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TYROSINASE INHERITANCE IN *STREPTOMYCES SCABIES*

I. GENETIC RECOMBINATION

KENNETH F. GREGORY AND JAY C. C. HUANG[1]

Department of Microbiology, Ontario Agricultural College, Guelph, Ontario, Canada

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ABSTRACT

GREGORY, KENNETH F. (Ontario Agricultural College, Guelph, Ontario, Canada), AND JAY C. C. HUANG. Tyrosinase inheritance in *Streptomyces scabies*. I. Genetic recombination. J. Bacteriol. **87**:1281-1286. 1964.—Mutants derived from *Streptomyces scabies* strain A26 recombined with other derivatives of A26, but not with nine other strains of *S. scabies* nor with eight strains of other streptomycetes. Most of the spore progeny of heterogenomic mycelia formed from complementary diauxotrophic strains of *S. scabies* A26 were capable of forming tyrosinase (*tye*+), provided either of the parents was *tye*+. About 99.8% of these spores carried the nutritional markers of either one or the other of the two parents. All recombinant classes between nutritional and streptomycin susceptibility markers were likewise predominantly *tye*+. We suggest that the *tye*+ characteristic is carried in a small genetic unit, which is unlinked to most other genes and capable of replicating faster than the rest of the *S. scabies* genome.

Although there is no morphological evidence for a sexual cycle in the genus *Streptomyces*, genetic recombination has been demonstrated to occur in several species in this genus (reviewed by Braendle and Szybalski, 1959; Bradley, 1962). *S. scabies*, an etiological agent of potato scab, was observed to undergo hyphal fusions (Gregory, 1956). When nutritionally deficient mutants of *S. scabies* A26 were plated together on minimal medium, prototrophic colonies developed (Gregory, 1959). Most of the spores produced by these colonies carried the nutritional requirements of one or the other of the two parents, although the nutritional independence character of the colonies could be perpetuated by mycelial transfers. These colonies were, therefore, considered to be heterokaryotic or, more precisely, heterogenomic, because the streptomycetes lack a limiting nuclear membrane (Moore and Chapman, 1959; Hopwood and Glauert, 1960).

Among the progeny of such heterogenomic mycelia were stable prototrophs, clones with single nutritional deficiencies, and clones with deficiencies derived from both parents. This last category occurred at a frequency of about 1×10^{-3} per spore plated, and was considered to provide conclusive evidence for genetic recombination (Gregory, 1959). When the ability to produce tyrosinase (*tye*+), a characteristic of "wild" *S. scabies* strains, was used as an unselected marker, a remarkable bias toward *tye*+ was observed in the spores of heterogenomic mycelia. The two types of progeny which resembled the parental strains in nutritional requirements were both mostly *tye*+, regardless of which parent contributed this marker. Although tyrosinase-deficient (*tye*−) mutants arose spontaneously in the strain studied at a frequency of about 0.2% of the spores plated, the reverse mutation to *tye*+ was never detected. This fact, together with the lack of a demonstrable selective advantage of *tye*+ over *tye*− under the conditions used, tended to eliminate mutation or selection as a cause of this bias. These data led Gregory and Shyu (1961) to postulate that a factor concerned with the production of tyrosinase in *S. scabies* was cytoplasmically inherited.

The present paper gives further details on the behavior of this factor during genetic recombination in *S. scabies*.

MATERIALS AND METHODS

All mutant strains (Table 1) were derived from *S. scabies* A26, a strain originally isolated from a potato scab lesion and of confirmed pathogenicity for Katahdin potatoes (Gregory and Vaisey, 1956). The parent strain grows well in a medium lacking growth factors, is inhibited by 0.5 μg of

[1] Present address: Electron Microscope Laboratory, Ontario Veterinary College, Guelph, Ontario, Canada.

TABLE 1. *Mutant strains of Streptomyces scabies used*

Strain no.	Mutant characteristics*
A26-30	*arg-1⁻ bio-2⁻ tye⁺*
A26-34	*nic⁻ bio-1⁻ tye⁻*
A26-37	*arg-1⁻ met⁻ str-r tye⁺*
A26-42	*nic⁻ arg-2⁻ tye⁺*
A26-43	*nic⁻ lys⁻ tye⁺*
A26-44	*arg-3⁻ sur⁻ tye⁺*
A26-53	*arg-1⁻ met⁻ tye⁻*
A26-54	*nic⁻ bio-1⁻ str-r tye⁺*
A26-59	*arg-1⁻ met⁻ str-r tye⁻*

* *Arg⁻*, *bio⁻*, *lys⁻*, *met⁻*, *nic⁻*, and *sur⁻* indicate nutritional requirements for arginine, biotin, lysine, methionine, nicotinic acid, and a reduced form of sulfur, respectively; *str-r* indicates resistance to 20 μg of streptomycin per ml; *tye⁻* indicates inability to produce tyrosinase. Numerical suffixes distinguish different mutations for the same nutritional requirements.

streptomycin per ml, and produces tyrosinase. Tyrosinase production is revealed by the formation of a dark-brown pigment surrounding colonies grown on media containing either protein or free L-tyrosine. The similar pigment formed by *S. lavendulae* was shown to be melanin (Mencher and Heim, 1962).

A glucose-asparagine-mineral salts medium (Gregory and Vaisey, 1956), prepared with washed agar and double-distilled water in acid-washed flasks, served as the "minimal" medium. The same medium supplemented with 0.1% casein hydrolysate (Casamino Acids) and 0.3% yeast extract (Difco) served as the "complete" medium, except in experiments with strain A26-59, where the medium of Hickey and Tresner (1952) was required to achieve good sporulation. To permit recognition of tyrosinase production by individual colonies, 0.04% L-tyrosine was usually added to the media.

All incubations were at 30 C for 4 to 7 days. Spore suspensions were prepared by scraping the surface spores from 6-oz bottle slants into sterile 0.02% "Tween 20" (Atlas Powder Co., Wilmington, Del.). Spore suspensions were washed three times by centrifugation and resuspension in Tween 20.

RESULTS

Determinations of the tyrosinase-producing capabilities of spores produced by heterogenomic ("heterokaryotic") mycelia from many different combinations of *tye⁻* plus *tye⁺* strains of *S. scabies* showed that the great majority of these spores were *tye⁺*, regardless of which parent had contributed this marker. In many cases, the incidences of *tye⁻* strains among these progeny were no greater than could be accounted for by spontaneous mutation. In other instances, a significantly larger number of *tye⁻* spores were formed. In one case, 83% of the progeny bearing the nutritional markers of the *tye⁻* parent (arginine and methionine; confirmed by replica plating) were *tye⁺* (Table 2). The incidence of nonparental *str-r* recombinants (ca. 0.2%) was similar to the frequency of *bio⁻met⁻* recombinants, another common class observed in this cross. Most of the colonies developing on the nonsupplemented medium were found to be heterogenomic clones rather than recombinants.

Scoring of the tyrosinase capabilities of genetic recombinants isolated from mixed growth, on complete media, of strains of *S. scabies* with complementary selective markers similarly revealed a strong bias toward the *tye⁺* phenotype. The results of two experiments in which strain A26-59 (*arg⁻met⁻str-r tye⁻*) was crossed with its original parent A26 (*arg⁺met⁺str-s tye⁺*) are shown in Table 3. Mixed cultivation on complete medium minimized the possibility that prototrophic recombinants might have increased their relative frequency in the heterogenomic

TABLE 2. *Phenotypes of spores derived from a single heterogenomic colony of Streptomyces scabies A26-53 (arg⁻ met⁻ str-s tye⁻) plus A26-54 (nic⁻ bio⁻ str-r tye⁺)*

Supplement to medium*	Colony count per ml of spore suspension†	
	tye⁺	*tye⁻*
None	32	0
Nicotinic acid + biotin	8.2×10^2	0
Arginine + methionine	5.3×10^3	1.1×10^3
Arginine + methionine + streptomycin	10	0
Complete medium	5.7×10^3	1.3×10^3

* L-Tyrosine was added to all media at a concentration of 0.04%. Nicotinic acid was added at a concentration of 1 μg/ml; biotin, 0.01 μg/ml; L-arginine, 50 μg/ml; L-methionine, 50 μg/ml; streptomycin, 20 μg/ml.

† Means of triplicate plates.

TABLE 3. *Inheritance of tyrosinase capability among streptomycin-resistant recombinants of Streptomyces scabies*

Culture plated	Count per ml of spore suspension*			
	Parental phenotypes		Recombinant phenotypes	
	arg⁺ met⁺ str-s tye⁺	*arg⁻ met⁻ str-r tye⁻*	*arg⁺ met⁺ str-r tye⁺*	*arg⁺ met⁺ str-r tye⁻*
A26	3.8×10^8	0	0	0
A26-59	0	1.8×10^8	0	0
Mixed growth, A26-59 + A26 (Expt 1)	6.0×10^6	2.1×10^8	5.7×10^3	$<1 \times 10^2$
Mixed growth, A26-59 + A26 (Expt 2)	1.1×10^5	1.3×10^7	8.3×10^3	1.7×10^3

* Means of triplicate plates.

clones subsequent to their formation because of a selective effect of the medium. A large excess of unmated parental types was, of course, recovered from such crosses. In both experiments, large numbers of *arg⁺met⁺str-r* recombinants were recovered. In the first experiment, virtually all of these recombinants were *tye⁺*; in the second experiment, 83% were *tye⁺*. These results could be explained by assuming a close linkage between the *tye* and *str* loci, except that data from heterogenomic clones (e.g., Table 2) showed that the *str-r* characteristic was incorporated into less than 0.2% of the clones receiving the *tye⁺* characteristic when these two markers were contributed by the same parent.

Analyses of ten of the prototrophic *str-r tye⁺* colonies from the preceding experiment were undertaken to determine whether these were true recombinants or heterogenomic clones. Seven of these ten colonies produced only *arg⁺met⁺str-r tye⁺* spores, and are, therefore, assumed to be genetic recombinants (Table 4). Colony 8 gave results typical of a heterogenomic clone, because most of the spores carried the markers of the two parents (with the exception that all colonies were *tye⁺*). Spores from the remaining two colonies produced more colonies on minimal medium plus streptomycin than would be expected from heterogenomic clones. It is not clear whether these colonies were mixtures of recombinants and heterogenomic clones, or whether they had a complex nuclear character like that of the heteroclones described by Hopwood, Sermonti, and Spada-Sermonti (1963) in *S. violaceoruber* (formerly *S. coelicolor*). None of these colonies produced spores with the identical phenotype of the A26-59 parent, because all the spores were *tye⁺* (except for the 0.2 to 0.3% incidence of *tye⁻* spores arising by mutation in all *tye⁺* cultures).

Neither heterogenomic clones nor recombinants were produced when auxotrophic *str-r* and prototrophic *str-s* cultures were plated together directly onto minimal medium containing streptomycin. A comparison of the results of such platings with the incidence of prototrophic colonies arising from mixed platings of diauxotrophic strains on minimal agar is shown in Table 5. The few colonies developing on the streptomycin-containing medium resulted from spontaneous mutations of *str-s* to *str-r*.

The ability of strain A26-59 to undergo genetic recombination with nine unrelated wild-type strains of *S. scabies* and with eight culturally similar, tyrosinase-producing streptomycetes isolated from soil was tested by growing the pairs of strains together on complete medium and by plating the resulting spores on selective media.

TABLE 4. *Analysis of ten prototrophic, streptomycin-resistant colonies obtained from mixed growth of Streptomyces scabies A26 (arg⁺ met⁺ str-s tye⁺) and A26-59 (arg⁻ met⁻ str-r tye⁻)*

Colony no.	Colonies per ml of spore suspension*		
	arg⁺ met⁺ str-s tye⁺	*arg⁺ met⁺ str-r tye⁺*	*arg⁻ met⁻ str-r tye⁺*
1–7	0	1.4×10^2–1.7×10^5	0
8	5.3×10^4	6.6×10^1	2.3×10^6
9	1.5×10^5	3.0×10^4	6.0×10^6
10	2.3×10^5	1.1×10^5	2.1×10^7

* All colonies recovered were *tye⁺*; hence, no colony with the phenotype of the A26-59 parent (*arg⁻ met⁻ str-r tye⁻*) was recovered.

TABLE 5. *Yields of heterogenomic colonies from mixed platings of mutant strains of Streptomyces scabies*

Medium	Culture	Genotype	Colonies per 10^6 spores of parent present in lowest numbers	
			Plated separately	Plated together
Minimal medium*.	A26-43	nic⁻ lys⁻	0	434
	A26-44	arg-3⁻ sur⁻	0	
	A26-43	nic⁻ lys⁻	0	93
	A26-30	arg-1⁻ bio-2⁻	0	
	A26-42	nic⁻ arg-2⁻	0	20
	A26-44	arg-3⁻ sur⁻	0	
	A26-34	nic⁻ bio-1⁻	0	36
	A26-37	arg-1⁻ met⁻	0	
Minimal + streptomycin†..	A26-59	arg⁻ met⁻ str-r tye⁻	0	
	X A26 (1)	str-s	0.0015	0.0015
	X A26 (2)	str-s	0.0069	0.0085
	X A26 (3)	str-s	0.0080	0.0089

* Means of duplicate plates. Approximately 10^6 spores of each parent were spread on the surface of each plate.

† Means of triplicate plates. Typical data from three of eight experiments done. Approximately 10^9 spores of each parent were spread on the surface of each plate.

TABLE 6. *Tyrosinase phenotype of major recombinant classes recovered from mixed growth of A26-34 (nic⁻ bio⁻ str-s tye⁻) and A26-37 (arg⁻ met⁻ str-r tye⁺)*

Determination	Nutritional and resistance phenotype					Count per ml of spore suspension	
	nic	bio	arg	met	str	tye⁺	tye⁻
Recombinants	+	+	+	+	r	190	0
	−	+	+	+	r	3,250	200
	+	+	+	−	r	400	20
	+	−	+	−	s	426	0
Parental spores recovered	−	−	+	+	s	—	168,000
	+	+	−	−	r	49,000	—

If either parent was not recovered at a concentration of 10^4 or more spores per ml of suspension, the cross was repeated. From mixed growth of A26-59 and its wild-type parent, so many *str-r tye*⁺ recombinants resulted that they could be counted on complete medium plus streptomycin, even in the presence of the A26-59 parent. Their frequencies ranged from 0.02 to 12% (mean = 3.7%) in eight experiments, and did not correlate with the proportions of the two parents present. Prototrophic *str-r* recombinants were recovered in much smaller numbers (see Table 3). In no case was either type of recombinant detected from A26-59 plus any of the other 17 strains.

In a detailed analysis of the classes of recombinant spores formed by mixed growth of strains A26-34 (*nic⁻bio⁻str-s tye⁻*) and A26-37 (*arg⁻met⁻ str-r tye⁺*), Shyu (1961) found significant numbers of recombinants in 4 of the 30 (2^5 − two parent types) possible recombinant classes derived from the five loci for selected markers (four nutritional markers and streptomycin-resistance). The distribution of the unselected marker *tye*⁺ in these four types of recombinants is shown in Table 6. It is evident that from 94 to 100% of each recombinant class was *tye*⁺. No single selected marker appeared in all of these four classes except *arg*⁺, a characteristic contributed by the *tye*⁻ parent. Therefore, the predominance of *tye*⁺ in all of these classes was not a result of linkage between *tye*⁺ and any of the selected markers.

DISCUSSION

The results show that genetic recombination occurs between mutant strains of *S. scabies* A26. The incidence of new phenotypes isolated from the progeny of mixed growth of genetically marked strains was much greater than could be accounted for by mutation. For example, the frequency of true-breeding *arg*⁺ *met*⁺ *str-r* spores produced by growth of an *arg*⁺ *met*⁺ *str-s* strain with an *arg*⁻ *met*⁻ *str-r* strain was at least ten thousand times greater than the frequency of *str-r* mutants arising from the first strain, whereas the frequency of prototrophic mutants from the second strain was too low to be measured. The recovery of stable clones with nutritional deficiencies inherited from each of the two parents is further evidence for genetic recombination. Failure to recover recombinants from mixed growth of the test strain, *S. scabies* A26-59, with any of nine other strains of *S. scabies*, or of eight unidentified strains which culturally and morpho-

logically resembled *S. scabies*, indicates that this species is highly selective in its ability to undergo interstrain genetic recombination. Interspecific recombinations in streptomycetes were reported by Alačević (1963), but in other studies interspecific recombination could not be demonstrated (Braendle and Szybalski, 1959). Interstrain matings were reported in *S. rimosus* (Alikhanian and Mindlin, 1957), *S. aureofaciens* (Alikhanian and Borisova, 1961) and, in some cases, with *S. fradiae* (Braendle, Gardiner, and Szybalski, 1959) and *S. violaceoruber* (Bradley, 1962). In the last-named species, a compatability system controlling heterokaryon formation was described. In our studies, all combinations of mutant strains derived from strain A26 were capable of genetic interaction, provided conditions allowing intimate mixing of spores and mycelia (e.g., surface plating) were used.

The existence of streptomycin-resistant heterogenomic clones carrying *str-s* genes was unexpected, because streptomycin sensitivity was reported to be dominant to streptomycin resistance in streptomycetes (Bradley and Lederberg, 1956; Braendle and Szybalski, 1959). The large excess of *str-r* to *str-s* genes present in these clones (ratios from 40:1 to 91:1) probably accounts for the apparent dominance of *str-r*.

The determinant for tyrosinase production behaved differently from the other markers studied. All recombinant classes selected on the basis of other markers were predominantly tye^+. The recovery of a higher proportion of spores from the tye^- parent than from the tye^+ parent, after mixed growth, indicated that the tye^+ phenotype had not been selectively favored by the growth conditions. The bias toward tyrosinase-positive progeny was even more striking among the spores of heterogenomic mycelia. Although the nutritional and streptomycin-resistance markers of the two parents separated into the two parental phenotypes in ca. 99.8% of the conidia formed, the tye^+ characteristic was found in the majority of these spores. Because this same phenomenon occurred when either parent contributed tye^+, the results can not be explained on the basis of an unequal contribution of genes by two different mating types.

Hopwood et al. (1963) accounted for anomalous recombination phenomena in *S. violaceoruber* by postulating the occurrence of terminal chromosomal deletions from one or both parental genomes, subsequent to hyphal fusion, with the formation of merozygotes and partially heterozygous clones. It seems unlikely that a terminal deletion including the *tye* locus, occurring during the formation of a heterogenomic clone, can account for the results reported here, because crosses which were identical except for the polarity of the tye^--tye^+ markers always yielded a high incidence of tye^+ and never a loss of tye^+. It is improbable that the occurrence of a tye^- gene would specify a terminal deletion of the chromosome bearing this mutated gene, as opposed to the one bearing the wild-type allele.

Attempts to obtain infective transfer of tye^+ were unsuccessful (Gregory and Shyu, 1961). X-irradiation of the conidia from tye^+ progeny of "heterokaryons" which carried the nutritional markers of the tye^- parent gave an exponential rate of killing, indicating that no substantial amount of the genome was diploid. Tye^+ clones recovered from heterogenomic mycelia were as stable with regard to the tye^+ characteristic as were wild strains, so that a putative partial diploid would have to be a very stable one.

The data are compatible with the hypothesis (Gregory and Shyu, 1961) that the tye^+ gene is borne by a small genetic unit (plasmid) capable of being replicated faster than most of the other genes. The fact that some heterogenomic clones produced a significant number of tye^- spores suggests that the ratio of tye^+ to other genetic determinants was not extremely great.

Bradley (1958) observed instances in heterogenomic mycelia of *S. violaceoruber* where recessive genes from different germ plasms behaved as if each made up the majority of the nuclear population. He suggested (Bradley, 1962) that this may have been owing to the presence of more than one linkage group or of a single linkage group made up of separable elements that could replicate, even though not part of an aggregated genome. This situation would be similar to the postulated mode of inheritance of tyrosinase capability in *S. scabies*.

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