THE ISOLATION OF RED YEAST-LIKE FUNGI IN A DIAGNOSTIC LABORATORY

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RED yeast-like fungi, usually of the genus *Rhodotorula*, comprise about 2–6 per cent. of yeasts isolated in clinical specimens (Mackenzie, 1961; Stenderup and Pedersen, 1962). In routine hospital laboratories such isolates usually have not been identified beyond the genus level; nor has care always been taken to separate *Rhodotorula* spp. from similar species of *Cryptococcus* or *Sporobolomyces*. This practice has arisen partly because species of *Rhodotorula* rarely cause disease in man and partly because of the lack of general agreement as to how strains of *Rhodotorula* should be classified.

The increasing number of infections in our hospital due to micro-organisms of low virulence, as well as the known ability of *Rhodotorula* spp. to cause infection (Louria, Greenberg and Molander, 1960; Shelburne and Carey, 1962; Louria *et al.*, 1967; LEEBER and Sher, 1969) prompted our decision to attempt identification of all red yeast-like fungi isolated from clinical specimens. A recently published scheme for the identification of *Rhodotorula* and *Sporobolomyces* was used (Phaff, 1970; Phaff and Ahearn, 1970). This scheme appears in the new edition of an authoritative text and may perhaps achieve general agreement as a means of identification. The scheme is straightforward and easy to use; with its aid, all 58 red yeast-like fungi isolated during a 29-mth period of investigation could be identified. Our experience suggests that the scheme should also prove useful in other clinical laboratories.

MATERIALS AND METHODS

Clinical specimens. Over the period from May 1968 to the end of September 1970, all red yeast-like fungi from clinical specimens were identified. Techniques for liquefaction of sputum, selection of media and separation of yeast into genera have been described previously (Jennings, Bennett and Young, 1968). The only modification of approach was that inositol utilisation was substituted for starch production in separating *Cryptococcus* from *Rhodotorula* (Phaff and Spencer, 1969).

Speciation of the genus *Rhodotorula* was done according to the scheme of Phaff and Ahearn (1970). Substrate utilisation was detected by the use of auxanograms (Jennings *et al.*). Plates were read after 48 hr and again after 7 days' incubation at 28°C. Ability to grow in the absence of vitamins was tested in broth (Difco Vitamin-free Yeast Base). After incubation at 28°C for 1 wk, the broth was subcultured into the same medium and observed for another week. The single isolate of *S. salmonicolor* was identified by the criteria of Phaff (1970). Ballistospore production was assessed by the technique of Carmo-Sousa and Phaff (1962). Tests were also made for two characteristics common to the pathogen *C. neoformans*: viz., growth at 37°C and pathogenicity for mice (Jennings *et al.*).

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RESULTS

During the 29-mth period of study, 8062 routine specimens from patients were cultured for fungi; *Rhodotorula* spp. were isolated from 57 (0·71 per cent.) and *S. salmonicolor* from a single specimen. There was no difficulty in allotting any of the strains isolated to one or other of the species listed in the taxonomic scheme used. The commonest source of *Rhodotorula* spp. was faeces, 2·6 per cent. of samples containing these fungi. The identity and source of the isolates is given in the table. Although the organisms were generally considered

TABLE
Number, identity and source of members of the genus *Rhodotorula*
isolated from 8062 clinical specimens

Source	Number of specimens examined	Number (and percentage) yielding <i>Rhodotorula</i> sp.	Number of isolations of <i>R.</i>				
			<i>aurantiaca</i>	<i>glutinis</i> var. <i>glutinis</i>	<i>minuta</i> var. <i>texensis</i>	<i>rubra</i>	<i>pilimanae</i>
Urine	1727	13 (0·75)	1	1	3	8	0
Stool	1193	31 (2·6)	0	0	3	27	1
Blood and bone marrow	2111	2 (0·094)	1	0	0	1	0
“Respiratory”*	883	6 (0·68)	0	0	1	5	0
Miscellaneous†	2148	5 (0·23)	1	0	0	4	0

* Sputum, bronchial washing, saliva, tracheal aspirate.

† Skin, throat, wound, perineum, liver.

to be commensals, there were five patients in whom the possibility of infection was considered. In addition to the two isolations of *Rhodotorula* from blood, *S. salmonicolor* was isolated from a specimen of cerebrospinal fluid and *R. rubra* from a liver biopsy specimen from one patient and from a wound in another. In all five cases, the diagnosis of a mycosis was eventually discarded and no antifungal therapy was given.

The presence of these fungi on or in the human body raised the question whether or not such organisms can multiply at body temperature. Definite growth at 37°C on Sabouraud agar was noted with one isolate of *R. aurantiaca*, six of *R. minuta* var. *texensis* and 27 of *R. rubra*. Two of the 57 clinical isolates reacted positively in the test of neurotropism and pathogenicity generally used in the identification of *C. neoformans*. Although none of the 57 pairs of mice inoculated with the isolates died or appeared ill during the 2 wk after intravenous inoculation, one mouse each in two different pairs had positive brain cultures at the end of this period. Both mice had been inoculated with *R. rubra*.

DISCUSSION

The diversity of findings reported in published work in this field renders it difficult to say how our results compare with those of others. The total

frequency of red yeast-like fungi we observed was 0·7 per cent., compared with 1·6 per cent. in a very similar series of specimens (Mackenzie, 1961). A low frequency has been reported in stool (0·26 per cent.; Windisch and Staib, 1955) and in the normal mouth (0·9 per cent.; Seebacher *et al.*, 1971), whereas a much higher frequency has been noted in cultures from skin (Cramer and Koch, 1963; Sonck and Somersalo, 1963; Seebacher *et al.*) and bile (Staib, 1958).

Most of the red strains of yeast-like fungi isolated were *R. rubra*. This agrees with the majority of published accounts (Huxley and Hurd, 1956; Staib; Mackenzie; Seebacher *et al.*). Although such isolates were usually referred to in publications as *R. mucilaginosa*, this species is no longer considered distinct from *R. rubra* (Phaff and Ahearn, 1970). The occurrence of *R. aurantiaca*, *R. glutinis* and *R. minuta* in clinical specimens has been noted previously (Staib) though such isolations have been infrequent. The absence of previous reports of *R. pilimanae* in clinical specimens probably reflects the disordered state of the taxonomy of this genus and in particular the absence of this specific name in the taxonomic scheme of Lodder and Kreger-van Rij (1952). *S. salmonicolor* has rarely been isolated from man, the only instance known to us being a report of four isolations from normal skin (Huxley and Hurd). The reported occurrence of this species in air (Menna, 1954; Huxley and Hurd) and its isolation from air in our own institution suggests that its occurrence by chance in a clinical specimen may be expected.

SUMMARY

A recently published scheme of investigation was used to identify all isolates of red yeast-like fungi from clinical specimens over a 29-mth period. In 8062 specimens we found 45 strains of *Rhodotorula rubra*, seven of *R. minuta* var. *texensis*, three of *R. aurantiaca*, one of *R. glutinis* var. *glutinis*, one of *R. pilimanae* and one of *Sporobolomyces salmonicolor*. The commonest single source of these fungi was stool, of which 2·6 per cent. of specimens yielded *Rhodotorula* spp. None of the strains isolated appeared to be the cause of an infection. The routine identification of red yeast-like fungi could be made without difficulty, and techniques employed are recommended to other hospital laboratories wishing to provide this service.

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