A New Method for the Identification of Biochemical Mutants of Micro-organisms

A RAPID method for the identification of biochemical mutants of *Ustilago maydis* has been devised. The method is suitable for sporing or non-mycelial micro-organisms which form discrete colonies on agar.

Thirty-six possible requirements for single growth-factors are tested. Twelve plates of minimal medium are each supplemented with different combinations of six growth-factors with the arrangement shown in Table 1. A mutant strain with a single biochemical requirement when inoculated on all twelve plates will grow on two of them, one of plates 1–6 and one of plates 7–12. Thirty-six combinations are possible; each combination indicates a different requirement. The number of requirements tested can be varied by altering the number of plates and the arrangement of growth-factors among them.

Table 1

		Plates 1	2	3	4	5	6
Plates	7	adenine	biotin	phenylalanine	alanine	arginine	leucine
	8	hypoxanthine	folic acid	serine	cysteine	ornithine	glycine
	9	cytosine	pantothenic acid	tryptophan	threonine	aspartic acid	*iso*leucine
	10	guanine	pyridoxin	tyrosine	thiosulphate	proline	histidine
	11	thymine	thiamin	*p*-amino benzoic acid	methionine	glutamic acid	lysine
	12	uracil	riboflavin	nicotinic acid	choline	inositol	valine

An inoculating instrument with twenty-five points is used for transferring cells from each of twenty-five different mutant colonies growing on complete agar medium to each of the twelve plates. The multiple inoculator consists of twenty-five bolts ($\frac{1}{2}$ in., 10 *BA*) fitted to double-thickness zinc gauze. A short handle allows the instrument to be sterilized by dipping in methylated spirit and flaming. In preparing the master plate of complete medium the mutants are inoculated in positions corresponding to the points of the inoculating instrument and then incubated until a suitable growth has occurred. The Lederbergs' technique of replicating colonies with velvet or damp filter paper would be an equally suitable method for carrying out the inoculations[1].

So far as possible the arrangement of growth-factors allows for the detection of likely double or alternative requirements. Mutants requiring, for example, both *iso*leucine and valine for growth will only grow on plate 6, and the precise requirement must be tested with different combinations of the six growth-factors in that plate. Mutants with alternative requirements will grow on more than two plates. For example, those using methionine or cysteine will grow on plates 4, 8 and 11, and their requirements can be detected at once.

When large numbers of mutants are to be identified, this technique is more efficient than previous methods in which the field of search is progressively reduced[2,3]. With one or a few mutants auxanographic techniques are preferable[4,5].

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[1] Lederberg, J., and Lederberg, E. M., *J. Bact.*, **63**, 399 (1952).
[2] Lederberg, J., *Meth. Med. Res.*, **3**, 5 (1950).
[3] Pontecorvo, G., *et al.*, "Adv. in Gen.", **5**, 141 (1953).
[4] Lederberg, J., *J. Bact.*, **52**, 503 (1946).
[5] Pontecorvo, G., *J. Gen. Microbiol.*, **3**, 122 (1949).

The Snail's Foot as a Langmuir Trough

THE importance of the surface film of protein on stationary or slowly moving bodies of water as a food for small aquatic animals had apparently not been realized until the recent work of Goldacre[1], who made film-pressure measurements on ponds, lakes and rivers and found on all of them unimolecular layers of protein in higher or lower states of compression. A concomitant study of the behaviour of small aquatic animals led him to the conclusion that they ate this protein in large amounts. Observations of the movements of dust particles in a film during its ingurgitation by a tadpole indicated that this animal might well eat its own dry weight of protein, spread as a monolayer at the air–water interface, in one day.

I have recently acquired, through the courtesy of Mr. G. Ashby of the Zoological Society of London, some water snails of the South American species *Pomacea canaliculata*. Studies of the behaviour of these animals, of which my largest specimen weighs 52 gm., have shown that they not only eat surface films of protein, but also have the technique of compressing them to fibrous masses which are then eaten without a disproportionately high intake of water.

The phenomenon is manifested in the following manner. A snail in the usual resting position, adhering to the wall of the tank or to a floating plant just below the water surface, detaches the anterior portion of its foot and extends and curves it in such a manner as to form, either alone or with the tank wall, a funnel with its rim in the surface. Rapid undulatory movements are then seen to pass posteriorly from the periphery of the foot, while from the motion of particles in the adjacent water surface it is clear that the material constituting the surface film is being drawn towards the mouth of the funnel. Within a few seconds the presence of a collapsed protein film is evident at the water surface within the funnel. As the protein accumulates, the water-level is forced down and after, say, 10 sec., the funnel is almost empty, with denatured protein tightly packed in the narrow end. After a short period of further accumulation, the animal brings its mouth forward and downward and eats the protein.

The funnel is not refilled from above during compression of the film since the foot is rendered hydrophobic by the protein and the contact angle between foot and water is hence increased. The protein is probably anchored to the mucin layer of the foot by its polar side-chains, the hydrophobic, non-polar groups being directed predominantly towards the air. The strongly anionic character of the mucin is shown by the avidity with which, *in situ*, it takes up basic dyes such as methylene blue.

This behaviour is very much more frequent in young snails than in adults. With the latter, film compression sometimes occurs, as it were, absent-mindedly, while the animal is engaged in a clearly