Prakash, V. Mutants excreting lysine, histidine and methionine in N. crassa.

Many of the protein amino acid analogues or antimetabolites, being growth inhibitory, hove been reported as toxic. Mutant strains resistant to the amino acid analogues L-thiosine (S-ß-aminoethyl-L-cysteine), DL-2 thiazolalanine and L-ethionine hove been isolated. These mutants overproduce ond accumulate, respectively, lysine, histidine and methionine in the culture medium.

A conidial suspension in liquid minimal medium at pH 7.8 containing 1 x 10^7 conidia/ml (conidia harvested from 72 hour-old sensitive Malayan wild-type strain UM-29) was treated with 2.8 mg/ml N-methyl- N'-nitro-N-nitrosoguanidine for four hours at 25°C. After the mutagenic treatment, the conidia were immediately plated on sorbose minimal agar medium at 37°C containing the appropriate amino acid analogue (0.5 mg/ml L-thiosine, 0.5 mg/ml DL-2 thiazolalanine or 0.5 mg/ml L-ethionine) and incubated at 25°C for 48-72 hours to isolate mutants resistant to the analogues. It has been possible to isolate a large number of such mutants.

Table 1 presents the amino acid analogue resistant mutants in which no significantly detectable difference in growth rates or mycelial weights from the sensitive parent wild-type UM-29 was observed as long as other nutrient limitations were not imposed. Each of the three categories of mutants was found to be sensitive to the toxic effect of the other amino acid analogues.

Table 1. Growth of wild-type and resistant strains on amino acid analogues*.

Strain	Control	L-thiosine**			DL-thiazolalanine**			L-ethionine**		
		0.1	0.3	0.5	0.1	0.3	0.5	0.1	0.3	0.5
Wild type UM-29	56	21	5	-	17	0.4	-	11	0.2	-
L- thiorine-resistant										
TR-6	55	61	59	58	15	0.9	-	10	0.3	-
TR-23	58	49	55	55	12	0.6	-	16	0.5	-
TR-56	49	53	53	49	14	0.7	-	12	0.2	-
TR-104	54	55	53	51	14	0.3	-	15	0.2	-
DL-2 thiozoolonine-resistant										
TAR-I 1	64	19	3	-	59	56	54	10	0.7	-
TAR- 19	53	19	5	-	54	50	51	12	0.4	-
TAR-37	55	23	5	-	57	52	45	13	0.8	-
TAR-62	57	23	5	-	58	55	54	16	0.6	-
TAR-79	60	17	2	-	58	59	60	11	0.3	-
TAR-105	54	21	4	-	53	56	59	14	1.1	-
TAR-141	49	19	3	-	53	57	55	14	0.5	-
L-ethionine-resistant										
ER-31	55	11	1	-	15	0.7	-	62	56	57
ER-53	50	14	3	-	16	0.4	-	53	53	51
ER-67	54	13	4	-	11	1	-	52	55	48
ER-83	62	18	4	-	10	0.2	-	59	57	63
ER- 132	57	18	2	-	12	0.6	-	53	54	57

* The figures are overages of two independent experiments and are expressed as mycelial dry wt in mg from 96-hour cultures grown in 20 ml minimal medium at 28°C. ** Concentration in mg/ml. - = no growth.

Auxanographic feeding tests indicated overproduction of specific amino acids and their secretion into the liquid culture medium. A partition tube apparatus consisting of two tubular sections separated by a Gelman metrical membrane filter (Grade GA-8, 0.2μ porosity) was used. The porosity of the membrane was such thot it allowed free flow of the liquid culture medium without hyphae o, conidia passing from one section to the other. 30 ml of liquid minimal medium war charged in each of the tubular sections and autoclaved. An "excretor mutant" was inoculated in one section and 72 hours later the "double auxotroph indicator strain" was inoculated in the other.

The mutants iroloted all proved to be heavy feeders across the membrane, as they supported full growth of the corresponding indicator strain. The four thiorine-resistant mutants supported the growth of the lysine-requiring double auxotrophic strains lys-1(33933); lys-3(28815) and lys-2(37101); lys-5(D56 85), but did -at feed the histidine- o, methionine requiring indicator strains. The seven thiazoalanine-resistant strains supported the growth of the three histidine-requiring *strains his-1*(C85); his-3(K446) and his-4(C141); his-7(K227) and his-2(C94); his-5(K52). They did not feed the lysine- o, methionine-requiring indicator strains. All five of the ethionine-resistant strains supported the growth of the four double auxotrophic methionine-requiring strains me-1(38706); me-10(PD' and me-3(36104); me-P(CI24) and me-7(39103); me-8(P53) and me-5(9666); me-6(35809`, whereas they did not feed the lysine-. quiring or histidine-requiring indicator strains.

The excretion of amino acids into the medium by these mutants is indicative of control deficiencies resulting in impaired regulation of biosynthesis. Since such mutants differ from the wild type only in analogue-resistance and the excretion of the individual amino acids, the mutations producing these effects must be specific for the amino acid over-production and excretion without leading to a general breakdown in the cells.

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