ネズミチフス菌のアルギニン感受性突然変異体の分離法

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SHORT COMMUNICATION

DEVICE OF A METHOD FOR ISOLATION OF ARGININE SENSITIVE MUTANTS IN *SALMONELLA TYPHIMURIUM*

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Nutrient sensitive mutants are useful in studying regulatory mechanisms of expression of genetic informations but their isolation is not easy as compared with the isolation of auxotrophs because of the lack of suitable selective media. In the case of arginine-sensitivity of *Salmonella typhimurium*, too, SJ1001 (*ars-1*, formerly *arg-s-1*) obtained from the wild strain LT2 (Ishidsu 1963, 1964a, 1964b) has been the only one mutant so far isolated and detailed genetical analysis on this phenotype had to await the isolation of more mutants.

The growth of SJ1001 in liquid medium is arrested at a certain optical density level specific to the uracil concentration when insufficient amount of uracil is supplemented with excess arginine (Ishidsu 1964b). This implies that arginine sensitive mutants of *ars-1* type might be distinguishable from insensitive parents on solid medium by their colony sizes if the two compounds are supplemented at appropriate concentrations, just as auxotrophs can easily be distinguished from prototrophs on enriched minimal medium. A reconstitution experiment, in which SJ1001 and LT2 were mixed and plated on minimal medium supplemented with arginine and uracil at various concentrations, gave results in accordance with the above expectation (Table 1). The two colony types were most easily discriminated from each other on the medium supplemented with 0.01 mM uracil and 1 mM arginine. None of the large but almost all of the minute colony formers were arginine sensitive. On this medium, uracil auxotrophs and arginine-uracil double auxotrophs would also form minute colonies. But sensitive mutants are easily discriminated from them by further tests.

Penicillin screening was found also effective in enriching the minor population of sensitive mutants. Cells of SJ1001 and LT2, fully starved for uracil in minimal medium supplemented with 1 mM arginine for 2 hours, were mixed at the ratio of 1 to 1,000, respectively, and treated with penicillin (500 units/ml, 90 min) after lowering total cell density to about 10^7 cells/ml. The ratio of the former to the latter surviving the screening increased to 4 to 1 (1.5×10^3 to 3.9×10^2 cells/ml, respectively) as examined on the selective medium mentioned above.

By combining these methods, isolation of new arginine sensitive mutants was tried. LT2 was treated with 100 μg/ml *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Aldrich) for 1 hour in nutrient broth. After sequential cultivations in minimal medium and then in the minimal supplemented with 1 mM arginine and 1 mM uracil, cells were starved for

Table 1. Reconstitution experiment to find out a selective medium for arginine sensitive mutants[a]

Uracil concentrations (mM)	Arginine concentrations (mM)									
	0		0.01		0.1		1		10	
	L	M	L	M	L	M	L	M	L	M
0	566	0	290	0[b]	310	0[b]	310	0[b]	—[e]	—[e]
0.001	797	0	687	0	239	225[c]	272	344[c]	247	325[c]
0.01	731	0	803	0	239	371[d]	337	395[d]	335	386[d]
0.1	658	0	700	0	517	0	614	0	761	0
1	683	0	788	0	720	0	726	0	592	0
10	695	0	790	0	764	0	644	0	634	0

[a]LT2 and SJ1001 were grown in minimal medium and mixed at the 1 to 1 ratio. After the $1/2\times10^{-6}$ dilution by saline, 0.1 ml aliquots of the diluted mixture were plated on each plate. Large (L) and minute (M) colonies showing up after 27 hour incubation were scored separately. Number of colonies on 3 plates are totalized in each case.

[b]Under a binocular, number of very tiny colonies could be observed.

[c]Minute colonies were really minute and counting was not easy.

[d]Minute colonies were moderate size. They were very easily distinguished from large colonies without being missed.

[e]Not examined (—).

uracil for 2 hours in the minimal with 1 mM arginine. Penicillin screening was applied in the same starvation medium at 500 units/ml for 90 min. The treated cells were plated on the selective medium after 1/10 dilution by saline. Minute colonies were picked and examined further. In total, seven arginine sensitive mutants of independent origin were isolated. Unexpectedly, five of them were sensitive also to uracil.

There have been found nutrient sensitive strains or mutants in various microorganisms (Tatum 1946; Lacroute *et al.* 1965; Piérard *et al.* 1965; Dalal *et al.* 1966; Cosloy and McFall 1970; Armstrong and Ishiwa 1971). Most of the chemicals to which sensitivities have been found are the end products either of a branched biosynthetic pathway or of one of the resembling pathways that proceed in parallel series catalyzed by a single set of enzymes. In all the cases, the sensitivities are overcome by the chemical(s) synthesized in pairs with the substance that causes the sensitivity. Therefore, a medium supplemented with two substances, one inhibits the growth but the other compensates the former's effect, at appropriate concentrations could be effective as a selective medium for a desired nutrient sensitive mutant and such a mutant could be isolated according to the fundamentally same procedure as has been applied to the isolation of auxotrophs.

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