Nutrient Requirements for Production of Pycnidia of *Botryodiplodia theobromae* Pat.

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INTRODUCTION

Difficulty has been encountered in obtaining sporulation of *Botryodiplodia theobromae* in culture (Olofinboba, 1967; Ogundana, 1968). Recently Ekundayo & Haskins (1969) and Alasoadura (1970) demonstrated that the fungus required illumination to produce pycnidia. Moreover, the intensity of its sporulation was influenced by the composition of the culture medium, an aspect considered in more detail below.

METHODS

An isolate of *Botryodiplodia theobromae* Pat. from yam (*Dioscorea rotundata*) was used.

The basal medium consisted of (g/l): sucrose, 30; K_2HPO_4 , 1.0; $NaNO_3$, 3.0; $MgSO_4 \cdot 7H_2O$, 0.5/l. 'Analar' grade chemicals were used. Cultures were grown in 250 ml Erlenmeyer flasks, each containing 25 ml medium and incubated at 30 °C in chambers with 'day-light' fluorescent tubes giving a light intensity of 2153 lux at the culture surface (Ekundayo & Haskins, 1969). The production of pycnidiospores on the various media was assessed after 7 days by removing pycnidia with an inoculating needle and breaking these open in 10 ml distilled water. The pycnidiospore suspension was filtered through three layers of muslin to remove mycelial fragments and the number of spores estimated from counts in a haemocytometer.

RESULTS AND DISCUSSION

The effects of various combinations of the nutrients in the basal medium on pycnidiospore production are indicated in Table 1. Growth was sparse on sucrose alone and no pycnidia were produced. The provision of $NaNO_3$ in addition to sucrose resulted in the production of small pycnidia (90-120 μm in diam.), which showed that a utilizable carbon source and NO_3^- radical were needed for pycnidium formation. The provision of Na_2SO_4 or of $MgSO_4 \cdot 7H_2O$ in addition to sucrose + $NaNO_3$ did not enhance pycnidium production. The addition of K_2HPO_4 to a medium containing sucrose + $NaNO_3$, resulted in production of large globose pycnidia (190 to 230 μm in diam.) similar to those which were produced on potato dextrose agar. This indicated that the K^+ or HPO_4^{2-} radicals or both were needed, in addition to a carbon source and NO_3^- ion, for the formation of full-size pycnidia. Pycnidiospores from small and large pycnidia were the same in size (32.0 to 35.5 $\times$ 13.0 to 15.0 μm) but the large pycnidia contained more.

When K_2HPO_4 was replaced by Na_2HPO_4 as a constituent of the basal medium profuse mycelia growth occurred but no pycnidia were produced. This showed that K^+ was essential for pycnidium production, and that Na^+ did not replace K^+ .

The effect of varying the concentration of K^+ , supplied as K_2HPO_4 , was investigated. In these experiments no compensation was made for variations in HPO_4^{2-} concentrations.

Table 1. *Effect of various combinations of the components of the basal medium on sporulation of Botryodiplodia theobromae, at 30 °C.*

Medium	Size of pycnidia	$10^{-6} \times$ spore density/culture	Average spore size (μm)
Sucrose	(None)	0	—
Sucrose NaNO_3	Tiny*	19.8	34×14
Sucrose NaCl	(None)	0	—
Sucrose $\text{NaNO}_3 + \text{Na}_2\text{SO}_4$	Tiny	28.3	33×14
Sucrose $\text{NaNO}_3 + \text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	Tiny	27.8	34×15
Sucrose $\text{NaNO}_3 + \text{MgSO}_4 \cdot 7\text{H}_2\text{O} + \text{K}_3\text{HPO}_4$	Large†	261.3	34×14
Sucrose $\text{NaNO}_3 + \text{MgSO}_4 \cdot 7\text{H}_2\text{O} + \text{Na}_2\text{HPO}_4$	(None)	0	—
Sucrose $\text{NaNO}_3 + \text{K}_2\text{HPO}_4$	Large	258.2	34×14
Sucrose $\text{NaNO}_3 + \text{MgSO}_4 \cdot 7\text{H}_2\text{O} + \text{KCl}$	Large	328.5	34×14

* Tiny pycnidia were 90 to 120 μm in diam.

† Large pycnidia were 190 to 230 μm in diam.

Increasing K^+ in the sequence 5, 10, 25, 50, 125 mg/l resulted in the following, corresponding increases in pycnidiospore production (as $10^{-6} \times$ spore density/culture): 13.0, 52.8, 67.5, 206.1, 373.0. At 250 and 375 mg K^+ /l there was a decline in spore density to 225 and 92 respectively and at higher levels of K^+ (500, 750 and 1000 mg/l) no pycnidia were produced. The mean spore size at all K^+ levels from 5 to 375 mg/l was $34 \times 15 \mu\text{m}$.

In another experiment K^+ was supplied in KCl ; corresponding figures for concentrations over the range 5 to 125 mg/l were 11.3, 42.5, 55.0, 182.5 and 280.0 respectively; they rose to 365.0 at 250 mg K^+ /l, then declined to 145, 15 and 2.5 at 375, 500 and 750 mg K^+ /l respectively, and at 1000 mg K^+ /l no pycnidia were produced. Since there was no source of phosphate in this experiment, the slightly lower response to K^+ concentration might either have been the result of phosphate deficiency in the medium or due to pH, which would have tended to be higher with K_2HPO_4 .

REFERENCES

- ALASOADURA, S. O. (1971). Culture studies on *Botryodiplodia theobromae* Pat. *Mycopathologia et mycologia applicata* **42**, 153–160.
- EKUNDAYO, J. A. & HASKINS, R. H. (1969). Pycnidium production by *Botryodiplodia theobromae* I. The relation of light to the induction of pycnidia. *Canadian Journal of Botany* **47**, 1153–1156.
- OGUNDANA, S. K. (1968). *Physiological studies on soft rot of yams in storage*. M.Sc. Thesis, University of Lagos, Nigeria.
- OLOFINBOBA, M. O. (1967). *Botryodiplodia blue-stain of wood*. Ph.D. Thesis, University of Ibadan, Nigeria.