	---
Debaryomyces vanrijii	4	2	---	---	--	---	2(2)	--	4(3)	---	---	---
Cryptococcus laurentii	2	---	---	---	2(1)	---	---	--	---	---	---	---
*Candida albicans**	1	---	---	---	--	---	---	2	---	---	---	---
Candida steelytica	1	---	---	---	--	---	---	--	---	---	1	--
Pichia quilliermondii	1	---	---	---	--	---	---	--	---	1	---	--

* = yeast human pathogen, ** = Number of samples in which yeast species is dominant.

Table 2. Physiological and morphological properties of the isolated yeast species.

Species	Number of strains tested (269)	Fermentation					Assimilation												Growth at		Building of		
		glucose	galactose	maltose	sucrose	lactose	galactose	sorbose	ribose	xylose	arabinose	rhamnose	sucrose	maltose	lactose	mannitol	succinate	citrate	37℃	42℃	ascospres	true mycelium	pseudomycelium	pellicle
Debaryomyces hansenii	48	96*	58	54	75	0	100	67	96	100	54	38	100	100	25	100	100	100	33	0	100	0	42	54
Rhodotorula mucilaginosa	42	0	0	0	0	0	90	62	52	95	52	10	100	100	0	76	71	33	48	0	0	0	0	0
Trichosporon beioelii	38	0	0	0	0	0	100	90	100	79	100	58	90	90	100	100	100	68	63	32	0	100	100	100
Kluyveromyces marxianus	34	100	59	41	53	6	100	65	77	82	47	0	100	100	47	88	82	65	82	75	100	0	100	100
Torulaspora delbrueckii	24	100	33	33	33	0	100	75	0	83	0	0	83	92	0	58	25	0	50	0	100	0	100	100
Saccharomyces cerevisiae	20	100	50	40	50	0	80	0	0	0	0	0	100	100	0	50	40	0	60	20	100	0	60	60
Candida blankii	18	89	56	78	56	0	100	100	100	100	100	100	100	100	100	100	100	100	0	100	100	100		
Hansenula polymorpha	12	100	0	0	67	0	83	67	83	83	67	0	100	100	0	100	50	67	100	100	100	0	0	0
Cryptococcus albidus	10	0	0	0	0	0	100	100	80	100	100	100	100	100	100	100	80	80	0	0	0	0	0	0
Clavispora lusitaniae	8	100	50	75	25	0	100	100	75	100	0	100	100	100	0	100	75	75	100	75	100	0	100	100
Rhodotorula aurantiaca	6	0	0	0	0	0	100	0	0	0	0	0	33	0	0	33	33	0	0	0	0	0	0	0
Debaryomyces vanriji	4	50	0	0	50	0	100	100	50	50	100	0	100	100	0	100	0	50	100	0	100	0	50	50
Cryptococcus laurentii	2	0	0	0	0	0	100	0	100	100	100	100	100	100	100	100	100	100	100	0	0	100	100	100
Candida albicans	1	100	100	100	100	0	100	100	100	100	100	0	100	100	0	100	100	100	100	100	0	0	100	100
Candida steolytica	1	100	100	100	100	0	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0	0	100	100
Pichia quilliermondii	1	0	0	0	0	0	100	100	100	100	100	0	100	100	0	100	100	100	100	100	100	0	100	100

1987).

Table 1 shows that the red yeast species *Rhodotorula mucilaginosa, Rhodotorula aurantiaca* and *Cryptococcus laurentii* were isolated from fresh water samples in low frequency(21% of the total isolates). Their occurrence in low frequency in fresh water could be used as an indicator for water pollution as previously shown by Spencer *et al.*(1970, 1974b); Simard(1971) and Hagler and Mendoca-Hagler(1981). Also, *Trichosporon beigelii*, a filamentous arthro-spore-forming yeast of basidiomycetious affinity, which has been suggested as a pollution indicator(Hinzelin & Lectard, 1976, 1978), was isolated frequently in this study particularly from fresh water samples collected from Maghagha.

Sewage water: Results presented in table 1 showed that, all the tested sewage samples contained yeast cells and that, yeast cell counts ranged between $3\times10^4/l$ and $1.6\times10^7/l$. It is also clear from table 1 that, the total count of yeast cells as well as the spectrum of yeast species in sewage from station B is higher than those from station A. *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* were isolated in considerable numbers from sewage of station B but were missed in sewage of station A. These variations are expected since station B receives various wastewaters coming from different sources of higher populated areas indoor the city, whereas station A receives wastewater from low population areas. Cooke *et al.*(1960), Cooke & Matsuura(1963, 1969), Spencer *et al.*(1974 b) and Hinzelin and Lectard(1979) have shown that yeast counts in sewage, although highly variable, are much higher in sewage than in fresh waters. Counts up to $2\times10^8/l$ yeasts/l have been reported form sewage and $10^5/l$ were observed in domestic sewage.

Debaryomyces hansenii, Rhodotorula mucilaginosa, Trichosporon beigelii and *Kluyveromyces marxianus* were dominant and were represented by high number of yeast cells. Except for *Kluyveromyces marxianus*, the remaining 3 species were previously recorded as prevalent species in sewage by Hagler and Ahearn(1987).

Wastewater from sugar-cane factory: Our investigations on yeast flora of wastewater from sugar-cane factory show that, yeast cell counts ranged between $1.5\times10^6/l$ and $2.6\times10^7/l$(Table 1). Yeast counts were much higher than those of sewage. Wastewater from sugar-cane factory contains manu residues of sugar industry and is acidic with pH value between 4.8 and 5.8 which promote flurishing of yeasts. *Saccharomyces cerevisiae* was the most dominant species followed by *Kluyveromyces marxianus* and *Trichosporon beigelii*(Table 1). *Saccharomyces spp.* were reported by Ahearn *et al.* (1968) as dominant species in fresh water receiving sugar-cane wastes.

The physiological and morphological properties of the ioslated yeast species are presented in table 2. It is clear that, glucose fermenting ability, building of ascospores, truc mycelium, pigmented colonies and arthrospores represented differential merkmals in our choosen merkmal set for the most dominant species.

It is worthmentioning that *Trichosporon beigelii, Rhodotorula mucilaginosa* and *Candida albicans*, which are known to be human pathogens(Rosalinde *et al.*, 1987), were isolated in this study from some fresh water samples, sewage water and wastewater of sugar-cane factory. Their occurrence in fresh water samples may represent health hazard to human and care should by taken to avoid this pollution.

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References

Ahearn, D. G. 1973. In "Estuarine Microbial Ecology: The Belle W. Baruch Library in Marine Science, Number 1".(L. Harold Stevenson and R. R. Colwell, eds), pp. 433-440. University of South Carolina Press, Columbia, South Carolina.

Ahearn, D. G.; Meyers, S. P.; Cook, W. L. and Hansen, G. 1969. Antonie van Leeuvenhoek, Supplement. **35**: D19.

Ahearn, D. G., Roth, F. J. Jr. and Meyers, S. P. 1968. *Marine Biology,* **1**: 291.

Barnett, J. A.; Payne, R. W. and Yarrow, D. 1983. Yeasts, characteristics and identification. Cambridge University Press, Cambridge.

Capriotti, A. 1962a. *Archiv fur Microbiologie* **41**: 147.

Capriotti, A. 1962b. *Archiv fur Microbiologie* **42**: 407.

Colwell, R. R., Walker, J. D., Conrad, B. F. and Seesman, P. A. 1976. *Developments in Industrial Microbiology* **17**: 269.

Cooke, W. B. 1965. *Mycologia* **57**: 696.

Cooke, W. B. 1970. United States Department of the Interior, Federal Water Pollution Control Administration, Robert. A. Taft Water Research Center, Cincinnati, Ohio.

Cooke, W. B. and Matsuura, G. S. 1963. *Protoplasma* **57**: 163.

Cooke, W. B. and Matsuura, G. S. 1969. *Ecology* **50**: 659.

Cooke, W. B., Phaff, H. J., Miller, M. W., Shifrine, M. and Knapp, E. 1960. Mycologia **52**: 210.

Crow, S. A., Cook, W. L., Ahearn, D. G. and Bourguin, A. W. 1976. In "Proceedings of the Third International Biodegradation Symposium"(J. M. Sharpley and A. M. Kaplan, eds.), pp. 93-99. Applied Science Publishers, London.

Fell, J. W. 1967. Bulletin of Marine Science of the Gulf and Caribbean, **17**: 454.

Fell, J. W. 1974. In "Effects of the Ocean Environment on Microbial Activities"(R. R. Colwell and R. Y. Morita, eds.) pp. 510-523. University Park Press, Baltimore.

Hagler, A. N. and Ahearn D. G. 1987. Ecology of aquatic yeasts. In "The yeasts(Rose A. H. and Harrison J. S., eds), vol. 1, pp. 181-205.

Hagler, A. N. and Mendoca-Hagler, L. C. 1981. *Applied and Environmental Microbiology* **41**: 173.

Hagler, A. N., and Mendoca-Hagler, L. C., Schrank, A. and Santos, E. A. 1981. Abstracts of the Annual Meeting of the American Society for Microbiology, **81**: 175.

Hinzelin, F. and Lectard, P. 1976. *Hydrobiologia,* **49**: 27.

Hinzelin, F. and Lectard, P. 1978. *Hydrobiologia,* **61**: 209.

Hinzelin, F. and Lectard, P. 1979. *Hydrobiologia,* **69**: 121.

Hoppe H. G. 1972. *Kiel Meersforschung* **28**: 219.

Kreger van Rij N. J. W. 1984. The Yeasts, a Taxonomic Study. 3 Aufl. Groningen, The Netherlands, Elsevier Science Publishers B. V. Amsterdam.

Kriss, A. E., Mishustina, I. E., Mitskevich, N. and Zemtsova, E. V. 1967. "Microbial Population of Oceans and Seas"[Russian publication, 1964, translated by K. Syers(G. E. Fogg, ed.)]. Edward Arnold, London.

Kriss, A. E., Runkina, E. A. and Tikhonenko, A. S. A. 1952. *Zhurnal Obshchei Biologii.* **13**: 232.

Lodder, J. 1970. The Yeasts, a Taxonomic Study. 2nd ed. North-Holland Publish. Co. Amsterdam.

Meyers, S. P., Ahearn, D. G. and Cook, W. L. 1970. *Mycologia* **62**: 505.

Meyers, S. P., Ahearn, D. G., Gunkel, W. and Roth, F. J. Jr. 1967. *Marine Biology* **1**: 118.

Noel, J. G. and Simard, R. E. 1973. Abstracts of the Annual Meeting of the American Society for Microbiology **73**: 33.

Rosalinde, H. J., Louvois de. and Mulhall, A. 1987. Yeasts as Human and Animal Pathogens. In "The Yeasts".(Rosa, A. H. and Harrison, J. S., eds) vol. I, pp. 207-281.

Simard, R. E. 1971. *Marine Pollution Bulletin* **2**, 123.

Simard, R. E. and Blackwood, A. C. 1971a. *Canadian Journal of Microbiology* **17**: 197.

Simard, R. E. and Blackwood, A. C. 1971b. *Canadian Journal of Microbiology* **17**: 353.

Spencer, J. F. T., Gorin, P. A. J. and Gardner, N. R. 1969. Antonie van Leeuvenhoak 35 supplement, Yeast Symposium, p. 17.

Spencer, J. F. T., Gorin, P. A. J. and Gardner, N. R. 1970. *Canadian Journal of Microbiology* **16**: 1051.

Spencer, J. F. T., Gorin, P. A. J. and Gardner, N. R. 1974a. *Canadian Journal of Microbiology* **20**: 949.

Spencer, J. F. T., Gorin, P. A. J. and Gardner, N. R. 1974b. *Canadian Journal of Microbiology* **20**: 993.

Vaatanen, P. 1980a. *Applied and Environmental Microbiology* **40**: 48.

Vaatanen, P. 1980b *Applied and Environmental Microbiology* **40**: 55.

Van Uden, N. and Ahearn, D. G. 1963. *Antonie van Leeuvenhoak* **29**: 308.

Van Uden, N. and Fell, J. W. 1968. In "Advanced in Microbiology of the Sea"(M. R. Droop and E. J. F. Wood, eds). vol I, pp. 167-201. Academic press, New York.

Viviana, M. A. and Tortorano, A. M. 1976. *Bulletin de la Societe Francaise de Mycologie Medicale* **5**: 109.

Wollett, L. L., Hedrick, L. R. and Tarves, M. G. 1970. *Antonie van Leeuwenhoak* **36**: 347.