Genetic Recombination in *Streptomyces*

THE Actinomycetes have properties intermediate between those of the fungi and those of the bacteria. As in practically all bacteria and in many fungi, a sexual cycle is not known to occur in the Actinomycetes. In some bacteria and Fungi Imperfecti[1], however, there are parasexual processes[2], that is, processes leading to genetic recombination otherwise than via sexual reproduction. The present communication reports the discovery of a recombination process in an Actinomycete, *Streptomyces coelicolor* Reiner-Müller.

The two starting strains used were kindly supplied by Dr. A. Tonolo. One of them produces a deep blue pigment[3] diffusing into the agar ; the other, a mutant from the first, does not produce it. Both grow well on a chemically defined minimal medium[4] containing only glucose and inorganic salts.

Two multiple mutants were isolated by successive ultra-violet irradiations of spores : one with requirements for methionine and histidine and producing blue pigment (5 *me hist*) from the first strain, and one with requirements for proline and glutamic acid and not producing the blue pigment (14 *pr glu pigm*) from the second strain. The mutants grew well either on the minimal medium supplemented with the respective requirements, or on a 'complete medium'[5] containing peptone, yeast extract, etc.

The two multiple mutant strains, 5 *me hist* and 14 *pr glu pigm*, were streaked separately and together on slants of agar media made up of mixtures of the minimal and the complete media (in proportions varying from 3 : 1 to 1 : 1). Separately the two strains grew very little in these mixtures ; but the mixed inoculum produced abundant growth in two or three days, with intense blue pigmentation of the agar and abundant sporulation. A suspension in distilled water of spores from the mixed culture was filtered, and the spores, washed by centrifugation, were plated on the minimal medium. For 'viable counts' part of the same suspension was also plated, after suitable dilution, on the complete medium. An unexpectedly large number of the spores produced growth on the minimal medium. The time for which the mixed culture was grown before harvesting the spores and plating them on the minimal medium was varied from three to nine days with the results shown in Table 1.

As a control of the experiment reported in the first line of the table, 12×10^6 spores of strain 14 *pr glu pigm* alone, plated on the minimal medium, produced no growth ; neither did $24 \cdot 7 \times 10^6$ spores of strain 5 *me hist* alone. Even a mixed suspension of spores of the two strains (34×10^6 spores) obtained from separate cultures produced no growth. Clearly a period of mixed growth is necessary for spores producing prototrophic colonies to arise.

Table 1

Mixed culture medium (min./ complete)	Age of mixed culture (days)	No. of spores from mixed culture plated on the min. medium (corrected for viable counts)	Colonies growing on the min. medium ('prototrophic')	Prototrophic colonies per cent of spores plated
1/1	4	46×10^6	10,800	0·023
3/1	3	$3 \cdot 4 \times 10^6$	260	0·008
3/1	4	4×10^6	0	0
3/1	6	580	250	43
3/1	7	7,000	910	13
3/1	9	4,000	780	19

Table 2

Mixed culture medium (min./ complete)	Age of mixed culture (days)	Supplements added to the min. medium for plating spores from mixed culture	Rate of recombination (per cent)	No. of colonies tested	Recombinant types obtained No.	Type
1/2·3	7	proline, histidine	3·1	36	3	*pr*
					4	*hist*
					29	+
1/2·3	7	glutamic, methionine	1·4	482	1	*me*
					481	+
1/1	16	histidine, glutamic	19	168	9	*hist*
					7	*pigm*
					136	+
1/1	16	methionine, proline	4·6	300	34	*pr*
					2	*pr me*
					264	+

The symbols indicate the requirement or the absence of blue pigment (*pigm*). + indicates the wild type, that is, no requirement and presence of blue pigment.

Recombinant strains presenting requirements of one or both of the parent strains were identified and selected by the following method. Spores from mixed cultures were plated on the minimal medium partially supplemented with the substances required by the parent strains. All the possible combinations of substances required by the parent strains were tried except those which would permit the growth of either or both parent strains.

A number of colonies showing growth on these media were tested for their nutritional requirements, and the presence or absence of the blue pigment noted. Table 2 gives details of the results.

Thus, three types of recombinants occurred among the strains isolated : (*a*) with all the 'wild' characters of the starting strains ; (*b*) with some 'wild' characters of one strain and some mutant characters of the other strain ; (*c*) with mutant characters from both strains (*pr me*).

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Rate of Metabolism in Tardigrades during Active Life and Anabiosis

SOME species of tardigrades are noted for the ability to survive complete desiccation[1]. When dried, the animals are able to withstand extreme temperatures and lack of oxygen. The experiments described here were made with the purpose of contributing to the knowledge of the metabolism of tardigrades during the period of anabiosis.

First, the rate of metabolism during anabiosis was measured and compared with that during active life[2]. Two species were used : *Macrobiotus hufelandi* Schultze, a moss-inhabiting species, able to survive