RECIPROCAL CROSSES IN STREPTOMYCES COELICOLOR[1]

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THE interaction of two complementary growth-factor dependent mutants, i.e., complementary auxotrophs, of *Streptomyces coelicolor* to form growth-factor independent progeny, i.e., prototrophs, was interpreted as gene recombination by SERMONTI and SPADA-SERMONTI (1955) and by BRAENDLE and SZYBALSKI (1959), but as heterokaryosis by BRADLEY (1957). The former workers concluded that gene recombination was operative because (1) nonparental auxotrophs were also isolated from mixed cultures of the two parental types and (2) all of the recombinant classes tested were found to be stable. Conversely, BRADLEY (1958a) obtained infrequent dissociates regularly from nonparental auxotrophic recombinants and from prototrophic recombinants; only parental dissociates were stable. Recently SERMONTI and SPADA-SERMONTI (1959) derived diverse types from recombinant clones.

In order to elucidate the recombinational process, BRAENDLE and SZYBALSKI (1957) carried out "reciprocal crosses" using streptomycin susceptibility as an unselected marker. They found that when streptomycin resistance was coupled with proline and glutamate independence, and streptomycin sensitivity was coupled with methionine and histidine independence, all of the recombinants were drug resistant. Contrariwise, when streptomycin resistance was coupled with methionine and histidine independence, and streptomycin sensitivity with proline and glutamate independence, almost all of the recombinants were inhibited by streptomycin. Although this correlation could have resulted from gene recombination, interpretations based on the data of BRAENDLE and SZYBALSKI were inconclusive because progeny arising from the cross involving the two streptomycin resistant parental strains were not analyzed.

This investigation was concerned with reciprocal crosses using streptomycin susceptibility and actinophage susceptibility as unselected markers. The progeny of sensitive by sensitive crosses and of sensitive by resistant crosses were sensitive. Conversely, the progeny of resistant by resistant crosses were resistant. These results prove that prototrophs derived from mixed cultures of complementary auxotrophs are not simple haplophase recombinants. These findings are consistent with the hypotheses that these prototrophs are heterokaryons or heterozygous diploids.

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MATERIALS AND METHODS

The strains used in this study were mutants derived from *S. coelicolor* strain WAc-199 and *S. coelicolor* strain 1 (Table 1). The former wild type strain was isolated from a Wisconsin soil sample whereas the latter wild type strain, and two mutant derivatives, 5 and 23, were generously provided by DR. G. SERMONTI, Istituto Superiore di Sanita, Rome. The epithet, *S. coelicolor*, has been retained here even though DR. H. KUTZNER and DR. S. WAKSMAN, Rutgers University, suggested that these organisms should be designated *S. violaceoruber* (private communication).

Stock cultures were grown at 30°C on complete medium of the following composition: glucose, 20 gms; agar (Difco), 15 gms; yeast extract (Difco), 1 gm; casitone (Difco), 1 gm; $MgSO_4 \cdot 7H_2O$, 0.5 gm; KNO_3, 2 gms; K_2HPO_4, 2 gms; deionized water, 1 L. The salts of nitrate and phosphate were autoclaved at 121°C for 12 minutes as one double-strength solution, and the remaining components as the second double-strength solution. The two aliquots were mixed immediately before dispensing into petri plates. Minimal medium was made by deleting yeast extract and casitone from the complete medium formula. Growth-factor supplements, i.e., amino acids and uracil, were added to the minimal medium, when indicated, at a concentration of 10 mg/L.

Auxotrophic mutants were obtained by indirect selection subsequent to treatment with ultraviolet irradiation (BRADLEY and LEDERBERG 1956). Streptomycin resistant variants and actinophage resistant variants were obtained by direct selection (BRADLEY 1958a). Streptomycin resistant mutants grew well on complete medium to which 25 mg/L dihydrostreptomycin sulfate had been added. Actinophage susceptibility was determined on peptone-yeast extract medium by cross brushing the organism to be tested against a three cm streak of 10^7 to 10^8 phage particles (BRADLEY 1959a). Sensitive streptomycetes were lysed at the phage-host

TABLE 1

Description of principal cultures

Strain	Ancestor	Phenotype*
1	wild type from G. SERMONTI, Rome	prototrophic, blue V^s S^s
5	1	met-his-blue V^s S^s
23	5	met-his-blue V^s S^r
199	wild type from Wisconsin soil sample	prototrophic, blue V^s S^s
202	199	cys-pro-pink V^s S^s
203	199	arg-ura-blue V^s S^s
209	202	cys-pro-pink V^r S^s
210	203	arg-ura-blue V^r S^s
214	203	arg-ura-blue V^s S^r

* V^s/V^r: sensitive/resistant to actinophage MSP-10; S^s/S^r: sensitive/resistant to 25 mg/L dihydrostreptomycin sulfate; met-, his-, cys-, pro-, arg-, and ura-: required added methionine, histidine, cystine, proline, arginine, and uracil respectively.

intersection whereas resistant streptomycetes grew uninterrupted across the phage streak.

Recombinants were isolated by the following procedure: colonies from two auxotrophic strains, grown separately on complete medium, were cut from the agar and minced; sufficient fragmented mycelium of each parent was plated together on complete agar plates to yield confluent growth; after 4–6 days at 30°C, a sample of the mixed growth was transferred to minimal medium agar by replica plating (LEDERBERG and LEDERBERG 1952); colonies that developed on minimal medium after 4–6 days were purified by three serial transfers on complete medium; prototrophy was verified by replating the purified recombinants to minimal medium.

RESULTS

Streptomycin susceptibility as an unselected marker

The progeny resulting from crosses involving two streptomycin sensitive parents ($S^s \times S^s$) were invariably streptomycin sensitive (Table 2). Conversely, the recombinant progeny of two streptomycin resistant parents were invariably streptomycin resistant. Prototrophic recombinants obtained from parents differing with respect to streptomycin susceptibility were completely inhibited by 25 mg/L dihydrostreptomycin sulfate, or yielded a few vigorously growing colonies (Figure 1). From the cross of strain 23 by strain 210 (met-his-blue $S^r \times$ arg-ura-blue S^s) 365 recombinant colonies were inhibited completely by streptomycin,

TABLE 2

Results of reciprocal crosses employing streptomycin susceptibility and actinophage susceptibility as unselected markers

Strains crossed	Unselected markers	Phenotypes of purified recombinants			
		V^sS^s	V^rS^s	V^sS^r	V^rS^r
5 × 210	$S^s \times S^s$	283	0	0	0
23 × 214	$S^r \times S^r$	0	0	469	0
5 × 214	$S^s \times S^r$	215 + 175*	0	0	0
23 × 210	$S^r \times S^s$	365 + 136*	12	1	0
202 × 203	$V^s \times V^s$	344	1	0	0
209 × 210	$V^r \times V^r$	0	373	0	0
202 × 210	$V^s \times V^r$	197 + 166†	0	0	0
209 × 203	$V^r \times V^s$	534	19	0	0

Complementary auxotrophs were grown together on complete medium for 4–6 days; prototrophic recombinants were selected by replica plating to minimal medium; each recombinant colony was purified by three serial subcultures on complete medium; prototrophy was verified by replating to minimal medium.

* A few S^r colonies developed (less than ten percent of the inoculum).

† Scant growth at the phage-host intersection.

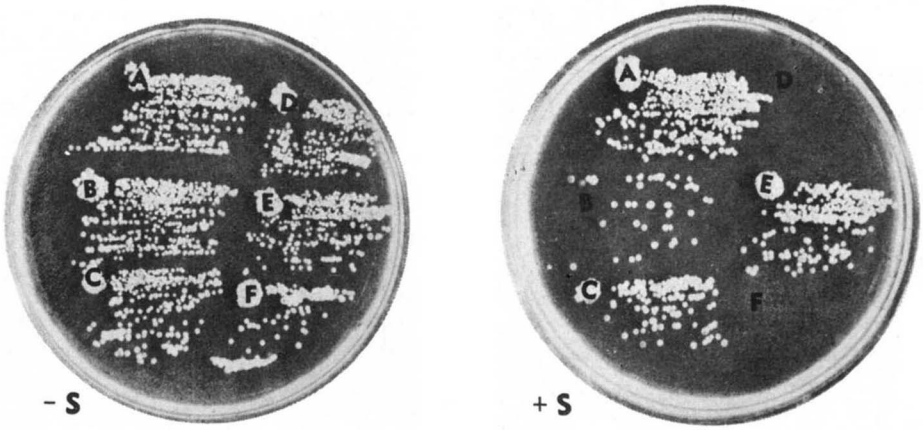

FIGURE 1.—Assay for susceptibility to streptomycin. Strain 23 (parental S^r), designated as A, grew equally well on complete medium (—S) and medium containing 25 mg/L dihydrostreptomycin sulfate (+S). Strain 210 (parental S^s), designated as F, grew on complete medium only. B is an incompletely susceptible recombinant; C and E, which are from the same recombinant colony, are totally resistant; D is a completely susceptible recombinant.

but 1–10 percent of the inoculum from 136 recombinant colonies grew on complete medium containing streptomycin. Only one colony consisted solely of streptomycin resistant plating units. From the cross of strain 5 by strain 214 (met-his-blue S^s × arg-ura-blue S^r) 215 streptomycin sensitive colonies and 175 incompletely susceptible colonies were found.

During serial replating of prototrophs, whether drug sensitive or resistant, parental types were recovered. The variety of dissociates, their frequency and stability, confirmed previous findings (BRADLEY 1958b).

Actinophage susceptibility as an unselected marker

According to expectation, progeny from crosses involving two actinophage sensitive parents were sensitive. Corresponding crosses between two actinophage resistant parents yielded virus resistant recombinants. Prototrophic recombinants obtained from parents differing with respect to phage susceptibility were usually inhibited completely by virus, or were slightly resistant. Rarely recombinants were fully resistant to actinophage. From crosses of strain 209 by strain 203 (cys-pro-pink V^r × arg-ura-blue V^s), 534 recombinants were found to be totally sensitive whereas 19 were entirely resistant to virus. In the reciprocal cross, i.e., strain 210 by strain 202 (arg-ura-blue V^r × cys-pro-pink V^s), 197 of the purified prototrophs were very susceptible to actinophage, but 166 grew scantily with an excess of phage (Figure 2). As before, recombinant colonies gave rise to parental dissociates infrequently.

DISCUSSION

Stable prototrophic progeny can arise from mixed cultures of two complementary auxotrophs as a result of (1) mutation, either spontaneous or induced,

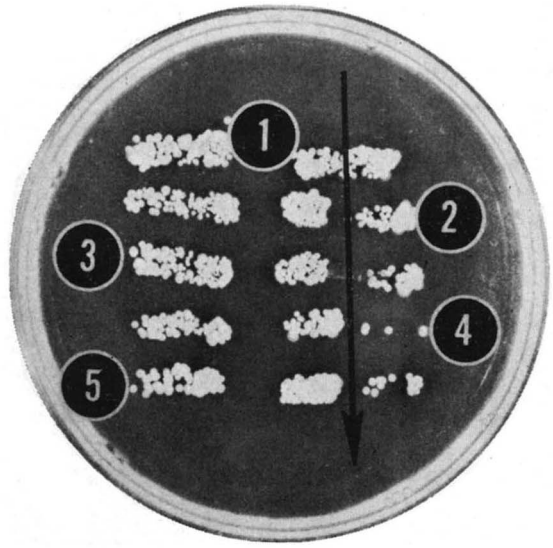

FIGURE 2.—Assay for susceptibility to actinophage. The arrow indicates the site of viral inoculum. Cross streak No. 1 is strain 210 (parental V^r); No. 2 is strain 202 (parental V^s); No. 3 is an incompletely susceptible recombinant; No. 4 and No. 5 are very susceptible recombinants.

to nutritional independence; (2) fusion of haploid nuclei to form diploid nuclei, which segregate immediately to form haploid recombinant nuclei; (3) fusion of haploid nuclei to form stable diploid nuclei; (4) gene recombination between diploid nuclei; (5) establishment of heterokaryosis which is perpetuated through heterokaryotic spores; (6) interactions between part of a nucleus and an intact nucleus to form a partially diploid nucleus that may or may not be stable. Spontaneous mutation is not responsible for the origin of the observed prototrophs because the rate of prototroph formation in two membered cultures exceeds greatly the rate at which the multiple marked parents singly revert to the wild type. Culture filtrates of individual parents or of mixed cultures do not increase significantly the rate of reversion; therefore the induction of mutations to prototrophy by products of one parent or products from interactions between the parents is improbable. Inasmuch as cell contact is necessary for prototroph formation, actinophage mediated transduction and nucleic acid mediated transformation are not responsible for the origin of the recombinants. At the present time, too few characteristics have been studied to rule out merozygosis (hypothesis No. 6) conclusively. Recovery of novel phenotypes and parental types from recombinants is inconsistent with simple haplophase gene recombination; however the possibility exists that these dissociates or segregants represent a unique fraction of the population or are an artifact resulting from unintentionally selective media and subjective selective biases.

SERMONTI and SPADA-SERMONTI (1956) noted that even on partially supplemented media, prototrophic recombinants constituted an overwhelming proportion of the recombinant population. BRADLEY and ANDERSON (1958) and BRAD-

LEY, ANDERSON and JONES (1959) found that when actinophage susceptibility or streptomycin susceptibility was used as an unselected marker, all of the recombinants were sensitive. If haplophase recombination were operative, these findings would be interpreted in terms of linkage. The present investigation demonstrated that in crosses involving a streptomycin sensitive parent and a resistant parent, the recombinants were antibiotic sensitive regardless of how the nutritional requirements and drug susceptibility were coupled in the parents. Similar results were obtained with respect to actinophage susceptibility.

These results are contrary to those reported by BRAENDLE and SZYBALSKI (1957) who reported that streptomycin susceptibility seemed to be linked very strongly to glutamic acid. Inasmuch as BRAENDLE and SZYBALSKI did not cross the two streptomycin resistant strains, it is not established that the two resistant strains were the result of identical mutational events. Moreover, streptomycin sensitivity was assumed to be invariably dominant to resistance but no supporting data were provided. The crosses reported by these workers therefore are not valid reciprocal crosses.

It must be emphasized that even though sensitive by sensitive and resistant by resistant crosses were performed in this investigation, the sameness of the mutations for resistance has not been unquestionably established. These results do show that simple haplophase gene recombination is not the principal genetic process in this system. These data do not differentiate between stable diploidy and stable heterokaryosis. The consistent recovery of parental dissociates and dissociates having novel phenotypes, and the variable expressivity of unselected markers is indicative of a complex series of genetic interactions (BRADLEY 1959b).

HOPWOOD (1959) working with another strain of *S. coelicolor* has found complementary recombinant phenotypes in equal numbers among the progeny of crosses. The discrepancies between his results and the results reported here may be a manifestation of strain differences. As HOPWOOD has pointed out, *S. coelicolor* is in fact a heterogeneous group of organisms. Genuine strain-to-strain differences in behavior, therefore, are not unexpected.

SUMMARY

Complementary, growth-factor dependent, mutant pairs of *Streptomyces coelicolor* interacted to form growth-factor independent recombinants. When both parents were streptomycin sensitive or actinophage sensitive, the selected prototrophic recombinants were sensitive. Similarly, when both parents were drug resistant or virus resistant, the recombinant progeny were resistant. When the parents differed with respect to susceptibility to streptomycin or actinophage, recombinants were sensitive, irrespective of the coupling in the parental strains. Failure to recover both unselected phenotypes in reciprocal crosses proved that haplophase gene recombination was not the principal process operative. These results are consistent with the hypotheses that the recombinants are heterokaryons or heterozygous diploids.

LITERATURE CITED

BRADLEY, S. G., 1957 Heterokaryosis in *Streptomyces coelicolor*. J. Bacteriol. **73**: 581–582.

1958a Genetic analysis of segregants from heterokaryons of *Streptomyces coelicolor*. J. Bacteriol. **76**: 464–470.

1958b Genetics of antibiotic-producing streptomycetes. Univ. Minn. Med. Bull. **30**: 146–154.

1959a Cross resistance patterns of mutants resistant to streptomyces—phages. Mycologia **51**: 125–131.

1959b Mechanisms controlling variation in streptomycetes. Annals. N.Y. Acad. Sci. **81**: 899–906.

BRADLEY, S. G., and D. L. ANDERSON, 1958 Compatibility system controlling heterokaryon formation in *Streptomyces coelicolor*. Proc. Soc. Exptl. Biol. Med. **99**: 476–478.

BRADLEY, S. G., D. L. ANDERSON, and L. A. JONES, 1959 Genetic interactions within heterokaryons of streptomycetes. Annals. N.Y. Acad. Sci. **81**: 811–823.

BRADLEY, S. G., and J. LEDERBERG, 1956 Heterokaryosis in Streptomyces. J. Bacteriol. **72**: 219–225.

BRAENDLE, D. H., and W. SZYBALSKI, 1957 Genetic interaction among streptomycetes: heterokaryosis and synkaryosis. Proc. Natl. Acad. Sci. U.S. **43**: 947–955.

1959 Heterokaryotic compatibility, metabolic cooperation, and genic recombination in Streptomyces. Annals. N.Y. Acad. Sci. **81**: 824–851.

HOPWOOD, D. A., 1959 Linkage and the mechanism of recombination in *Streptomyces coelicolor*. Annals. N.Y. Acad. Sci. **81**: 887–898.

LEDERBERG, J., and E. M. LEDERBERG, 1952 Replica plating and indirect selection of bacterial mutants. J. Bacteriol. **63**: 399–406.

SERMONTI, G., and I. SPADA-SERMONTI, 1955 Genetic recombination in Streptomyces. Nature **176**: 121.

1956 Gene recombination in *Streptomyces coelicolor*. J. Gen. Microbiol. **15**: 609–616.

1959 Genetics of *Streptomyces coelicolor*. Annals. N.Y. Acad. Sci. **81**: 854–861.