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Mutants of *Aspergillus nidulans* unable to use choline-0-sulphate

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SUMMARY

Mutants of *Aspergillus nidulans* unable to use either the choline moiety or the sulphate moiety of exogenous choline-0-sulphate have been selected. Choline-0-sulphate non-utilizing (*csu*) mutations have no other apparent pleiotropic effects, but it has not yet been established whether they lead to loss of choline sulphatase (and thus of the ability to utilize endogenously produced choline-0-sulphate) or to loss of a specific transport system for choline-0-sulphate or to loss of both.

1. INTRODUCTION

Although choline-0-sulphate occurs widely throughout the plant kingdom and is extensively stored in a number of higher fungi including various *Aspergillus* species (De Flines, 1955; Harada & Spencer, 1960; Itahashi, 1961; Lindberg, 1955*a*, *b*; Nissen & Benson, 1961; Spencer & Harada, 1960; Stevens & Vohra, 1955; Woolley & Peterson, 1937), little is known of its precise metabolic role. The *in vivo* synthesis of choline-0-sulphate in fungi has been shown to require participation of ATP-sulphurylase, adenosine-5′-phosphosulphate kinase, and 3′-phosphoadenosine-5′-phosphosulphate choline sulphotransferase (Hussey *et al.* 1965; Spencer & Harada, 1960; Spencer *et al.* 1968). Utilization of endogenous choline-0-sulphate requires choline sulphatase whereas utilization of exogenous choline-0-sulphate requires a specific permease as well (Bellenger *et al.* 1968; Hussey *et al.* 1965; Scott & Spencer, 1968; Spencer *et al.* 1968). Mutants of *Neurospora crassa* at the *cys*-3 locus lack choline sulphatase and choline-0-sulphate permease along with aryl sulphatase and two sulphate permeases (Marzluff, 1970*a*, *b*; Metzenberg & Parson, 1966), probably as the result of the loss of some regulatory component of a positive control system (Marzluff & Metzenberg, 1968). Murray (1965) has found that *cys*-3 ascospores show poor germination, but it is not clear whether this is a result of the defects in choline-0-sulphate metabolism or an independent effect of *cys*-3 mutations. However, no mutants specifically unable to utilize endogenously produced choline-0-sulphate have been reported, despite the usefulness of such a mutant in elucidating the role of choline-0-sulphate. Here the selection and characterization of mutants of *Aspergillus nidulans* defective in the utilization of exogenous choline-0-sulphate are presented. It is not yet known whether these mutants are also defective in the utilization of endogenous choline-0-sulphate.

2. MATERIALS AND METHODS

(i) *Chemicals*

Choline-0-sulphate was synthesized by reacting choline (as the chloride salt) with excess chlorosulphonic acid, adding distilled water to destroy the excess chlorosulphonic acid, and precipitating with a large excess of absolute ethanol. The precipitate of choline-0-sulphate was washed free of sulphuric and hydrochloric acids and unreacted choline chloride with absolute ethanol and recrystallized from 85 % ethanol. Identification of the product was confirmed by its infra-red spectrum, identical to that reported by Takebe (1960), and by its precipitation with phosphotungstic acid.

Napthol AS BI sulphate, fast red TR salt, calcium choline-0-phosphate chloride, and potassium glucose-6-sulphate were purchased from the Sigma Chemical Co., St Louis, Missouri.

(ii) *Selection of mutants*

The *A. nidulans* strain *bi*A-1 *ad*E-20 *su*A-1*ad*E-20 *s*B-3 *cho*A-1 *cha*A-1 (biotin-requiring, adenine-requiring, suppressed for adenine requirement, sulphite-requiring, choline-requiring, chartreuse conidial colour) was obtained from the Fungal Genetics Stock Center, Humboldt State College, Arcata, California, and used for the induction and selection of mutants. Choline-0-sulphate repairs both sulphite requirements resulting from loss of the sulphate permease (at the *s*B locus) and choline requirements in *A. nidulans* (Arst, 1968; Hussey *et al.* 1965). The testing of choline-0-sulphate as a sulphur source on medium solidified with agar necessitates the use of sulphate permease-less strains because agar itself contains enough sulphate to support normal growth of s^+ strains.

N-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis was performed by the method of Adelberg, Mandel & Chen (1965) as adapted by Darlington & Scazzocchio (1967). Mutants able to grow on medium containing 1 mM sulphite and 100 μM choline but not on medium containing 100 μM choline-0-sulphate were selected using the replica plating technique of Mackintosh & Pritchard (1963). Some of the mutants isolated were able to use choline-0-sulphate as a choline source but not as a sulphur source. These have blocks in the conversion of intracellular sulphate to sulphite (Hussey *et al.* 1965) and were discarded. No mutants able to use choline-0-sulphate as a sulphur source but not as a choline source were found. In addition to mutants unable to utilize choline-0-sulphate as a choline or sulphur source, other mutants to which choline-0-sulphate is toxic were obtained. These were recognized by their poor growth on media containing choline and sulphite in addition to choline-0-sulphate, whereas choline-0-sulphate non-utilizing mutants grow normally on such media.

(iii) *Detection of aryl sulphatase activity*

There are probably two aryl sulphatases in *A. nidulans* (Siddiqi, Apte & Pitale, 1966) but biochemical evidence shows that they are distinct from choline sulphatase (H. McDonnell & B. Spencer, as quoted by Scott & Spencer, 1968). Aryl sulphatase activity was detected histochemically after 24 h growth at 37 °C by flooding Petri dishes with 10 ml of 50 mM tris–50 mM maleate buffer at pH 6·0 containing 10 mg napthol AS BI sulphate and 50 mg fast red TR salt and incubating overnight at 37 °C. Sulphate was the only sulphur source present in the growth medium, and *s*B mutants, which exhibit leaky growth on sulphate (Arst, 1968), accumulate an orange precipitate whereas s^+ strains do not. This is consistent with the finding by Siddiqi *et al.* (1966) and of Hussey & Spencer (1967) that aryl sulphatase synthesis is repressed by a metabolite of sulphate in *A. nidulans*. Therefore aryl sulphatase histochemical activities were compared between *s*B single mutants and *s*B, choline-0-sulphate non-utilizing double mutants.

(iv) *Media and supplements*

Media and supplements as described by Cove (1966) and modified after Pontecorvo *et al.* (1953) were used. Growth tests were performed on the nitrogenless solid medium described by Cove (1966). Usually 5 mM urea was used as a nitrogen source. However, for testing utilization of sulphur sources, 10 mM nitrate was used as nitrogen source because nitrate interferes with utilization of sulphate by *s* mutants and reduces their leaky growth. Selenate, chromate and molybdate toxicities were tested as described previously (Arst, 1968; Arst, MacDonald & Cove, 1970).

(v) *Genetic analysis*

Genetic techniques were modified after Pontecorvo *et al.* (1953) and McCully & Forbes (1965). Dominance relationships of choline-0-sulphate non-utilizing mutations were tested in diploids and heterocaryons homozygous for the *s*B-3 and *cho*A-1 mutations.

3. RESULTS AND DISCUSSION

Four mutations, designated *csu* (choline-0-sulphate utilization) which prevent utilization of exogenous choline-0-sulphate as choline or sulphur source were selected. They do not complement with each other in heterocaryons. Genetic analysis and dominance studies were carried out with only one mutation, *csu*A-6, while growth and histochemical tests were also performed on the other three mutations, *csu*A-1, -3 and -8. *csu*A-6 is located in linkage group V and is therefore unlinked to any known locus (Arst, 1968; Dorn, 1967; Gravel *et al.* 1970) affecting sulphate or choline metabolism. It is recessive in both diploids and heterocaryons.

The *csu* mutations do not affect histochemical aryl sulphatase activity, nor do they affect utilization of glucose-6-sulphate (at 2 mM) as sulphur source or of choline-0-phosphate (at 50 μM) as choline source. Moreover, they do not affect toxicities of selenate, chromate, or molybdate in the presence or absence of *s*B mutations (cf. Arst, 1968; Arst *et al.* 1970), and they do not affect the leakiness of the *s*B-3 or *cho*A-1 mutations (cf. Arst, 1968) in the presence or absence of exogenous choline-0-sulphate. Ascospores and conidiospores of *csu* strains germinate normally, and no other pleiotropic effects have been found. It has been possible to detect *csu* mutations *in vivo* only in the presence of a *cho* or *s*B mutation.

Further work will be necessary to show whether the *csu*A mutants are defective in choline sulphatase, choline-0-sulphate uptake, or both. However, if they do lack choline sulphatase, then utilization of endogenously formed choline-0-sulphate would be a completely dispensable function – at least in all conditions tested here – and, moreover, catabolism of endogenous choline-0-sulphate would not account for the leakiness of *s*B and *cho*A mutations or for any part of the tolerance to toxic sulphate analogues shown by the wild type.

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