

This Week's Citation Classic

CC/NUMBER 20
MAY 19,1980

Martin J P. Use of acid, rose bengal, and streptomycin in the plate method for estimating soil fungi. *Soil Sci.* **69**:215-32, 1950. [Univ. California Citrus Experiment Station, Riverside, CA]

The use of a combination of rose bengal at a concentration of about 1-30,000 and streptomycin at 30 µg per ml in fungus plating media to prevent growth of bacteria and restrict size of colonies was found to be far superior to the older standard procedure of acidification. [The *SCI*[®] indicates that this paper has been cited over 220 times since 1961.]

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January 16, 1980

"During this century the agar plate method has probably been the most common method used for isolation and relative enumeration of fungi in soils and other natural materials containing organic residues. Because bacteria, including streptomycetes, are much more numerous than fungi in most of these materials it is necessary to add a substance to the plating medium which will prevent the growth of bacteria but still allow a maximum number of fungi to form colonies. Prior to about 1950 most fungal plating media were acidified to prevent bacterial growth. Inasmuch as some fungi are acid-sensitive and are fast-growing species that often cover the plates before slower-developing species form colonies, various individuals have looked for a better method. N.R. Smith and V.T. Dawson¹ in

1944 observed that the dye rose bengal inhibited the growth of most bacteria and reduced the size of many fungal colonies. Also, about this time several antibiotics became available. This provided an opportunity for various investigators to test new materials and combinations for bacterial growth-preventing effectiveness while maintaining near neutral pH of medium.

"My work involved the testing of various dyes, antibiotics, and other compounds at different concentrations and in different combinations to see if a compound or combinations could be found which would allow a great variety and number of fungi to form colonies on the plates. It was found that a combination of rose bengal at a concentration of about 1-15,000 to 1-30,000 and streptomycin at 30 µg per ml was the best of all tested. Compared to the standard acidification procedure, the number of fungal colonies was increased up to 100% and the kinds of fungi by 14%. Testing numerous fungal strains showed the primary cause of increased colony numbers was related to acid sensitivity of some dominant species, e.g., *Stysanus stemonites*, rarely isolated using acidified media, accounted for up to 35% of colonies developing on plates with rose bengal and streptomycin.

"The procedure was developed, or with some modifications was tried, by numerous microbiologists and most liked it. As a result, it was quickly adopted by microbiologists in all parts of the world; hence the original paper has been frequently cited."

1. **Smith N R & Dawson V T.** The bacteriostatic action of rose bengal in media used for the plate counts of soil fungi. *Soil Sci.* **58**:467-71. 1944.