A NEW MODIFIED CDA MEDIUM FOR SELECTIVE ISOLATION OF *RAMICHLORIDIUM PINI* AND SUPPRESSION OF *SCLEROPHOMA PYTHIOPHILA*

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ABSTRACT

Czapek Dox Agar modified by Oxoid (CDA) suppressed significantly the growth of *Sclerophoma pythiophila* (Cda.) Hohn compared to that on either of Malt Extract Agar, Potato Dextrose Agar or Oat Meal Agar. The effects of the individual ingredient of CDA on the growth of the fungi were also assessed. It was found that magnesium glycerophosphate of CDA was toxic to *S. pythiophila* but not to *Ramichloridium pini* de Hoog and Rahman. An increase of the dose of this salt from 0.5 to 4.0 g/l of CDA medium resulted a further significant suppression of *S. pythiophila* compared to the growth of the fungus on CDA and supported considerably higher growth of *R. pini* upto ten days. The new modified CDA medium was effective to act as a medium for selective isolation of *R. pini* and suppression *S. pythiophila.*

INTRODUCTION

Necessities of investigators with diverse interests in fungi have led to the formulation of new, and modifications of existing media to suit the specific need of the researchers during the past. Thus, 185 fungal culture media have been listed by Booth (1971). Tsao (1970) has reviewed a large number of selective media and stressed the increasing need for evolving more and more selective media. Most of the selective media so far evolved have dealt with the isolation of fungi from soil. Only a very small number of media deal with the isolation of fungi from infected plant parts.

Rahman (1982) reported *Sclerophoma pythiophila* (Cda.) Hohn to be the most common associates of *Ramichloridium pini* de Hoog and Rahman. During isolation

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of fungi from lodgepole pine shoots on a number of media, Czapek Dox Agar (Oxoid) suppressed *S. pythiophila* to some extent. *R. pini* is the causal pathogen for lodgepole pine shoot dieback in Scotland (Rahman 1982 ; de Hoog *et al.* 1983). *S. pythiophila* is quite ubiquitous on coniferous shoots and needles. A medium, on which this fungus may be suppressed while supporting normal growth of *R. pini,* is likely to be very useful to subsequent researchers. Hence, it was attempted to develop a medium for suppression of *S. pythiophila* and normal isolation of *R. pini.*

MATERIALS AND METHODS

The fungi used in this investigation were *S. pythiophila* (Herb. IMI No. 254034) and *R. pini* (CBS 761.82). Culture media used were Czapek Dox Agar modified by Oxoid (CDA), 2% Malt Extract Agar (MA), Potato Dextrose Agar (PDA) and Oat Meal Agar (OMA) as suggested by Booth (1971). CDA was found to suppress significantly the growth of *S. pythiophila* compared to the other media mentioned. CDA medium was then chosen for further investigation to assess if the reduced growth of *S. pythiophila* was due to toxicity or deficiency and whether it could be further manipulated. CDA medium included (a) ferrous sulphate 50 mg ; (b) sodium nitrate 2 g : (c) magnesium glycerophosphate 0.5 g ; (d) sucrose 30 g : (e) potassium sulphate 250 mg ; (f) potassium chloride 250 mg ; (g) Agar Oxoid (no 3) 12 g and (h) distilled water 100 ml. Hence, in the second step, by excluding ingredients a, b, c, d, e and f from CDA, media A, B, C, D, E and F were respectively prepared. Presence of magnesium glycerophosphate in CDA was associated with irregular and distorted growth of *S. pythiophila.* Therefore, in the third stage modified CDA media G, H, I, J, K and L were prepared which contained 0.2, 0.5, 1.0, 1.5, 2.0 and 4.0 g of magnesium glycerophosphate/1 respectively.

All the media were autoclaved at 1089 g/cm^2 (15 psi) for 20 minutes. The pH of the media was adjusted to 6.0 and approximately 20 ml medium was poured into standard 85 mm diameter petridish. Mycelial agar plug inocula of 7 mm diameter from one month old cultures of the fungi were used as standard inocula. A single inoculum was placed in the centre of a dry plate with the mycelial surface in direct contact with the medium. A standard replication of 5 plates was used. The plates were incubated at 18°C in dark and were observed after 5, 10 and 20 days. Linear growth of agar (Ryan *et al.* 1943 ; Fawcett 1925), was used. Two records of diameter growth were taken at right angles for each colony at every observation for regular colonies. Maximum and minimum diameters were measured in case of irregular growth to get the average.

RESULTS

The limits of the mean diameter growth of *S. pythiophila* and *R. pini* on the media used have been summarized in Table–1. It is obvious that CDA medium significantly suppressed the diameter growth of *S. pythiophila* compared to growth on either of MA, PDA, or CDA medium. The diameter growth of *S. pythiophila* in pure culture on MA medium varied from 2–3 times than that of *R. pini* (Table–1). This causes quick suppression of mycelial growth of *R. pini* from an infected tissue if that was colonised by *S. pythiophila.* The result was undesirably low recovery of *R. pini* unless the material was really in an early stage of colonisation (Rahman 1982).

Table 1. Diameter growth of *Sclerophoma pythiophila* and *Ramichloridium pini* on MA, PDA, OMA and CDA and new modified CDA media A to L after 5, 10 and 20 days.

Agar Media	Mean colony diameter in mm±t. se at P=0.05					
	S. pythiophila			*R. pini*		
	5	10	20	5	10	20
MA	24.9±0.2	44.4± 0.7	83.7± 0.8	10.8±0.3	20.8±0.3	29.5±0.5
PDA	20.2±0.4	45.1± 0.3	85.0± 0.1	10.0±0.1	19.8±0.2	28.6±0.9
OMA	21.5±0.2	42.0± 0.8	72.0± 2.0	10.1±0.2	20.2±0.1	23.0±0.9
CDA	7.0±0.0	17.2± 2.7	50.1±10.4	10.5±0.4	19.5±0.3	24.4±0.8
A	8.2±0.7	23.0± 8.7	74.0± 7.2	7.7±0.5	11.6±0.5	25.4±0.5
B	9.9±0.3	14.2± 5.2	43.9±12.3	7.4±0.5	10.6±0.5	18.0±0.6
C	10.8±1.0	19.3± 1.7	44.8± 9.6	8.1±0.4	11.1±0.5	19.9±0.5
D	13.8±0.3	22.2± 2.7	41.1± 2.7	8.1±0.2	10.3±0.7	19.6±0.5
E	8.8±1.4	13.9± 4.5	60.9± 3.7	7.4±0.4	11.4±0.7	25.6±0.6
F	9.4±0.5	17.4± 8.8	63.0±16.3	7.5±0.7	12.8±1.1	27.1±0.4
G	7.0±0	15.5±11.3	54.5± 9.8	12.4±0.5	15.0±0	29.7±0.8
H	12.4±5.1	25.7±10.6	73.8± 6.4	12.4±0.6	15.6±0.3	30.0±0.4
I	10.1±2.8	18.0± 4.6	74.6±14.4	11.6±0.5	14.6±0.5	28.2±0.3
J	10.1±6.2	23.1±15.3	68.9±19.0	10.5±0.4	13.3±0.6	26.0±2.1
K	7.1±0.3	11.7± 4.4	45.9±12.5	9.4±0.5	14.2±0.3	26.2±0.7
L	7.0±0.0	9.4± 2.3	40.0± 6.2	9.5±0.8	13.6±0.7	26.8±1.2

Examination of the diameter growth data of both the fungi on media A, B, C, D, E and F revealed that magnesium glycerophosphate in CDA was primarily responsible for the suppression of *S. pythiophila*, the mechanism of which has not been studied. Within 10 days irregular and largely variable colonies of *S. pythiophila* developed on all the media except C and D. This is represented by the fact that the standard deviations of the colony diameters were much more pronounced in case of the remaining media. Thus, because of higher standard deviation,

higher t. so values were obtained (Table 1). On medium D, the colonies of *S. pythiophila* were of very faint growth and that was expected as the carbon source (i. e., sucrose) was absent. On medium C, lacking in magnesium glycerophosphate, *S. pythiophila* produced profuse mycelial growth in uniform and less variable colonies up to 10 days. Subsequently, although variation increased, profuse mycelia production continued. This indicated that the fungus did not suffer from the deficiency of magnesium glycerophosphate. On the other hand, except in case of medium D, in all other media presence of this salt is associated with irregular and malformed colonies. This suggested that the salt was toxic to *S. pythiophila*.

To test the above findings, the fungi were grown on CDA and C, H, I, J, K and L media and it was found that diameter growth of *S. pythiophila* was significantly lower on medium 1. compared to CDA or any other medium up to 10 days. In a separate trial, mean dry weight of 10 days' old colonies of *S. pythiophila* on MA, CDA and medium L at 18°C was found to be 155, 55 and 16 mg respectively. This also showed that in total mycelia production by *S. pythiophila* medium L differed from CDA significantly. On the other hand, diameter growth of *R. pini* was not only quite comparable to that on MA, CDA and medium L, but on medium L diameter growth of *R. pini* was significantly higher than that of *S. pythiophila* (Medium L in Table-1). Thus, inocula infected by both *R. pini* and *S. pythiophila* when plated on this medium, the former will form a colony before the latter can initiate any growth. Hence, the new modified CDA, containing 4 g. in place of 0.5 g of magnesium glycerophosphate, is considered as a selective medium for suppression of *S. pythiophila* and isolation of *R. pini*.

DISCUSSION

Development of culture media for the selective isolation of specific fungi or any micro-organism is generally based on the principle of selective exclusion of undesired micro-organisms, permitting the preferential establishment of the desired fungi on the isolation medium (Tsao 1970). In the present study the same basic principle has been followed to suppress the growth of the undesired fast growing *S. pythiophila*.

It may be noted that although magnesium is generally used as magnesium sulphate, and phosphate as a salt of potassium, generally a concentration of 0.5 to 1.0 g/l of medium is of more common use in fungal culture media (Booth 1971). Crabill (1972) and Scheffer and Walker (1953) used a much higher concentration, i. e., 5.0 g of potassium hydrogen phosphate/litre of medium and 2.5 g of magnesium sulphate/litre of medium to suit their specific needs. The use of magnesium glycerophosphate could not be traced in any of the 185 fungal culture media listed by Booth (1971). The only use found was in Czapek Dox Agar (Oxoid) wherein 0.5 g/l magnesium glycerophosphate of medium was used. It is, therefore, considered that in the new modified CDA medium, the higher concentration of magnesium glycerophosphate is unlikely to matter, and future researchers on shoot dieback of lodgepole pine associated with *R. pini* will find the new modified CDA medium extremely useful.

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