SHORT ARTICLES

ENHANCEMENT OF GAS PRODUCTION IN A STRAIN OF *SHIGELLA FLEXNERI* SEROTYPE 6 BY R PLASMIDS

CHRISTINE E. R. DODD AND DOROTHY JONES

*Department of Microbiology, School of Medical Sciences, University of Leicester, Leicester
LE1 7RH*

SUMMARY. The introduction of four different R plasmids into an aerogenic strain of *Shigella flexneri* serotype 6 resulted in changes in the amount of gas produced and in the range of carbohydrates from which this occurred. The possible causes of these changes and their implications for bacterial identification are discussed.

INTRODUCTION

Members of the genus *Shigella*, as currently defined, do not produce gas from glucose except for some biotypes of *Shigella flexneri* serotype 6 (Enterobacteriaceae Subcommittee, 1958). These biotypes were originally designated the "Newcastle bacillus" (Clayton and Warren, 1929) and the "Manchester bacillus" (Downie, Wade and Young, 1933). The Newcastle biotype ferments glucose and dulcitol, often with the production of gas, and the Manchester biotype produces acid and gas from glucose, dulcitol and mannitol. Subsequently other aerogenic strains of the genus *Shigella* have been described; these include a variant of *S. dysenteriae* serotype 3 (Stypulkowska, 1964), a strain of *S. boydi* serotype 13 (Rowe, Gross and van Oye, 1975), and a "gasogenic variety" of *S. dysenteriae* serotype 2000-53 (Szturm-Rubinsten *et al.*, 1970).

The occasional occurrence of unusual phenotypic characters amongst strains of a bacterial genus may be associated with the presence of a plasmid. Examples of this in enterobacteria include lactose fermentation by strains of *Salmonella* (Falkow and Barron, 1962) and *Proteus* (Falkow *et al.*, 1964) and H₂S production by strains of *Escherichia coli* (Ørskov and Ørskov, 1973; Magalhães and Vêras, 1977) and *S. sonnei* (Farmer *et al.*, 1976). The relationship, examined in this paper, between the presence of R plasmids and the production of gas by shigellae has not previously been reported.

MATERIALS AND METHODS

Bacterial strains. The test strains are listed in table I. The four transconjugant strains derived from *S. flexneri* serotype 6 strain NCTC9780 (E90R), strains E901, E906, E907 and E908, were obtained as described below. The genotypes of the donor *E. coli* strains and the plasmids are also given in table I.

Isolation of transconjugant strains. Overnight cultures in Nutrient Broth No. 2 (Oxoid) of the recipient strain *S. flexneri* serotype 6 (strain E90R) and the four *E. coli* donor strains (E136, E137, E138 and E141) were diluted 100-fold in fresh broth and incubated for 4–6 h.

Plates of Blood Agar Base (Oxoid) containing chloramphenicol 10 µg/ml and spread with 0.2 ml of a preparation of bacteriophage λ *vir* containing 3.4×10^9 particles/ml were flood-seeded with 1.5 ml of each donor culture. The bacteriophage λ *vir* was included as a counterselective agent against all donor strains. The plates were allowed to dry and then *S. flexneri* strain E90R was spotted on the lawns and the plates were incubated overnight at 37°C. Any colonies that

Received 22 Dec. 1980; accepted 19 Feb. 1981.

TABLE I
Characteristics of test strains

Strain*	Designation	Genotype†	Source
<i>Recipient strain</i> <i>S. flexneri</i> serotype 6 aerogenic Newcastle variety	E90R		NCTC9780
<i>Donor strains</i> <i>E. coli</i> strain: J53.1F ⁻ (R723) J53.1F ⁻ (R724) J53.1F ⁻ (S-a) J62 F ⁻ (R222)	E136 E137 E138 E141	K-12(F ⁻) <i>pro</i> 22 <i>met</i> 63 <i>sup</i> am <i>nal</i> (<i>str tet cat sul</i>) K-12(F ⁻) <i>pro</i> 22 <i>met</i> 63 <i>sup</i> am <i>nal</i> (<i>str tet cat sul</i>) K-12(F ⁻) <i>pro</i> 22 <i>met</i> 63 <i>sup</i> am <i>nal</i> (<i>str cat kan sul</i>) K-12(F ⁻) <i>lac</i> 28 <i>proC</i> 23 <i>his</i> 51 <i>trp</i> 30 (<i>str tet cat sul</i>)	Dr R. Hedges Dr R. Hedges Dr R. Hedges Dr R. Hedges
<i>Transconjugant strains</i> <i>S. flexneri</i> serotype 6 E90R (R222) E90R (R723) E90R (R724) E90R (S-a)	E901 E906 E907 E908		See text See text See text See text

* Numbers in parentheses, e.g., (R723) refer to plasmids used to produce transconjugants.

† *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, naladixic acid.

grew on these plates were treated as presumptive transconjugants. They were streaked on to chloramphenicol plates and individual colonies were then tested for growth on a selective minimal medium that allowed growth of the donor but not the recipient strain. The M9 minimal medium of Clowes and Hayes (1968) was supplemented with proline and methionine for presumptive transconjugants derived from matings with *E. coli* strains E136, E137 and E138 and with proline, tryptophan and histidine for those derived from matings with *E. coli* strain E141. All supplements were added to give a final concentration of 50 µg/ml.

Any colonies that grew on the chloramphenicol medium but not on the selective minimal media were assumed to be transconjugant strains of *S. flexneri*. They were designed E901, E906, E907, E908 (table I).

Computation of similarity between transconjugant strains and parent strain. The four transconjugant strains (E901, E906, E907, E908) and the parent strain (E90R) were tested for 192 morphological, physiological and biochemical characters (Dodd, 1979). These tests included the production of gas from adonitol, dulcitol, glucose, glycerol, inositol, maltose, mannitol and starch during incubation for 14 days at 37°C. Gas production from carbohydrates was tested in a medium containing Bacteriological Peptone (Oxoid) 10 g/L, NaCl 5 g/L and bromothymol blue (E. Gurr Ltd) 0.04 g/L; after sterilisation a 10% (w/v) filter-sterilised solution of the carbohydrate was added aseptically to give a final concentration of 1% (w/v). The medium was dispensed in 4-ml amounts in 4 × 0.5-inch test tubes containing an inverted Durham tube. Before inoculation the Durham tubes were inspected for any trace of trapped air. The tubes were incubated at 37°C and inspected daily for 14 days for production of acid and gas.

Gower's similarity coefficient (S_G ; Gower, 1971) between the parent strain and each transconjugant strain was calculated to determine the phenetic similarity and expressed as percent similarity (%S) between each of the transconjugant strains and the parent.

RESULTS

The percent similarity between the transconjugant strains and *S. flexneri* serotype 6 strain E90R based upon 192 phenetic characters were: strain E901, 96.4%S; strain E906, 96.6%S; strain E907, 96.4%S; strain E908, 95.3%S. These indicate a close phenetic relationship between the parent strain and each transconjugant. The differences are accounted for by the antibiotic resistances conferred by the plasmids acquired from the donor strains (see table I) and the changes in gas production from carbohydrates.

The differences in gas production from the various carbohydrates between the parent strain *S. flexneri* serotype 6 strain E90R and the transconjugant strains are given in table II. The parent strain (E90R) produced only a small bubble of gas from dulcitol, but all the transconjugant strains produced significantly more gas from this carbohydrate; the bubble filled the whole curve of the Durham tube. Moreover, the transconjugant strains produced gas from some other carbohydrates (table II). The pattern of gas production from the test carbohydrates was different for each transconjugant. The amount of gas produced and the range of substrates

TABLE II
Gas production from carbohydrates by strains of Shigella flexneri serotype 6

Substrate	Gas production by strain				
	E90R	E901	E906	E907	E908
Dulcitol	+	+++	+++	+++	+++
Glucose	—	—	—	++	—
Glycerol	—	++	++	++	—
Maltose	—	++	—	++	++

+ = Small bubble of gas; ++ = moderate gas production; +++ = gas production sufficient to fill curvature of Durham tube; — = no gas production.

from which this occurred were altered by the introduction of R plasmids into *S. flexneri* serotype 6 strain E90R.

In similar experiments (see *Methods*) the same plasmids were introduced into a strain of the Manchester variety of *S. flexneri* serotype 6 (NCTC4720) and into an anaerogenic "Newcastle" strain (NCTC9781) but there was no change in gas production. Any increase in the amount of gas produced by transconjugants of the "Manchester" strain (NCTC4720) might have been masked because the parent strain produced significant amounts of gas. However, there was no change in the range of carbohydrates from which gas was produced.

DISCUSSION

Our results show that the introduction of four different R plasmids into an aerogenic strain of *S. flexneri* serotype 6 altered its ability to produce gas from carbohydrates. This is another example of a plasmid affecting a taxonomically important character in a way that might lead to the misidentification of a bacterial isolate. It is unlikely in this case that such changes in gas production would lead to the misidentification of strains of *S. flexneri* serotype 6 because aerogenic strains of *S. flexneri* serotype 6 are known and they may produce large amounts of gas (Carpenter, 1961). However, similar changes might be produced in other biotypes and serotypes of *Shigella* normally considered to be anaerogenic. Identification problems would then arise.

When Carpenter (1961) observed that recent isolates of aerogenic strains of *S. flexneri* serotype 6 produced much larger amounts of gas than the characteristic "very small volume (or tiny bubble)", she suggested that the change was due to the use of modern peptones. However, the present studies suggest that it may have been the presence of R plasmids that caused the changes, because Carpenter's (1961) observations coincided with the increasing prevalence of plasmids conferring resistance to antibiotics amongst enteric bacteria (Watanabe, 1963).

The mechanism by which gas production by *S. flexneri* serotype 6 is altered by these R plasmids is not clear. The presence on each plasmid of a gene, or genes, determining the character, seems unlikely because the transfer of these plasmids to two other strains of *S. flexneri* serotype 6 did not result in any change in gas production. Furthermore, it is unlikely that four different R plasmids would influence the same character. More complex non-specific interactions between the R plasmid and the host bacteria are a more probable explanation. These could result in alterations in membrane permeability or changes in the enzymes of the metabolic pathways.

The effect that the presence of plasmids may have on bacterial taxonomy because they encode for characters used in classification and identification is only now being investigated (Jones, 1978; Dodd, 1979; Harwood, 1980). Our work indicates that there may be other aspects to this problem and that a plasmid may influence characters that are not specifically plasmid encoded. Such "plasmid-influenced" characters may be as important in the identification of bacterial strains as "plasmid-encoded" characters and may be more difficult to identify because their expression may be influenced by the intracellular organisation of the host bacterium.

C.E.R.D. is indebted to the Medical Research Council for a research studentship. We thank Dr R. W. Hedges (Royal Postgraduate Medical School, Hammersmith Hospital, London) for the strains of *E. coli* and the plasmids.

REFERENCES

- CARPENTER, K. P. 1961. The relationship of the Enterobacterium A12 (Sachs) to *Shigella boydii* 14. *J. gen. Microbiol.*, **26**, 535.
CLAYTON, F. H. A. AND WARREN, S. H. 1929. An unusual bacillus recovered from cases presenting symptoms of dysentery. *J. Hyg., Camb.*, **28**, 355.
CLOWES, R. C. AND HAYES, W. 1968. Experiments in Microbial Genetics, Blackwell Scientific Publications, Oxford.

- DODD, C. E. R. 1979. A taxonomic study of the genus *Shigella* with reference to the influence of R plasmids. Ph.D thesis, University of Leicester.
- DOWNIE, A. W., WADE, E. AND YOUNG, J. A. 1933. An organism resembling the Newcastle type of dysentery bacillus associated with cases of dysentery. *J. Hyg., Camb.*, **33**, 196.
- ENTEROBACTERIACEAE SUBCOMMITTEE OF THE NOMENCLATURE COMMITTEE OF THE INTERNATIONAL ASSOCIATION OF MICROBIOLOGICAL SOCIETIES. 1958. Supplement to the third Report of the Shigella Group. *Int. Bull. bact. Nomencl. Taxon.*, **8**, 93.
- FALKOW, S. AND BARON, L. S. 1962. Episomic element in a strain of *Salmonella typhosa*. *J. Bact.*, **84**, 581.
- FALKOW, S., WOHLHIETER, J. A., CITARELLA, R. V. AND BARON, L. S. 1964. Transfer of episomic elements to *Proteus*. II. Nature of lac⁺ *Proteus* strains isolated from clinical specimens. *J. Bact.*, **88**, 1598.
- FARMER, J. J., RIDDLE, C. F., STARGEL, M. D., IIDA, H., AIKAWA, T., ACHANZAR, D. AND TAYLOR, W. I. 1976. Unusual Enterobacteriaceae: H₂S⁺ *Shigella sonnei*, one authentic and one false positive due to contamination with the obligate anaerobe *Eubacterium lentum*. *J. clin. Microbiol.*, **3**, 206.
- GOWER, J. C. 1971. A general coefficient of similarity and some of its properties. *Biometrics*, **27**, 857.
- HARWOOD, C. R. 1980. Plasmids. In Microbial classification and identification, edited by M. Goodfellow and R. G. Board, Academic Press, London, p. 27.
- JONES, D. 1978. Composition and differentiation of the genus *Streptococcus*. In Streptococci, edited by F. A. Skinner and L. B. Quesnel, Academic Press, London, p. 1.
- MAGALHÃES, M. AND VÉRAS, A. 1977. Properties of pPE 1572 (Hys-Raf), a plasmid governing hydrogen sulphide production and raffinose fermentation in *Escherichia coli*. *J. gen. Microbiol.*, **99**, 445.
- ØRSKOV, I. AND ØRSKOV, F. 1973. Plasmid-determined H₂S character in *Escherichia coli* and its relation to plasmid-carried raffinose fermentation and tetracycline resistant characters. *J. gen. Microbiol.*, **77**, 487.
- ROWE, B., GROSS, R. J. AND VAN OYE, E. 1975. An organism differing from *Shigella boydii* 13 only in its ability to produce gas from glucose. *Int. J. syst. Bact.*, **25**, 301.
- STYPULKOWSKA, H. 1964. An untypical strain of *Shigella dysenteriae* 3 isolated in Poland. *Medycyna dośw. Mikrobiol.*, **16**, 147 (in Polish).
- SZTURM-RUBINSTEN, S., PIÉCHAUD, D., D'HAUTEVILLE, H. AND SARRAT, H. 1970. Gasogenic variety of the *Shigella* provisional serotype 2000-53. *Annls Inst. Pasteur, Paris*, **119**, 320 (in French).
- WATANABE, T. 1963. Infective heredity of multiple drug resistance in bacteria. *Bact. Rev.*, **27**, 87.