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Taxonomical Studies on Red Yeasts in El-Minia City, Egypt

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ABSTRACT: 227 red yeast strains were isolated from air (60 isolates), plant flowers (45 isolates), soil (40 isolates), water (37 isolates) and dairy products (45 isolates). On the basis of 33 different physiological and morphological properties, the isolated strains were assigned to 6 species belonging to 4 genera. *Rhodotorula mucilaginosa* and *Cryptococcus albidus* were the most dominant species among red yeasts of the air, plant flowers, water and dairy products, whereas *Cryptococcus albidus* and *Rhodotorula glutinis* were prevailed in soil. *Cryptococcus laurentii* was represented by considerable number of strains, whereas the other spesies were of low occurrence. Noteworthy was the isolation of 2 different groups of isolates belonging to *Rhodotorula glutinis*. These groups were differentiated from each other on the basis of rhamnose, cellobiose and arabinitol assimilation and growth at 37℃. Systematic position of *Rhodotorula glutinis* was discussed.

KEYWORDS: Taxonomy, red yeasts.

Red yeasts represent a heterogenous group of yeast microflora. The members of this group belong to ascomycetes, *basidiomycetes* and *deuteromycetes* (Kreger van Rij, 1984). Moreover, they occupied different habitats (Hagler&Ahearn, 1987; Rhaff & Starmer, 1987; Haridy, 1987, 1991, 1992). These red yeasts are especially harmful in dairy products because of their ability to peptonize milk and decompose butterfat (Nissen, 1930). They grow on the surface of dairy products forming distinct red colonies (Cordes & Hammer, 1927; Walker & Ayres, 1970). Simard (1971) and Simard and Blackwood (1971a, 1971b) have proposed that the total counts of red yeasts could be used as an indicator of pollution in River waters. They comprise a higher proportion of the total yeast population in clean water than in polluted water. Generally in Egypt and particularly at El-Minia city, there is no study along that line. Therefore, we report on red yeast flora of some habitats at el-Minia city as a part of general survey of red yeasts in Egypt in an attempt to throw light on red yeast species presented and to determine their roles in these habitats.

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Materials and Methods

Collection of samples: Samples from cultivated soils, water, plant flowers, raw milk and dairy products such as yoghurt, cheese and whey were collected in the sterile conical flasks and then transferred directly to laboratory. Samples were mixed with sterile distilled water and series of dilutions were prepared. 0.5 m*l* portions were spread on plates containing yeast malt agar medium adjusted to pH 3.5 (Lodder, 1970). In case of air sampling, plates containing the above mentioned medium were exposed to air at 8 meters hight for 2 hours.

Isolation and identification of red yeast strains: Inoculated plates were incubated at 28℃ for 2-3 days. According to macro- and micromorphological characters of developing red colonies, 227 red strains were isolated, purified, preserved on agar slants and stored at 4℃. Identification of the isolated strains were performed according to standard keys of Lodder (1970), Barnett *et al.* (1983) and Kreger van Rij (1984).

Results and Discussion

Table 1 showed the distribution of isolated red

Table 1. Distribution of the isolated red yeast species in different habitats

Yeast species	Habitats					Total number of the isolates tested (227)
	air	water	plant flowers	soil	dairy products	
Cryptococcus albidus	27	13	15	18	11	84
Cryptococcus laurentii	2	4	9	--	3	18
Rhodotorula glutinis	2	5	6	18	9	40
Rhodotorula mucilaginosa	28	15	15	4	20	82
Sporidiobolus pararoseus	--	--	--	--	2	2
Bullera alba	1	--	--	--	--	1

yeast species in the different habitats. It was clear that *Cryptococcus albidus* and *Rhodotorula mucilaginosa* were the most dominant species among red yeasts of air, plant flowers and water, whereas *Cryptococcus albidus* and *Rhodotorula glutinis* were prevailed in cultivated soils. *Rhodotorula mucilaginosa* represented the dominant species in dairy products followed by *Cryptococcus albidus* and *Rhodotorula glutinis*. *Cryptococcus laurentii* was represented by a considerable number of strains. Other species were of low occurrence. Dominance of *Rhodotorula mucilaginosa* and *Cryptococcus albidus* in the air was previously reported by Di Manna (1955), Voros-Felkai (1966, 1967), Al-Doory (1967) and Haridy (1992), whereas their dominance in plant flowers was recorded by Phaff & Starmer (1987) and Haridy (unpublished results). Isolation of *Cryptococcus albidus, Rhodotorula mucilaginosa* and *Rhodotorula glutinis* from soil was reported by Capriotti (1958, 1963, 1967) and Monib *et al.* (1982), whereas their isolation from water was recorded by Ahearn (1973) and Hagler & Ahearn (1987). Occurrence of *Rhodotorula mucilaginosa* and *Rhodotorula glutinis* in raw milk and dairy products was reported by Haridy (1987, 1991).

Table 2 showed physiological and morphological characteristics of the isolated red yeast species. On the basis of 33 different properties, the isolated strains (227 isolates) were assigned to 6 species belonging to 4 genera. It was clear that *Cryptococcus albidus* and *Rhodotorula mucilaginosa* were the most dominant species among the red yeasts in El-Minia city followed by *Rhodotorula glutinis*. *Cryptococcus laurentii* was also represented by a considerable number of strains. Other species were of low occurrence.

Cryptococcus albidus and *Cryptococcus laurentii* were differentiated from each other by assimilation of melibiose and erythritol, growth at 37℃ and building of pseudomycelium and pellicle. Rodrigues (1984) differentiated these species on the basis of the characteristics above mentioned.

Assimilation of galactose, sorbose, ribose, arabinose, mannitol, galactitol and succinate represented the differential properties between *Rhodotorula mucilaginosa* and *Rhodotorula glutinis* (table 2). It was of interest that 40 red yeast strains belonging to *Rhodotorula glutinis* formed 2 different groups (I and II). Group I contained 15 strains which were isolated from soil. Strains of this group could assimilate rhamnose, cellobiose and arabinitol and grew at 37℃, whereas strains of group II showed negative results in these properties. These characters represented differences between these groups (table 2). Generally, these results showed the possibilities that either a soil biotop specific variety of *Rhodotorula glutinis* present or *Rhodotorula glutinis* represents a mixed taxa which could not be separated from each other by the present characteristics. The later possibility is relatively higher. Fell *et al.* (1984) reported that

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

Yeast species	Total number of isolates tested (227)	glucose fermentation	Assimilation																							Growth at				Building of				
			galactose	sorbose	ribose	xylose	arabinose	rhamnose	sucrose	maltose	trehalose	cellobiose	melibiose	lactose	raffinose	melezitose	erythritol	ribitol	arabinitol	mannitol	galactitol	inositol	gluconate	succinate	citrate	37℃	42℃	proteolytic activity	production of acids	ballistospores	basidiospores	true mycelium	pseudomycelium	pellicle
Cr. albidus	84	0	96[a]	64	77	100	100	73	100	96	73	96	0	100	100	100	0	64	64	100	41	50	96	100	64	0	0	0	0	0	0	0	0	0
Cr. laurentii	18	0	100	33	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	67	100	100	100	100	0	0	0	0	0	67	100	100
Rh. glutinis (GI)	15	0	100	100	100	100	100	100	100	100	100	100	0	0	100	100	0	100	100	100	100	0	100	100	67	100	0	0	0	0	0	0	0	0
Rh. glutinis (GII)	25	0	100	100	100	100	100	0	100	100	100	0	0	0	100	100	0	100	0	100	100	0	100	100	41	0	0	0	0	0	0	0	0	0
Rh. mucilaginosa	82	0	0	0	0	50	0	0	100	26	26	26	0	0	100	100	0	26	0	0	0	0	50	0	0	5	0	0	0	0	0	0	0	0
Sp. pararoseus	2	0	100	100	100	100	100	100	100	100	100	100	0	0	100	100	0	100	100	100	0	0	100	100	100	0	0	0	0	100	100	100	100	100
B. alba	1	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0	0	0	100	0	0	0	0

[a]=Percentage of positive reactions of the isolates *Cr.*=*Cryptococcus* *Rh.*=*Rhodotorula* *Sp.*=*Sporidiobolus* *B.*=*Bullera* GI=group I GII=group II

Rhodotorula glutinis is a complex of imperfect forms of at least 3 sexual species: *Rhodosporidium toruloides, Rhodosp. sphaerocarpum* and *Rhodosp. diobovatum*. Of those isolates of *Rhodotorula glutinis* that have been examined for G+C contents, there are two groupings : one with 60-61.2 mol % that includes imperfect forms of *Rhodosporidium toruloides*; the other group with a G+C of 66.8-67.8 mol % representing *Rhodosporidium diobovatum*.

Sporidiobolus pararoseus and *Bullera alba* were represented by one or two strains which did not enable us to throw light on their physiological behaviours and their systematic positions. Unexpected was the inability of the isolated yeast strains to hydrolyse proteins or to produce acids from glucose (table 2).

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