SHORT COMMUNICATIONS

Genetic Recombination in a Thermophilic Actinomycete, *Thermoactinomyces vulgaris*

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Thermoactinomyces vulgaris is strongly thermophilic, growing rapidly above 50° (Tsilinsky, 1899). Its spores have a multilayered outer integument (Cross, Walker & Gould, 1968; Dorokhova, Agre, Kalakoutskii & Krassilnikov, 1968), possess dipicolinic acid (Cross *et al.* 1968) and withstand boiling in aqueous suspension for considerable periods (Tsilinsky, 1899; Erikson, 1952; Cross, 1968). In all these features they resemble the spores of the eubacterial bacilli and Clostridia and differ from those of the mesophilic Streptomyces (Glauert & Hopwood, 1961; Rancourt & Lechevalier, 1964; Bradley & Ritzi, 1968; Wildermuth & Hopwood, 1970).

Thermoactinomyces vulgaris is one of two organisms causally implicated in the disease known as Farmer's Lung, a condition in which hypersensitivity to antigens of the organisms occurs as a result of inhaling vast quantities of their spores from mouldy hay (Pepys *et al.* 1963; Cross, 1968).

We had several motives for attempting to develop a useful system of genetic analysis in *Thermoactinomyces vulgaris*. One was to provide a genetic comparison with the mesophilic *Streptomyces coelicolor* from the point of view of spore morphogenesis (Hopwood, Wildermuth & Palmer, 1970), sexual biology (Hopwood, Harold, Vivian & Ferguson, 1969) and genome organization (Hopwood, 1967). Another was to open the way for a genetic approach to the biochemical basis of thermophily; we are not aware of any previous report of genetic recombination in a thermophilic bacterium. An obvious attraction of the organism for genetic studies is its very rapid growth rate.

The strains of *Thermoactinomyces vulgaris* used in our work had a nutritional requirement satisfied by hydrolysed casein in Czapek–Dox medium. Our 'minimal medium' (MM) therefore consisted of Czapek–Dox medium (Oxoid), containing 0.6% (w/v) Bacto vitamin-free Casamino-acids (Difco); the 'complete medium' (CM) consisted of MM supplemented with L-tryptophan (50 µg./ml.), adenine, guanine, thymine and uracil (10 µg./ml. each), biotin, nicotinamide, *p*-aminobenzoic acid, pantothenic acid, pyridoxin, riboflavin and thiamine (1 µg./ml. each). Auxotrophic mutants were isolated after exposure of spore suspensions in water to approximately 2000 ergs/mm.² ultraviolet light, plating on CM to yield 100 to 300 colonies per plate, and replicating by means of velvet to MM. Streptomycin-resistant mutants were isolated by spreading dense spore suspensions on CM containing 25 µg./ml. streptomycin sulphate. All cultures were incubated at approximately 52°.

We started with strain CUB76 of *Thermoactinomyces vulgaris* kindly supplied by Dr T. Cross, University of Bradford. Preliminary attempts to demonstrate genetic

recombination in mixed cultures of two mutant derivatives of CUB76 were not successful; a few colonies appeared on the selective plates, but the yield was no greater from the mixed culture than from the parental strains grown separately.

Since recombination was not demonstrated in CUB76, other wild-type strains of *Thermoactinomyces vulgaris* were isolated from soil samples, using the selective isolation conditions described by Cross (1968). Samples of soil from various localities were shaken in water and drops of the liquid were spread on plates of MM containing 25 µg./ml. sodium Novobiocin and 50 µg./ml. Actidione. The plates were incubated for 24 h at 52°, when colonies of *T. vulgaris* appeared on all plates. A single colony was chosen from each of several soil samples, and the resulting strains were used as the starting cultures for the isolation of auxotrophic and streptomycin-resistant mutants. Attempts were then made to select prototrophic streptomycin-resistant recombinants from mixed cultures of an auxotrophic streptomycin-resistant mutant with its wild-type progenitor. One wild-type, stock number 1227 (isolated from garden soil kindly collected by Mr G. R. Davies at Wymondham, Norfolk), gave clear evidence of recombination with its nicotinamide-requiring, streptomycin-resistant derivative, and attention was then confined to this strain and its mutant and recombinant descendants. Further auxotrophic mutants were isolated, including strains requiring adenine, riboflavin, thiamine, thymine, tryptophan or uracil. Mixed cultures of these strains yielded recombinants on selective media with frequencies in the region of one per 10^3 to 10^5 spores of parental phenotype; these frequencies were at least 100 times higher than could be explained by mutation of the markers concerned as judged by mutation frequencies in cultures of the parents grown separately.

A first attempt to characterize the process of genetic recombination in strain 1227 involved four-point crosses of the kind used by Hopwood (1959) to analyse recombination in *Streptomyces coelicolor*. The procedure was to plate samples of the same spore suspension, derived from a mixed culture of the two parents, on four selective media, on each of which two different markers were selected, leaving two markers unselected. A sample of recombinants was picked from each medium to plates of the same composition and classified into the four theoretically possible classes by replica plating to test media. Considering all four media together, nine out of the 16 possible combinations of the parental markers were theoretically recoverable, comprising both members of two complementary pairs and one member of five others. Neither member of the parental pair of genotypes could, of course, be selected.

The following four markers were used in a series of crosses: requirements for nicotinamide (*nic*), thiamine (*thi*) or uracil (*ura*) and resistance to streptomycin (*str-r*). Table 1 gives the results of the first cross, involving two mutant strains, *nic thi ura⁺ str-r* $\times$ *nic⁺ thi⁺ ura str-s*. Recombinants obtained by crossing these strains were used to make two further crosses with the same markers in different coupling arrangements: *nic⁺ thi ura str-r* $\times$ *nic thi⁺ ura⁺ str-s* and *nic thi⁺ ura str-r* $\times$ *nic⁺ thi ura⁺ str-s*.

The first conclusion from these crosses is that we are dealing with a true recombination process in which substitution of homologous segments of genetic material occurs, rather than with some kind of replication (Clark, 1967) in which genetic material from one strain is merely added to the genetic complement of the other. The most direct evidence for this conclusion is the isolation of certain classes of progeny (*nic⁺ thi ura str-r*; *nic thi⁺ ura str-r*; Table 1) expressing auxotrophic markers, which are unlikely to be dominant, inherited from both parents. Confirmation of this

conclusion came from the results of the crosses of pairs of recombinants. These pairs did not give rise to a majority of colonies displaying the phenotype of one of their own parents.

Table 1. *Results of crossing strains nic-1 thi-3 ura-1⁺ str-1 and nic-1⁺ thi-3⁺ ura-1 str-s*

Genotypes of progeny*	Numbers of recombinants of each genotype on selective media supplemented as indicated†			
	Nicotinamide uracil		Thiamine uracil	
	streptomycin	Nicotinamide	streptomycin	Thiamine
<i>nic thi</i> + <i>str-r</i>	—	—	—	—
+ + <i>ura str-s</i>	—	—	—	—
<i>nic</i> + + <i>str-r</i>	20	4	—	—
+ <i>thi ura str-s</i>	—	—	—	—
+ + <i>ura str-r</i>	57	—	73	—
<i>nic thi</i> + <i>str-s</i>	—	—	—	—
+ <i>thi</i> + <i>str-r</i>	—	—	23	12
<i>nic</i> + <i>ura str-s</i>	—	—	—	—
+ + + <i>str-s</i>	—	94	—	169
<i>nic thi ura str-r</i>	—	—	—	—
+ + + <i>str-r</i>	1	0	0	1
<i>nic thi ura str-s</i>	—	—	—	—
<i>nic</i> + <i>ura str-r</i>	1	—	—	—
+ <i>thi</i> + <i>str-s</i>	—	—	—	0
+ <i>thi ura str-r</i>	—	—	1	—
<i>nic</i> + + <i>str-s</i>	—	0	—	—
Total recombinants classified	79	98	97	182

* Arranged in complementary pairs; the top pair is parental; the bottom three pairs differ from both parents by two markers; the remainder differ from one parent by only one marker.

† Spores from the mixed culture were plated on the four selective media and samples of the resulting recombinant colonies were classified in respect of the non-selected markers (giving four possible genotypes on each medium). Total colony counts on the four media were approximately equal. A dash indicates that a particular genotype could not grow on the medium in question.

The second conclusion is that *Thermoactinomyces vulgaris* resembles all other prokaryotes so far studied in having partially diploid zygotes. The evidence for this is the finding that the great majority of the recombinants differed from one or other parent by only a single marker; these were the classes *nic thi*⁺ *ura*⁺ *str*—*r*, *nic*⁺ *thi*⁺ *ura str*—*r*, *nic*⁺ *thi ura*⁺ *str*—*r* and *nic*⁺ *thi*⁺ *ura*⁺ *str*—*s*, irrespective of the coupling of the markers in the three crosses. Had both parents contributed a complete genome to the zygotes, the observed result is incompatible with any of the three possible sequences of four markers on a single circular linkage group, any of the 12 sequences on a linear linkage group, or an arrangement involving more than one linkage group.

A final conclusion is that the process of genetic transfer involves fragments representing only a small fraction of the total genome, otherwise recombinants inheriting two markers from each parent should have been frequent, as they are in *Streptomyces coelicolor*. An alternative possibility is that the markers so far studied are carried on separate chromosomes, genetic transfer normally involving only one. In any event, the results preclude the assessment of linkage at this stage; in this they differ from the results of comparable crosses in *S. coelicolor* in which linkage was successfully demon-

strated by such crosses because the linkage map is comparatively short, and genetic transfer involves large portions of the genome (Hopwood, 1959, 1965, 1966, 1967); thus in *S. coelicolor* the situation did not arise in which all four markers in a cross could fail to show linkage.

We conclude that our strain of *Thermoactinomyces vulgaris* has a process of true recombination that should qualify it as a suitable organism for some of the genetic studies alluded to at the beginning of this note.

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REFERENCES

- BRADLEY, S. G. & RITZI, D. (1968). Composition and ultrastructure of *Streptomyces venezuelae*. *Journal of Bacteriology* **95**, 2358–2364.
- CLARK, A. J. (1967). The beginning of a genetic analysis of recombination proficiency. *Journal of Cellular Physiology* **70**, Suppl. 1, 165–180.
- CROSS, T. (1968). Thermophilic actinomycetes. *Journal of Applied Bacteriology* **31**, 36–53.
- CROSS, T., WALKER, P. D. & GOULD, G. W. (1968). Thermophilic actinomycetes producing resistant endospores. *Nature, London* **220**, 352–354.
- DOROKHOVA, L. A., AGRE, N. S., KALAKOUTSKII, L. V. & KRASSILNIKOV, N. A. (1968). Fine structure of spores in a thermophilic actinomycete, *Micromonospora vulgaris*. *Journal of General and Applied Microbiology* **14**, 295–303.
- ERIKSON, D. (1952). Temperature/growth relationships of a thermophilic actinomycete, *Micromonospora vulgaris*. *Journal of General Microbiology* **6**, 286–294.
- GLAUERT, A. M. & HOPWOOD, D. A. (1961). The fine structure of *Streptomyces violaceoruber* (*S. coelicolor*). III. The walls of the mycelium and spores. *Journal of Biophysical and Biochemical Cytology* **10**, 505–516.
- HOPWOOD, D. A. (1959). Linkage and the mechanism of recombination in *Streptomyces coelicolor*. *Annals of the New York Academy of Sciences* **81**, 887–898.
- HOPWOOD, D. A. (1965). A circular linkage map in the actinomycete *Streptomyces coelicolor*. *Journal of Molecular Biology* **12**, 514–516.
- HOPWOOD, D. A. (1966). Lack of constant genome ends in *Streptomyces coelicolor*. *Genetics, Princeton* **54**, 1177–1184.
- HOPWOOD, D. A. (1967). Genetic analysis and genome structure in *Streptomyces coelicolor*. *Bacteriological Reviews* **31**, 373–403.
- HOPWOOD, D. A., HAROLD, R. J., VIVIAN, A. & FERGUSON, H. M. (1969). A new kind of fertility variant in *Streptomyces coelicolor*. *Genetics, Princeton* **62**, 461–477.
- HOPWOOD, D. A., WILDERMUTH, H. & PALMER, H. M. (1970). Mutants of *Streptomyces coelicolor* defective in sporulation. *Journal of General Microbiology* **61**, 397–408.
- PEPYS, J., JENKINS, P. A., FESTENSTEIN, G. N., GREGORY, P. H., LACEY, M. E. & SKINNER, F. A. (1963). Farmer's Lung. Thermophilic actinomycetes as a source of 'farmer's lung hay' antigen. *Lancet* **ii**, 607–611.
- RANCOURT, M. W. & LECHEVALIER, H. A. (1964). Electron microscopic study of the formation of spiny conidia in species of *Streptomyces*. *Canadian Journal of Microbiology* **10**, 311–316.
- TSILINSKY, P. (1899). Sur les mucédinées thermophiles. *Annales de l'Institut Pasteur* **13**, 500–505.
- WILDERMUTH, H. & HOPWOOD, D. A. (1970). Septation during sporulation in *Streptomyces coelicolor*. *Journal of General Microbiology* **60**, 51–59.