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Rose Bengal-Malt Extract-Agar, a Simple Medium for the Simultaneous Isolation and Enumeration of Fungi and Actinomycetes from Soil

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Malt extract-agar for plate counts of fungi has become a successful standard medium (G. Müller, *Bodenbiologie*, Gustav Fischer Verlag, Jena, p. 230, 1965; C. H. Collins, *Microbiological Methods*, London, p. 121, 1967). Because bacteria and actinomycetes in soil are usually more numerous than fungi, these organisms used to be suppressed by acidifying the agar medium to *p*H 4 to 5, but this has been made unnecessary by the addition of rose bengal to suitable media (N. R. Smith and V. T. Dawson, Soil Sci. **58**:467, 1944). Counts of fungi were even higher in media containing rose bengal than in acidified media (J. P. Martin, Soil Sci. **69**:215, 1960).

Actinomycetes grow more slowly than do most bacteria and fungi, and hence they are likely to be masked in culture plates of ordinary media. Since the actinomycetes, as a group, are capable of growing in media containing low nitrogen, this property is used to prevent the development of the more rapidly spreading colonies of bacteria. Many different selective media for actinomycetes have been designed to reduce growth of fungi and bacteria, or to promote the development of actinomycetes (S. T. Williams and F. L. Davies, J. Gen. Microbiol. **38**:251, 1965).

In our studies on fungi and actinomycetes in periodically water-logged soils (gleys), a simple rose bengal-malt extract-agar was developed, suitable for the enumeration of both fungi and actinomycetes: commercial malt-extract (Bio-Malz), 20 g; K_2HPO_4, 0.5 g; Fe^{3+}, Mn, Cu, Zn, Mo, B, Co, 1 ppm each (added as soluble salts, not as nitrate); rose bengal, 1 part in 15,000; agar, 20 g; tap water, 1 liter; *p*H 6.0 to 6.2.

Fungi were incubated for 7 days, actinomycetes for 10 days (25 C). The addition of minor elements to the medium is recommended, since these minerals are known to promote the growth of actinomycetes (V. W. Cochrane, Ann. Rev. Microbiol. **15**:1, 1961). The incorporation of rose bengal in the medium not only suppressed the development of bacteria and reduced the spreading of molds, but, in addition, the compact colonies of actinomycetes appeared to be uniformly stained. Most of them were intense pink; a few were pink-orange. This feature clearly distinguished the actinomycetes from bacterial colonies, which either remained white or were slightly dyed. A great advantage of this medium is the ability to detect even small actinomycete colonies in the medium, owing to their characteristic round form and their uniform, intense pink color. In our studies on gleyed soils, fungal units were enumerated at a dilution of 10^{-4}, whereas the actinomycetes could be counted at a dilution of 10^{-5} (Table 1). Rose bengal-malt extract-agar was compared with a common 2% malt extract-agar for the quantitative determination of molds, and with three other well-known media for estimating the actinomycete population: Waksman's glucose-asparagine-agar (*see* G. Müller, *Bodenbiologie*, Jena, 1965, p. 229), von Plotho's glycerol-glycine-agar (O. von Plotho, Arch. Mikrobiol. **11**:33, 1940), and oatmeal-agar. The oatmeal-agar is prepared as follows: 20 g of oats is fermented for 36 hr (25 C) in 50 ml of tap water, autoclaved, and subsequently restored to a volume of 500 ml; 12 g of agar and a 500-ml solution, containing 0.05% K_2HPO_4, 0.02% $MgSO_4 \cdot 7H_2O$, 0.02% $FeCl_3$, 50 ml of soil extract, and 10 ml of Hoagland solution (H. Walter, *Grundlagen des Pflanzen-lebens*, Stuttgart, vol. 1, p. 211, 1962), are added; this solution is filtered through gauze; 10 g of $CaCO_3$ (per liter) is added; and the medium is sterilized by autoclaving.

The highest counts of actinomycetes were obtained on von Plotho's glycerol-glycine medium, closely followed by rose bengal-malt extract-agar (Table 1). However, on glycerol-glycine-agar, both actinomycetes and bacteria appeared as white colonies, and were thus difficult to distinguish. In contrast, only a few bacteria grew on rose bengal-malt extract-agar, allowing a rapid and easy enumeration or isolation of actinomycetes. The lowest number of colonies was registered on oatmeal-agar, and, in addition,

TABLE 1. *Plate counts of actinomycetes and fungi from profiles of Braunerde, gley, and pseudogley soil types*[a]

Soil type	2% Malt extract-agar	Rose bengal-malt extract-agar	Glycerol-glycine-agar	Glucose-asparigine	Oatmeal-agar
	Fungi		Actinomycetes		
Braunerde					
A_h	11.26	14.00	5.20	5.98	4.81	1.95
B_tA_1	2.88	3.30	1.32	0.72	1.32	0.72
G_0B_t	1.48	0.76	0.46	0.23	0.46	0.34
G_0	0.34	0.13	0.07	0.04	0.04	0.04
Gley					
A_h	11.50	11.50	4.60	3.68	4.14	3.68
A_hG_0	5.75	5.40	2.04	2.38	2.04	2.04
G_0	0.21	0.21	0.25	0.13	0.25	0.13
G_r	0	0	0.07	0.04	0.04	0.04
Pseudogley					
A_h	21.35	23.65	4.25	5.78	2.55	2.21
$S_{w(1)}$	1.93	2.17	0.78	1.30	1.04	0.52
$S_{w(2)}$	2.75	2.55	0.36	0.36	1.04	0.52
S_d	0.60	0.60	0.12	0.04	0.04	0.04
Total	60.05	64.27	19.52	20.68	17.69	12.31

[a] Samples are shaken for 1 hr with 0.18% sodium pyrophosphate as a soil-dispersing agent (H. Glathe and E. Ahrens, Landw. Forsch. 17:172, 1963). The counts shown for the actinomycetes should be multiplied by 10^6 and represent the numbers of organisms per gram of oven-dried soil. The counts for fungi should be multiplied by 10^4.

this medium was, complicated to make, and not well standardized. More fungi grew on rose bengal-malt extract-agar than on malt extract-agar.

Rose bengal-malt extract-agar may give an underestimate of the total numbers of actinomycetes in soil; however, when a comparative study of a large number of soils is required, this simple medium may be very convenient if both fungi and actinomycetes are the objects of study.

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