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A COMPARATIVE STUDY OF THE NUTRITION OF *PHYTOPHTHORA MEADII* & *P. PALMIVORA*

By

O. S. PERIES, A. DE S. LIYANAGE AND D. M. DANTANARAYANA

SUMMARY

The growth of Phytophthora meadii *and* P. palmivora *was compared in defined media with different carbon and organic-N sources and vitamins. P. meadii grew much better than P. palmivora on maltose. Of the organic-N compounds tested, L-glycine, L(-) histidine, L (+) isoleucine, L (-) phenylalanine and peptone were better for P. meadii and alpha alanine for P. palmivora. No significant growth differences were recorded on any of the vitamins tested.*

These results are discussed in relation to those of other workers. It is suggested that only comparative studies with several isolates of each species are likely to be of value to differentiate related Phytophthora species.

INTRODUCTION

The morphology of the Phytophthoras is extremely variable (Caten, 1971); and this often makes it difficult to distinguish related species like *P. arecae* (Coleman) Pethybridge, *P. meadii* McRae and *P. palmivora* (Butl.) Butl. All *Phytophthora* isolates from *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell.-Arg. in Sri Lanka, referred to the Commonwealth Mycological Institute, U. K. (CMI), have been identified as *P. meadii.*

Savage *et al.* (1968) and more recently Chee (1974) have suggested that the identification of *P. meadii* as a species should await further studies and that, for the present, it should be grouped under *P. palmivora,* and Merz, Burrel & Gallegly (1969) concluded that both these species should be grouped under *P. citrophthora* (Smith & Smith) Leonian. However, Waterhouse (1974) maintains that *P. meadii* is a good species and should be retained, lest its special physiological characteristics, which may be important, are lost sight of. It is important to establish the identity of a pathogen clearly to perfect control measures against it (Sansome, Brasier & Griffin, 1975). Therefore, the Rubber Research Institute of Sri Lanka (RRISL) has started systematic studies on the physiology and variation of *Phytophthora* cultures isolated from *Hevea* in this country, to facilitate their identification and add to the knowledge on the tropical species of this genus.

Much work has been done on the taxonomy, cytology, host-parasite relationships and the epidemiology of various species of *Phytophthora,* particularly *P. infestans;* however, little information is available on their mineral nutrition (Wills, 1954; Fothergill & Child, 1964). The nutrition of *P. meadii* has not been studied before, and studies on *P. palmivora* have shown that its different isolates have diverse nutritional preferences (Cameron & Milbraith, 1965; Roncadori, 1965; Singh, 1975). This study attempted to define a synthetic medium for the growth of *P. meadii* to facilitate a systematic study of its mechanism of variation, and thus the identification of the Phytophthoras pathogenic to *Hevea.* The nutrition of *P. meadii* and *P. palmivora* was then compared to find out whether there were any differences of possible taxonomic value.

MATERIALS AND METHODS

Isolates

P. meadii was isolated from a 2-year old *Hevea* seedling and *P. palmivora* from a cocoa pod. The identification of both fungi was confirmed by the CMI. Stock cultures were maintained under mineral oil, on Difco Lima Bean Agar (LBA) slants in test tubes, and sub-cultured

regularly every 6 months. A single zoospore isolate of each fungus, prepared as described by Hall (1959), was used throughout these studies. These isolates were maintained on LBA slants and sub-cultured at frequent intervals.

Media

Preliminary studies showed that thiamin was essential for the growth of both fungi, so the following basal medium (BM) was used: D-glucose, 10g; asparagine, 2.0g; thiamin, 0.5mg; KH_2PO_4, 1g; $MgSO_4.7H_2O$, 0.5g; Fe, 0.4 mg; Zn, 0.32 mg; Mn, 0.04 mg; glass distilled water to make up 1l. Asparagine and the micronutrients were added as 4.5 ml and 9 ml, respectively, of stock solutions of the amides and the salts $FeSO_4.7H_2O$, $ZnSO_4.7H_2O$ and $MnSO_4.4H_2O$.

For studies on the utilization of carbon, glucose was replaced by other compounds so as to provide the same amount of total C as in 10g of glucose.

The ability to grow on various sources of organic nitrogen was determined by replacing asparagine with equivalent quantities of each of the selected amino acids, which provided the same amount of total N as in 2g of asparagine. For studies on vitamin requirements, glucose was dissolved separately and boiled for 10 min with 5g/l activated carbon. This solution was filtered thrice through Whatman No. 42 filter paper, and made up into the BM to which the vitamins were added at 0.5 mg/l.

The pH of all media was determined with a Beckman pH meter and adjusted to 6.0 with N NaOH or N HCl, before autoclaving for 20 min. at 17.5 lb pressure. The pH after sterilization and the final pH were always recorded.

Inoculum and growth

The inoculum was taken from 4-5 day old cultures grown at room temperature (RT; 28±2°C) on BM. The mycelial mat was filtered off, washed five times in sterile distilled water, resuspended in 20 ml sterile distilled water and macerated by shaking the suspension with glass beads for 5 min. One loopful of this suspension, taken in a standard platinum loop, was used as the inoculum in all trials.

All glassware was acid washed, before use. Erlenmeyer flasks (100 ml) containing 20 ml of medium, 5 replicate flasks per treatment, were used throughout. The cultures were incubated in the dark at RT for 14 days, after which the mycelium from each flask was filtered under suction on 9 cm Whatman No. 42 filter paper, which was previously dried at 100°C for 5 h and weighed. The mycelium was washed five times in 250 ml glass distilled water, dried overnight at 60°C and at 100°C for 4 h the following morning. The mycelial dry weights given are the means of three replicates, correct to the nearest mg.

RESULTS

Carbon Sources

Among the monosaccharides tested, none of the pentoses supported an appreciable growth of either fungus (Table 1). D-Glucose and D (+) mannose were the best hexoses for the growth of both species. D (-) fructose supported better growth of *P. palmivora* and D (+) galactose that of *P. meadii;* although in each case the mycelial dry weight of the better growing species was only about a third of that on D-glucose. The other hexoses tested were barely utilized by the two fungi. Of the disaccharides, sucrose was found to be as good a source of C as glucose for both fungi, lactose and trehalose were unsuitable for both and maltose was far superior for *P. meadii* (95 mg), than for *P. palmivora* (7 mg). This was the most significant difference between the two species observed in the studies on carbon utilization. The trisaccharides, raffinose, and the sugar alcohols, D (-) mannitol and D (-) sorbitol, were unsuitable for both species.

Nitrogen sources

Only traces of growth were made by both fungi on BM without asparagine (control) and on the amino acids: L (-) cystine, DL-norleucine, DL-tryptophane and taurine (Table 2). This growth too may have been made on the N provided with the inoculum. All other N sources tested supported some growth of at least one of the test fungi, with L(-) proline being the best for both. Table 2 shows that several amino acids and the two amides, DL-asparagine and L (+) glutamine, were suitable sources of N for both fungi, although there were differences in the amounts of growth made by the two fungi on a number of them. This shows that *P. meadii* and *P. palmivora* can utilize N from different organic compounds, irrespective of their structure. In this respect, they resemble *P. fragariae*, which grew well on straight and branch-chained N compounds, cyclic amino acids, hydroxy acids, acid amides and dicarboxylic acids (Davies, 1959). However, both species grew poorly in all three sulphur-containing amino acids tested: cystine, methionine and taurine.

L-Glycine, L (-) histidine, L(+) isoleucine, peptone and L(-) phenylalanine all supported better growth of *P. meadii* than *P. palmivora* alanine being the only amino acid on which this position was reversed significantly. These nutritional differences would appear to be good characteristics to distinguish between the species, but their significance will be discussed later.

Vitamin requirements

The results (Table 3) show that both fungi grew well in the presence of thiamin, but poorly when it was replaced with any of the other vitamins tested. Therefore, both fungi seem to be deficient only for thiamin and can apparently synthesise the other vitamins in its presence, in the BM. Of the vitamins studied here, thiamin could be replaced at least partially, only by nicotinic acid, another B complex vitamin.

DISCUSSION

McKeen (1956) reported that a substance toxic to *Phytophthora* spp. was formed on prolonged autoclaving of media containing sugars and amino acids. This has been confirmed by Hall (1959) and several others. However, this effect was not observed in the present studies, where all media were autoclaved for 20 min at 17.5 lb pressure, and produced mycelial dry weights comparable with those reported by others who took precautions to avoid the production of toxins. Therefore, it appears that, either the toxic substances are not always produced or, if produced, not effective in all cases. This should be tested in preliminary studies in each case; as there is no necessity to take elaborate precautions to eliminate a toxin or prevent its formation, if the fungus being studied will grow well in media autoclaved in the normal manner.

Calcium has been shown to be essential for the growth of a few micro-organisms (Steinberg, 1948). Davies (1959), following up Fleetwood Walker's (1955) studies, confirmed that Ca ions were essential as a micro-nutrient for the growth of *P. fragariae*. Apparently, significant levels of Ca are not essential for all Phytophthoras, as both *P. meadii* and *P. palmivora* grew well on a basal synthetic medium, which at best could have contained only traces of Ca, as impurities in the analytical grade chemicals.

There are several references to the importance of pH in growth studies on the Phytophthoras. Roncadori (1956) found that, in the utilization of inorganic N by several species of *Phytophthora*, the initial growth in non-buffered media was accompanied by a rapid fall of pH to 3.0—4.0, which inhibited further growth. Singh (1975) buffered all his media at 6.5, as a precautionary measure. Our media were not buffered, but the results in Tables 1, 2 and 3 show that the pH rarely fell below 4.0 (only thrice), and good growth of *P. palmivora* was recorded at this pH by Singh (1975). Therefore, the differences in growth observed by us can be attributed to the composition of the media.

Few reports are available on the nutrition of *P. palmivora* and none on *P. meadii*. The studies of Rao, Desai and Kulkarni (1966 a, b) on *P. palmivora* were made on media solidified with agar, so that their results are not comparable with these. Further, there are certain fundamental shortcomings in their studies. They supplied equal weights of amino acids rather than their N equivalents and the composition of the agar would have interfered with the precision of their experiments. Roncadori (1965) observed differences in the utilization of C and N compounds between three isolates of *P. palmivora* and Singh (1975) reported that the two isolates of *P. palmivora* he studied had different preferences for C and N sources and reacted differently to the presence of ferric iron in the medium. Similar observations have been made on other species *e.g. P. infestans* (French, 1953). The present studies confirm these observations as our isolate of *P. palmivora* differed from those studied earlier in its nutritional preferences for C and N compounds.

The best sources of C for *P. meadii* in this study were: sucrose, maltose, mannose and galactose, in that order; and for *P. palmivora:* glucose, sucrose, mannose and fructose. The most significant difference we observed between the two species, in this respect, was that maltose was far superior for *P. meadii* than for *P. palmivora*, although Weststeijn (1964) found that maltose was the best C source for the isolate of *P. palmivora* he studied. Further, Roncadori (1965) found that xylose was a good C source and Singh (1975) that fructose was the best source of C for one of their isolates of *P. palmivora;* but our results differ (see Table 1). Likewise, there are differences in detail regarding the utilization of N compounds between our studies and those of others.

All sulphur-containing amino acids tested in this study supported very little growth of both fungi. This confirms the observations made by Wills (1954) on *P. parasitica*. However, Singh (1975) found that cystein was a satisfactory source of N for growth of *P. palmivora* although methionine was not, and Fothergill & Child (1964) found that *P. infestans* grew well on cystein and methionine. These results once again confirm the variability of the Phytophthoras. The reason for the unsuitability of the sulphur-containing amino acids observed in more than one study is not immediately clear. Sulphur is generally not toxic to the Phytophthoras, but a substance toxic to them may be formed on autoclaving these amino acids or when they are utilized initially by the growing fungi, thus inhibiting their further growth.

The literature cited here shows that the nutritional requirements of the Phytophthoras are variable, and different isolates of the same species can have specialized requirements. Leonian (1934) observed that mutants are often not stable and soon revert to the characteristics of the parent type. Therefore, the isolates that have been studied are apparently stable ones, and their nutritional requirements too would be expected to be stable. Consequently, a number of isolates of each species should be studied before arriving at any conclusion regarding the nutritional requirements of a species. Factorial experiments like those of Fothergill & Child (1964) should be undertaken, supplemented if necessary with adequate statistical designs, ensuring that the minimum number of isolates of each species is tested in comparative studies. In the absence of such experiments, the mere observation that an isolate of one *Phytohthora* species differed considerably from that of another species in its nutritional requirements, is clearly of little taxonomic value.

Taking the above into consideration, on the basis of the present studies, it would be useful to make a detailed study, laid out on sound statistical principles, to check on the utilization of fructose and maltose as carbon compounds and alpha alanine, L-glycine, L (-) histidine, L (+) isoleucine, peptone and L (-) phenylalanine as N sources for several isolates of *P. meadii* and *P. palmivora*. Such a study will show whether the difference between these species observed here, can be used as a basis for distinguishing between them.

The vitamin requirements of the two species do not appear to be of diagnostic value; however, it would be interesting to find out whether these fungi can synthesise thiamin *de novo* or from its component moieties, in the presence of any one or a combination of other vitamins. Surprisingly, such a study does not appear to have been done on any species of *Phytophthora*.

In spite of intraspecific variations there may still be gross nutritional differences between species of *Phytophthora*, as shown by Liong & Lin (1965), who found that *P. palmivora* can utilize starch as a carbon source for growth, whereas its related species cannot. Therefore, it is important to carry out detailed studies on the nutrition of this genus, as it is possible that a nutritional pattern unique to each species does exist; and if this is clearly identified, recognition between related species, so difficult now, will be facilitated.

TABLE 1. The utilization of carbon compounds by *P. meadii* (*Pm*) and *P. palmivora* (*Pp*)

	Carbon source	*Initial pH**	*Final pH*		*Dry Wt. (mg)*	
			Pm	*Pp*	*Pm*	*Pp*
Monosaccharides						
Pentoses	L(+) Arabinose	5.8	5.8	6.1	4	3
	D(—) Arabinose	5.7	5.4	6.2	1	1
	D(—) Ribose	5.6	4.8	6.0	1	1
	D — Xylose	5.7	5.4	5.3	4	2
Hexoses	D — Glucose	5.6	6.2	5.5	93	101
	D(—) Fructose	5.6	5.0	5.8	4	33
	D(+) Galactose	5.6	5.9	6.4	30	17
	D(+) Mannose	5.7	6.2	5.5	76	51
	L(+) Rhamnose	5.8	7.5	7.4	4	1
	L(—) Sorbose	5.6	4.2	4.2	0	0
Disaccharides						
	Lactose	5.6	6.7	7.4	4	3
	Maltose	5.6	6.7	7.1	95	7
	Sucrose	5.8	6.1	5.3	97	93
	Trehalose	5.6	7.0	7.1	6	2
Trisaccharides						
	Raffinose	5.8	6.9	7.3	5	8
Sugar alcohols						
	D(—) Mannitol	5.8	7.1	6.8	5	3
	D(—) Sorbitol	5.8	6.5	6.7	2	3

*pH after sterilization.

TABLE 2. Dry weight of mycelium (mg) produced by two species of *Phytophthora* on an artificial medium in the presence of various sources of nitrogen

Nitrogen source	*Initial pH**	*Final pH*		*Dry wt.*	
		Pm	*Pp*	*Pm*	*Pp*
Control (no N)	3.9	5.8	5.7	1	1
DL — Asparagine	5.6	6.2	6.4	89	90
D(+) Asparagine	5.7	5.6	4.9	32	27
DL — Aspartic acid	5.7	7.6	6.6	79	85
D(—) Aspartic acid	5.8	6.8	5.9	62	74
L(+) Aspartic acid	5.8	7.9	7.3	73	92
DL— Alanine	5.7	6.4	5.2	100	79
alpha— Alanine	5.7	5.4	4.8	22	50
L(+) Alanine	5.7	7.4	6.3	83	90
L(—) Arginine	5.8	4.1	4.4	77	95
L(+) Citrulline	6.0	5.6	4.8	51	43
L(—) Cystine	4.3	4.4	4.4	9	8
L(+) Glutamine	5.8	3.3	3.9	47	48
L(+) Glutamic acid	5.9	7.8	7.4	83	91
L— Glycine	5.8	7.5	4.8	81	34
Glycine anhydride	5.9	5.7	5.5	15	14
L(—) Histidine	6.0	5.7	5.3	85	15
L(+) Isoleucine	5.8	4.6	4.6	25	7
L(—) Leucine	5.9	4.4	4.6	17	12
Lycine monohydrochloride	5.7	4.6	4.8	18	9
L(—) Methionine	5.9	4.0	4.1	16	15
DL — Norleucine	5.8	4.4	4.6	7	3
DL — Norvaline	5.8	4.6	4.4	27	29
Peptone	5.8	4.6	4.9	78	23
L(—) Proline	5.9	7.2	6.8	111	96
DL — Phenylalanine	5.8	4.5	4.6	34	29
L(—) Phenylalanine	5.8	4.7	4.3	60	5
DL — Serine	5.8	5.7	5.2	83	76
L(—) Serine	5.8	7.6	7.7	89	72
Taurine	5.8	5.5	5.5	6	2
DL — Threonine	5.8	4.0	4.7	36	48
DL — Tryptophane	5.8	5.8	5.5	6	1
L(—) Tyrosine	5.9	5.0	4.7	42	51
L(+) Valine	5.8	4.6	4.3	33	24

*pH after sterilization.

TABLE 3. Growth of *P. meadii* and *P. palmivora* in the presence of different vitamins

Vitamin	*Initial pH**	*Final pH*		*Dry wt.*	
		Pm	*Pp*	*Pm*	*Pp*
Control	5.9	4.2	4.1	1	5
L — Ascorbic acid Vit C	5.8	4.3	4.3	10	11
Biotin Vit H	5.9	4.3	4.0	13	19
Calciferol Vit D_2	5.9	4.3	4.0	11	15
Cyanocobalamin Vit B_{12}	5.9	4.3	3.9	9	11
Folic acid Vit B_{12}	5.9	4.2	4.1	11	16
Meso-inositol	5.8	4.3	4.3	13	13
Nicotinic acid Vit B	5.9	4.2	4.2	57	31
Palmitate Vit A	5.9	4.0	4.0	18	14
Pantothenic acid	5.9	4.3	4.2	17	20
Para Aminobenzoic acid	5.8	5.7	4.4	11	13
Pyrimidin	5.9	4.3	4.3	9	12
Riboflavin Vit B_2	5.9	4.0	4.0	9	11
Thiamin Vit B	5.9	6.3	5.2	81	82
Thiazol	5.9	4.2	4.2	8	11

*pH after sterilization.

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