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Presence of Polyribosomes in Conidiospores of *Botryodiplodia theobromae* Harvested with Nonaqueous Solvents[1]

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Polyribosomes detected in extracts of spores harvested with water also were found in extracts prepared from spores harvested with nonaqueous fluids.

In an earlier report (2) we presented evidence that ungerminated conidiospores of the fungus *Botryodiplodia theobromae* contain polyribosomes, and (along with other lines of evidence) we concluded that these spores contain a latent messenger ribonucleic acid which is translated immediately upon the onset of germination in the absence of any detectable ribonucleic acid synthesis. Similar conclusions, based on related experimental approaches, have been reached by other investigators who examined spores of other fungi (1, 3-9). However, Lovett (personal communication; *In* D. J. Weber and W. M. Hess; ed., *The Fungal Spore: Form and Function*, in press) has pointed out that in most instances in which polyribosomes have been detected in ungerminated fungal spore extracts, the spores have been suspended in water at various steps in the spore harvesting procedure before ribosome extraction. In *B. theobromae*, for example, use of water as a harvesting medium is required for separation of spores from the parent pycnidia and mycelium. Lovett's criticism seems particularly justified in view of the high rates of cytoplasmic ribosome protein synthesis and cyanide-sensitive oxygen uptake which occur in *B. theobromae* spores incubated in water under nongerminating conditions (R. Brambl, manuscript in preparation). Furthermore, Mirkes (7) recently has shown that ungerminated *Neurospora crassa* conidia harvested in water appear to contain about 10 times more extractable polyribosomes than those nonhydrated conidia harvested by dry aspiration from the mycelium. The purpose of this present study was to establish whether polyribosomes could be extracted from *B. theobromae* spores that were harvested with nonaqueous fluids.

The methods of extraction and analysis of the spore ribosome fraction have been described in detail elsewhere (2); variations in centrifugation conditions are given in the caption of Fig. 1. The

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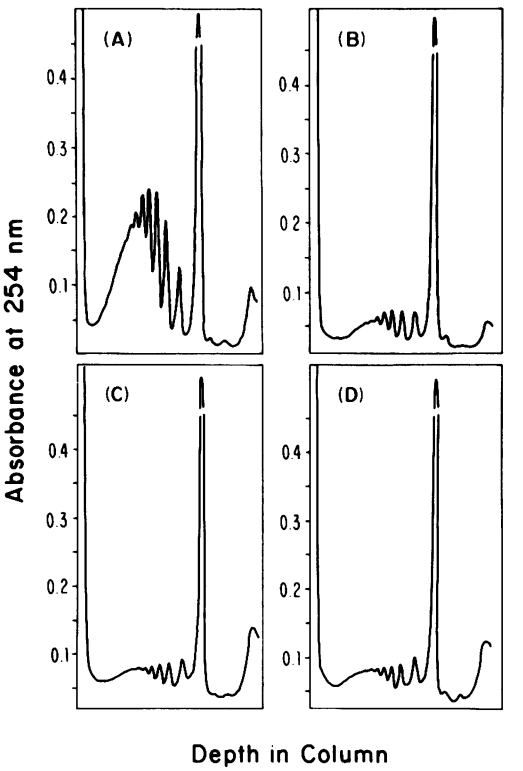

FIG. 1. *Sedimentation patterns of semipurified ribosomes obtained from germinated spores (A), or ungerminated spores harvested in (B) water, (C) FC-43, and (D) Soltrol 170. Direction of sedimentation is from right to left; the increase in absorbance at the bottom of the gradient is due to the potassium acid phthalate marker in the chase sucrose solution. The 105,000 × g ribosome fraction was obtained by centrifuging the ribosomes through a layer of 1.5 M sucrose. The sucrose density gradient columns (containing 125, 250, 375, and 500 g of sucrose/liter; 6.5, 10.0, 11.5, and 6.0 ml/layer, respectively) were centrifuged in a Beckman SW-27 rotor at 25,000 rpm for 210 min and then fractionated automatically, as described previously (2). Ribosome samples on each gradient column were equivalent to 200 μg of ribosomal ribonucleic acid.*

spore harvest water was replaced with either an inert fluorocarbon fluid, FC-43, obtained from the 3M Co., or a paraffinic oil, Soltrol 170, obtained from Phillips Petroleum Co. Neither fluid caused apparent damage to the spores; they germinated normally after harvest when suspended in a nutritionally complete incubation medium. The spores harvested in FC-43 or Soltrol 170 were exposed to the extraction buffer a maximum of 30 s before a 45-s mechanical disruption.

In Fig. 1A is shown the density gradient centrifugation pattern of the ribosomal fraction obtained from germinated spores, and Fig. 1B shows the pattern of the ribosome fraction from the water-harvested ungerminated spores with a characteristically low proportion of polyribosomes to the 81*S* monoribosomes. Most importantly, Fig. 1C and D show that the spores harvested in the nonaqueous fluids, FC-43 or Soltrol 170, do contain polyribosomes; these patterns are very similar to that obtained from the water-harvested spores. If the spores were first harvested in either FC-43 or Soltrol 170 and then left in water at 4 C for several periods up to 16 h, the subsequently extracted ribosome fractions were identical in composition to that of spores extracted directly after harvest with nonaqueous fluids.

It is clear, therefore, that polyribosomes are present in ungerminated *B. theobromae* spores which had not been harvested with water; the presence of these polyribosomes appears not to be a consequence of spore hydration.

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