PHYSIOLOGICAL PARAMETERS OF GROWTH IN SAPROLEGNIA PARASITICA COKER¹

by

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ABSTRACT

Persistent bacteria were separated from S. parasitica by means of the oligodynamic effect of a silver ring in a modification of Raper's technique.

Inoculation of fungal cultures was by means of mycelial macerate. Growth was measured by mycelial dry weight.

A chemically defined medium (standard medium) was developed which consisted of a mineral base (chlorides of magnesium, manganese, zinc, calcium, and iron) chelated with EDTA, supplemented with glucose, sodium glutamate, and methionine, and buffered at pH 7.0 with 0.01 M. KH₂PO₄.

Shaking culture methods supported increased growth rates and higher dry weight yields compared to stationary methods.

Excellent growth occurred between 15 to 30°C, in the standard medium and between pH 4.0 and 8.0 in the standard medium plus 0.01 M. sodium succinate and 0.01 M. TRIS used as additional buffers.

Significant phosphate toxicity was demonstrated at concentrations exceeding 0.05 M. Sodium succinate and TRIS, used as buffers at 0.01 M. each, were compatible with S. parasitica, whereas boric acid, sodium barbital, and sodium citrate inhibited growth under similar conditions.

Substitution of other carbon sources for glucose in the standard medium (on an equal carbon basis where possible) indicated that cellobiose, dextrin, fructose, glycercine, glycon, sodium lactate, and soluble starch supported significantly heavier growth than did the standard medium minus glucose; glycon had a greater yield than did the standard medium minus glucose; glycon had a greater yield than the standard medium. Arabinose, dulcitol, galactose, inulin, lactose, mannitol, mannone, raffinose, rhamnose, sorbitol, sucrose, and xylose neither stimulated nor inhibited growth; however, growth inhibition was produced by α-ketoglutaric acid, sodium citrate, and sodium succinate.

When fatty acids and lipids were substituted for glucose (on an equal carbon basis where possible), only butter, lard, oleo, and palmitic acid supported heavier growth of S. parasitica than the standard medium minus glucose. Stearic acid neither stimulated nor inhibited growth; acetic acid, butyric acid, formic acid, octanoic acid, and propionic acid significantly inhibited the growth of the fungus.

Various nitrogen sources were substituted for sodium glutamate in the standard medium (on an equal nitrogen basis where possible). Casein hydrolysate and gelatin produced yields higher than that developed in the standard medium; other nitrogen

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sources produced lesser yields but still greater than those from the standard medium minus sodium glutamate:

- Alanine, arginine, aspartic acid, and histidine (good nitrogen sources).
- Ammonium chloride, cysteine, leucine, serine, and urea (fair nitrogen sources).
- Glycine, isoleucine, lysine, methionine, phenylalanine, potassium nitrate, sodium nitrate, threonine, tryptophan, and valine (poor nitrogen sources).

When various sulfur sources were substituted for methionine in the standard medium (on an equal sulfur basis), only cysteine and cystine produced dry weights comparable to that which developed in the standard medium. The following were very poor sulfur sources yet supported more growth than did the standard medium minus methionine: sodium sulfide, sodium thiosulfate, and thiourea. The ability of the other sulfur sources to support growth was questionable: potassium persulfate, sodium bisulfite, sodium dithionate, sodium hydrosulfite, sodium sulfate, sodium sulfite, and sodium thiocyanate.

The standard medium contained only two nitrogen sources: sodium glutamate and methionine. Sodium glutamate served as a carbon source as well as a nitrogen source, but methionine could serve only as a source of sulfur.

INTRODUCTION

Except for the work of Kanouse (1932), Volkonsky (1933 a.), Leonian & Lilly (1938), and Lee (1962), the physiology of Saprolegnia parasitica Coker has virtually been neglected. Furthermore, studies by Scott & O'Bier (1962) concerning aquatic fungi associated with diseased fish and fish eggs have indicated the need for further investigations concerning physiological aspects of growth and reproduction in S. parasitica and related fungi. In view of the frequent occurrence of this species as a facultative parasite of fish (Scott & O'Bier, 1962) and the meager knowledge available concerning its physiology, S. parasitica was chosen as the subject of the present investigation.

Before attempting a critical study of saprolegniaceous fungi, it is necessary that they be grown in pure culture under standardized conditions. Scott & O'Bier (1962), like most students of the water molds, depended chiefly upon the use of hempseeds (Cannabis sativa) for the isolation and maintenance of these fungi in water culture. However, Scott, Powell & Seymour (1963) have indicated the desirability of improving the reproducibility of the medium by using a chemically defined substrate as a substitute for hempseed in the culture of Saprolegnia spp. for taxonomic and physiological studies.

In the present investigation, a more suitable defined medium was developed from that suggested by Scott et al. (1963) and Reischer (1951a.). This medium was advantageous for a physiological study of S. parasitica since it is reproducible, autoclavable, and stable in the presence of a variety of nutrients throughout a wide range of temperature and pH.

The objective of this study, therefore, was to grow pure cultures of S. parasitica in chemically defined media under controlled environmental conditions in order to observe the effect that changes in temperature, pH, and nutrients have on growth in this fungus.