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POLYAMINE DISTRIBUTION AND *S*-ADENOSYL METHIONINE DECARBOXYLASE ACTIVITY IN FILAMENTOUS FUNGI

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1. Introduction

The amines, putrescine, spermidine and spermine occur in a wide variety of organisms [1–3]. Although their cellular function is not known, they are invariably synthesized rapidly at the onset of growth when their intracellular concentrations are raised. It has been widely suggested that their synthesis is connected with nucleic acid synthesis and they are known to bind strongly to nucleic acids. The concentrations of the three amines vary widely from one organism and tissue to another. It has been generally thought that spermine was present in eukaryotes but absent from prokaryotes [see 4]. Recently Nickerson et al. [5] have measured the polyamine concentrations in a variety of fungi and their results suggested that spermine was present in yeasts but absent from filamentous fungi. However results from other laboratories have shown that spermine is present in *Aspergillus nidulans* [6,7] and *Neurospora crassa* [8,9]. Pösö et al. [10] have suggested, on the basis of measurements made in a variety of prokaryotes and eukaryotes that the absence of spermine, or its presence in very low concentrations in an organism may be related to the ability of putrescine to stimulate *S*-adenosyl methionine decarboxylase (EC 4.1.1.50), an enzyme participating in the biosynthesis of spermine. Those organisms having a putrescine sensitive *S*-adenosyl methionine decarboxylase contained spermine whereas those having putrescine insensitive *S*-adenosyl methionine decarboxylase lacked spermine.

We have studied the polyamine distribution and *S*-adenosyl methionine decarboxylase activities in a variety of filamentous fungi. We found that some filamentous fungi do contain spermine and are able to incorporate radioactive putrescine into spermine and that all the fungi we examined have *S*-adenosyl methionine decarboxylase activities which are sensitive to putrescine. Thus it appears that there is a correlation between the ability of an organism to synthesize spermine and the sensitivity of its *S*-adenosyl methionine decarboxylase to stimulation by putrescine.

2. Materials and Methods

Mucor hiemalis, Trichoderma viride and *Penicillium* sp. were obtained from Dr. N. Dix (Stirling University Biology Department), *Aspergillus nidulans* BWB 272 is from the Glasgow strain. Other organisms were obtained from the Mycological Institute, Kew, Surrey, England. They were routinely maintained on malt extract agar (Oxoid) containing 5% glucose and were subsequently grown in submerged culture for 56 56 h at 37°C in Vogel's medium [11] supplemented with vitamins at the following concentrations (mg/l): biotin, 0.023; cyanocobalamin, 0.005; folic acid, 0.12; riboflavin, 0.4; thiamin, 0.53; choline, 30.0; nicotinic acid, 3.7; and *p*-aminobenzoate, 0.13. [1,4-^{14}C]Putrescine dihydrochloride (50 mCi/mmol) and *S*-adenosyl-L-[carboxy-^{14}C]methionine (45 mCi/mmol) were purchased from the Radiochemical Centre, Amersham, Bucks., U.K. For polyamine measurements and incorporation studies the harvested mycelia were sonicated and extracted three times in cold 3% perchloric acid. After separation on Dowex 50 columns the polyamines were separated by paper electrophoresis [12]. The identity of the amines was

confirmed by TLC separation of their dansyl derivatives [13]. To measure [^{14}C]putrescine incorporation into spermidine and spermine 1.0 μCi [^{14}C]putrescine was added to 250 ml submerged culture at the time of inoculation. *S*-Adenosyl methionine decarboxylase assays were carried out by trapping $^{14}CO_2$ released after incubation of cell extracts with 0.025 μCi *S*-adenosyl-L-[1-^{14}C]methionine (0.42 μCi/μmol) for 1 h as described previously [14]. DNA was estimated by the diphenylamine reaction [15] and protein by the method of Lowry et al. [16] using bovine serum albumin as standard.

3. Results and Discussion

It can be seen from Table 1 that all the filamentous fungi investigated contain putrescine, spermidine and spermine but that the proportions of each of these amines vary not only between classes but also between species in the same genus. In each fungus spermidine is the predominant amine. The amount of spermine detectable in some species e.g. *N. crassa* is small but nevertheless detectable. This is in agreement with previous measurements for *N. crassa* reported by Viotti et al. [8] and Bowman and Davis [9] but appears to be at variance with Nickerson et al. [5]. The proportions of the three polyamines are known to vary considerably at different stages of growth in many organisms [4]. It is therefore possible that the small amounts of spermine may become difficult to detect at certain stages of growth. As the organisms used in this study were routinely maintained on malt agar which could contain traces of spermine measurement of the incorporation [^{14}C]-putrescine into spermidine and spermine were made to ascertain that the organisms were capable of de novo synthesis. It can be seen (Table 1) that the [^{14}C]putrescine becomes incorporated mainly in spermidine but also into spermine. Thus all the species investigated are able to synthesize spermidine and spermine from putrescine.

Since it has been previously suggested that the presence of spermine in an organism may be correlated with the presence of putrescine activated *S*-adenosyl methionine decarboxylase [10], we tested extracts from each fungus for putrescine activation of *S*-adenosyl methionine decarboxylase. The extracts

TABLE 1

Survey of polyamine content, [^{14}C]putrescine incorporation and *S*-adenosyl methionine decarboxylase in filamentous fungi
Methods were as described in Materials and Methods.

Organism	Polyamine content and [^{14}C]-putrescine incorporation nmoles μg DNA^{-1}; dpm μg DNA^{-1}			*S*-adenosyl methionine decarboxylase activity nmoles CO_2 h^{-1} mg protein^{-1}		
	Putrescine	Spermidine	Spermine	No additions	Plus putrescine [a]	Ratio $\frac{(+\text{putrescine})}{(-\text{putrescine})}$
Mucor hiemalis	6.9; 0.24	74; 2.86	6.3; 0.48	0.08	10.9	136
Rhizopus stolonifer	6.9; 0.07	42; 3.06	10.8; 1.09	0.45	19.9	44
Neurospora crassa	3.0; 0.05	44; 2.09	2.9; 0.06	0.16	56.5	353
Trichoderma viride	3.5; 0.07	31; 2.44	9.7; 1.08	0.70	13.2	19
Aspergillus nidulans	10.0; 0.29	21; 2.56	6.2; 0.77	0.04	6.75	169
A. niger	7.4; 0.07	38; 3.03	10.2; 0.93	1.86	8.40	4.5
A. oryzae	7.4; 0.10	61; 3.07	15.0; 1.10	0.22	12.1	55
Penicillium expansum	5.2; 0.20	17; 0.67	7.3; 0.53	0.11	4.75	43
P. fumiculosum	8.3; 0.16	23; 0.49	4.5; 0.23	0.75	2.62	3.5
P. nigricans	8.0; 0.36	46; 1.76	10.0; 0.12	0.09	10.5	117
Alternaria alternata	2.2; 0.09	51; 1.64	4.1; 0.20	0.05	15.6	312

[a] The putrescine concentration for the enzyme assays was 2 mM except for *Aspergillus oryzae, Penicillium nigricans* and *Rhizopus stolonifer* which were 1 mM putrescine.

used were obtained from disrupted cell supernatants after ammonium sulphate precipitation followed by extensive dialysis [14] to remove endogenous putrescine. All *S*-adenosyl methionine decarboxylases measured (Table 1) were strongly activated by the addition of 1–2 mM putrescine to the assay. The extent of activation varies between species and we have also found that in some cases there is inhibition at higher putrescine concentrations. Since it is possible that the increased activity of *S*-adenosyl methionine decarboxylase measured in the presence of putrescine might be accounted for by the removal of the product, decarboxylated *S*-adenosyl methionine by spermidine synthetase [17] we tested the effect of adding decarboxylated *S*-adenosyl methionine to the assay mixture. Although this caused some reduction in activity its removal by spermidine synthetase could not account for the very large stimulation of *S*-adenosyl methionine decarboxylase observed.

We therefore conclude that all the filamentous fungi examined here both synthesize spermine and possess putrescine activated *S*-adenosyl methionine decarboxylases; they are thus similar to yeasts in these respects. The data are also consistent with the suggestion of Pösö et al. [17] that in general organisms capable of spermine synthesis possess putrescine activated *S*-adenosyl methionine decarboxylases.

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