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Ornithine synthesis by an ornithine-deficient triple mutant

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Abstract

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Siderophores, small molecules that function in iron transport are produced by many microorganisms. Although there is much structural variability, most siderophores are either hydroxamates or phenolates-catecholates. Both major siderophores produced by Neurospora belong to the hydroxamate group and contain ornithine. Coprogen is secreted into the medium to scavenge for iron. Ferricrocin is an intracellular siderophore, whose probable function is iron storage.

Siderophore synthesis is regulated by a feedback system and is repressed if iron is present in the growth medium. This repression is not total, however, since we can detect both siderophores when Neurospora is grown in Vogel' minimal medium (MM) containing the usual amount of iron.

A mutant blocked in all pathways of ornithine synthesis should be unable to make siderophores. Such a mutant has been constructed by Rowland Davis (1970 J. Bacterial. 102: 799). The triple mutant, arg-5 ota, aga (FGSC #2744) lacks. enzymes in all known pathways leading to ornithine. G. Winkelmann reported that indeed, this mutant is unable to produce siderophores (1973 Arch. Mikrobiol. 88: 49).

A different picture has emerged from our studies, however. Using our very sensitive bioassay for siderophores (Horowitz et al. 1976 J. Bacteriol. 127: 135), we have found that even after four transfers on agar medium that contains no ornithine (Vogel's N-free salts, asparagine and glycerol; with arginine and putrescine (both filter sterilized) and ascorbic acid (300 µg/ml)added to the autoclaved medium), the conidia of the triple mutant still contain ferricrocin at approximately 5% of wild-type level. Since the normal amount of iron is present, the siderophore production system is not derepressed.

When stressed for iron, i.e.. growing in medium without added iron (Kappner et al. 1977 Arch. Microbial. 115: 323) the triple mutant produces a respectable amount of siderophores, both coprogen and ferricrocin (Table 1). Dry weights of siderophores in the Table are post-XAD-2 column chromatography. At this stage media siderophores are 25.70% pure, while intracellular ones are still less than 10% pure.

The production by arg-5, ota, aga under iron starvation conditions, of nearly 6 nmol of ornithine/mg dry weight in spermidine supplemented medium (about 3.5 nmol/mg dry weight in putrescine supplemented medium) suggests the following possible explanations: (a) an alternative pathway to ornithine synthesis exists which is derepressed under iron starvation conditions. (b) The mutant may be leaky. Under ordinary conditions this would not be detectable, but when stressed for iron, siderophore synthesis is so derepressed that enough of the enzymes are mobilized for production to occur. The tremendous derepression of siderophore synthesis that is possible is illustrated by examples cited by Emery (1971 Adv. Enzymol. 35: 125): organisms that produce siderophores in quantities that exceed the dry weight of the cells under deficient growth conditions.

TABLE 1

Siderophore production by arg-5, ota, aga and wild-type under iron-stressed conditions

Strains	Supplements[a] added to medium	Growth[b] (gm wet wt)	Total siderophores (mg)[c]		Total siderophore ornithine (μmoles)[c]	
			Medium	Intracellular	Medium	Intracellular
arg-5, ota, aga	arginine (50 μg/ml) putrescine (100 μg/ml)	11.2	9.7	3.1	1.51	0.61
arg-5, ota, aga	arginine (50 μg/ml) spermidine (100 μg/ml)	3.9	4.3	1.3	5.18	0.26
wild-type (74A)	None	13.3	116.1	18.0	424.08	13.33

a Arginine (recrystallized) and polyamines were filter sterilized and added to autoclaved medium.
b Mycelial wet wt of cultures grown in 500 ml medium for four days at 25° with shaking.
c Post XAD-2 column chromatography siderophores. Amounts are per 500 ml culture.
d Siderophores were hydrolyzed reductively with HI, and ornithine determined with a Beckman amino acid analyzer.

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