SHORT ARTICLES

GAIN OF ORNITHINE DECARBOXYLASE ACTIVITY BY A STRAIN OF *SHIGELLA FLEXNERI* SEROTYPE 6 ON THE INTRODUCTION OF AN R PLASMID

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SUMMARY. The introduction of R plasmid S-a into a strain of *Shigella flexneri* serotype 6 resulted in the strain gaining the ability to decarboxylate ornithine. The possible reasons for the phenomenon are discussed and the result is compared with that obtained previously when the introduction of R plasmids into a different strain of the same species resulted in enhanced gas production from a range of carbohydrates.

INTRODUCTION

The ability of plasmids to alter taxonomically important characters such as lactose fermentation (Falkow and Baron, 1962; Falkow *et al.*, 1964), citrate utilisation (Ishiguro and Sato, 1980) and H₂S production (Ørskov and Ørskov, 1973; Farmer *et al.*, 1976; Magalhães and Vêras, 1977) in members of the Enterobacteriaceae is widely documented. In a recent study (Dodd and Jones, 1981) we showed that gas production, a character important in the classification of *Shigella*, was affected by the presence of some R plasmids.

The ability to decarboxylate ornithine is another character important in the classification of *Shigella*. The currently recognised ornithine decarboxylase-positive members of the genus are strains of *S. sonnei* and of *S. boydi* serotype 13. Some ornithine decarboxylase-positive strains, which by other criteria could be accepted in the genus as new serotypes of *Shigella* species other than *S. sonnei*, have been described, e.g., *S. dysenteriae* provisional serotype 2000-53 (Edwards and Ewing, 1972). However, Edwards and Ewing (1972) suggest the exclusion of such strains from the genus on the basis of their ability to decarboxylate ornithine.

Where single characters are given discriminatory value in taxonomy, it is important that such characters are reliable and not readily altered, e.g., by the presence of plasmids. This paper describes the effect of an R plasmid on the ability of a strain of *S. flexneri* serotype 6 to decarboxylate ornithine.

MATERIALS AND METHODS

Bacterial strains. The test strains are listed in table I. The genotypes of the *Escherichia coli* strains and plasmids are also given in this table. The transconjugant strains derived from *S. flexneri* serotype 6 strain NCTC4760 (E89R)—strains E891, E896, E897, E898—were obtained by mating the parental type with the donor *E. coli* strains by the method described previously (Dodd and Jones, 1981).

Decarboxylase tests. The abilities of the test strains to decarboxylate four L-amino acids—ornithine, arginine, lysine and glutamine—were tested by method 2 of Cowan (1974). Decarboxylase Medium Base (Difco) 9g/L with 0.5% (w/v) of the appropriate amino acid hydrochloride was dispensed in 7-ml screw-capped bottles filled to the neck and sterilised at 121°C for 15 min. Before dispensing, the pH of the ornithine decarboxylase medium was

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TABLE I
Characters of test strains

Strain*	Designation	Genotype	Source
<i>Recipient strain</i>			
<i>S. flexneri</i> serotype 6 NCTC4760	E89R		National Collection of Type Cultures
<i>Donor strains</i>			
<i>E. coli</i> strain J53.1F ⁻ (R723)	E136	K-12(F ⁻) <i>pro</i> 22 <i>met</i> 63 <i>sup</i> <i>am</i> <i>nal</i> (<i>str</i> <i>tet</i> <i>cat</i> <i>sul</i>)	Dr R. Hedges†
<i>E. coli</i> strain J53.1F ⁻ (R724)	E137	K-12(F ⁻) <i>pro</i> 22 <i>met</i> 63 <i>sup</i> <i>am</i> <i>nal</i> (<i>str</i> <i>tet</i> <i>cat</i> <i>sul</i>)	Dr R. Hedges
<i>E. coli</i> strain J53.1F ⁻ (S-a)	E138	K-12(F ⁻) <i>pro</i> 22 <i>met</i> 63 <i>sup</i> <i>am</i> <i>nal</i> (<i>str</i> <i>cat</i> <i>kan</i> <i>sul</i>)	Dr R. Hedges
<i>E. coli</i> strain J62F ⁻ (R222)	E141	K-12(F ⁻) <i>lac</i> 28 <i>proC</i> 23 <i>his</i> 51 <i>trp</i> 30 (<i>str</i> <i>tet</i> <i>cat</i> <i>sul</i>)	Dr R. Hedges
<i>Transconjugant strains</i>			
<i>S. flexneri</i> serotype 6 E89R (R222)	E891		...
<i>S. flexneri</i> serotype 6 E89R (R723)	E896		...
<i>S. flexneri</i> serotype 6 E89R (R724)	E897		...
<i>S. flexneri</i> serotype 6 E89R (S-a)	E898		...

* Numbers in parenthesis, e.g., (R723), refer to plasmids used to produce transconjugants. The plasmids were isolated originally from *Shigella* spp.

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pro, *met*, *his*, *trp* = Requirement for, respectively, proline, methionine, histidine, tryptophan; *lac* = inability to produce acid from lactose; *sup* *am* = amber suppressor mutation; *str*, *tet*, *cat*, *sul*, *kan*, *nal* = the presence of genes encoding resistance to streptomycin, tetracycline, chloramphenicol, sulphonamide, kanamycin, nalidixic acid.

adjusted to 6.8 with 1N NaOH. Tests were incubated at 37°C and examined daily for 5 days for a positive decarboxylase reaction. All test cultures were compared with a basal medium control.

Estimation of similarity between transconjugant and parent strains. The four transconjugant strains (E891, E896, E897 and E898) and the parent strain were tested for 192 morphological, physiological and biochemical characters as described by Dodd and Jones (1982). The tests included the abilities of the strains to decarboxylate the four L-amino acids ornithine, arginine, lysine and glutamine. Gower's similarity coefficient (S_G ; Gower, 1971) was calculated between each transconjugant and the parent strain to determine their phenetic similarity. This was expressed as the percentage similarity (%S) between each of the transconjugant strains and the parent.

RESULTS

On the basis of 192 phenetic characters the percentage similarities between the parent strain *S. flexneri* serotype 6 (E89R) and the transconjugant strains were: E891, 94.8%S; E896, 94.5%S; E897, 94.8%S; E898, 94%S. This shows that there was a close phenetic similarity between the parent and progeny strains. The differences were due primarily to the changes in antibiotic sensitivities and, in the case of strain E898, by a change in ornithine decarboxylation.

The abilities of the parent and transconjugant strains to decarboxylate the four amino acids are shown in table II. All strains decarboxylated arginine. The parent strain and three of the transconjugants were unable to decarboxylate ornithine but strain E898 had gained this ability.

TABLE II

Decarboxylation of four L-amino acids by strains of Shigella flexneri serotype 6

Substrate	Decarboxylation by strain				
	E89R	E891	E896	E897	E898
Lysine	—	—	—	—	—
Arginine	++	+	++	++	++
Ornithine	—	—	—	—	+
Glutamine	—	—	—	—	—

++ = Strong positive; + = weak positive; — = negative.

The reaction was only weakly positive, but unequivocal and reproducible. The other two decarboxylase tests in which the parent strain E89R gave negative results remained negative in strain E898. Thus the change was specifically in ornithine decarboxylation and not, for example, a change in alkali production due to some other process, which would have affected equally the lysine and glutamine decarboxylase results.

DISCUSSION

The introduction of the R plasmid S-a into a strain of the Manchester variety of *S. flexneri* serotype 6 led to this strain gaining the ability to decarboxylate ornithine. The Manchester variety of *S. flexneri* 6 is aerogenic and the combination of this character with acquired ornithine decarboxylation ability presents a novel combination of characters in a member of *S. flexneri*. Such a strain might prove difficult to identify correctly by established identification schemes. The results also indicate that ornithine decarboxylation is not necessarily confined to strains of *S. sonnei* and *S. boydi* serotype 13 and that strains such as *S. dysenteriae* serotype 2000-53 may be considered as legitimate members of the genus.

There are several possible mechanisms by which the plasmid may have altered the ability of strain E89R to decarboxylate ornithine. A plasmid-encoded ornithine decarboxylase might have been present but this is unlikely because plasmid S-a is well characterised and we showed previously that its introduction into *S. flexneri* serotype 6 strain E90R did not produce the same

effect (Dodd and Jones, 1981). A second possibility is that the plasmid mobilised an ornithine decarboxylase gene from the *E. coli* chromosome and transferred it into the *Shigella* strain. The close phenetic similarity between strain E898 and its parent E89R suggests that a major genomic alteration such as might be expected in an *E. coli/Shigella* hybrid did not occur. If mobilisation of the *E. coli* chromosome had occurred, it would have had to entail incorporation of a small fragment specific for the enzyme. This may not be a reasonable supposition if the enzyme is induced by a positive control mechanism that requires the formation of a chromosomally-determined inducer.

A third possibility is that a cryptic ornithine decarboxylase gene exists in strain E89R and the introduction of the R plasmid S-a causes some non-specific change that results in induction of the enzyme. For example, if the strain lacks an appropriate transport system, the plasmid may cause a change in membrane permeability or may specify a transport system that could transport ornithine non-specifically, thus allowing low levels of ornithine to enter the cell and induce the enzyme. Strain E898 gives only a weak ornithine decarboxylase reaction which suggests that it is dependent on the occurrence of non-specific activities. Cryptic enzyme systems that are occasionally expressed are known in *Shigella*, e.g., the β -galactosidase gene is cryptic in *S. dysenteriae* serotype 1, but lactose-fermenting colonies sometimes develop (Edwards and Ewing, 1972).

In *E. coli*, ornithine may be decarboxylated by an alternative pathway involving arginine dihydrolase in certain conditions of growth (Greenberg, 1969). Therefore, a final possibility is that ornithine decarboxylation in the plasmid-carrying strain occurs by an alternative pathway as a result of the combined non-specific reactions of plasmid- and chromosomally-determined enzymes. An example of this concerning raffinose fermentation has been reported previously. When a *lac* plasmid is introduced into a host normally capable of fermenting sucrose, e.g., *Yersinia enterocolitica*, or a plasmid coding for sucrose fermentation is introduced into a strain normally capable of lactose fermentation, e.g., *E. coli*, non-specific activities of the lactose permease and sucrose invertase enable raffinose fermentation to occur (Cornelis, Luke and Richmond, 1978). Such reactions are generally weaker than those produced by the specific enzyme system.

It is interesting that the same plasmid has produced different phenetic character changes in two *S. flexneri* serotype 6 strains: the change in ornithine decarboxylation ability and the previously reported change in gas production from some carbohydrates (Dodd and Jones, 1981). This could be an effect based on strain variation similar to that seen in *E. coli* K-12, where the change to a requirement for purine when certain N plasmids were introduced has been shown to be strain dependent (Waleh and Stocker, 1981). Alternatively, it may reflect a greater taxonomic difference between the *S. flexneri* serotype 6 varieties than has previously been supposed. The taxonomic study by Dodd and Jones (1982) would support this suggestion. Whichever of these possibilities is the correct explanation, it is clear that the effect of a plasmid on the phenotype of its host may depend as much upon host-determined characters as on the genes carried by the plasmid.

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