SHORT COMMUNICATION

Plasmid-specified Sucrose Fermentation in *Salmonella arizonae*

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Thirty cultures of *Salmonella arizonae* 47:r:253 (Ar 23:24-25) were isolated over 7 months from the faeces of a captive reptile. All were unusual in their inability to produce a positive *o*-nitrophenyl- β -D-galactosidase reaction, and in their ability to ferment sucrose. These *S. arizonae* carried a plasmid having a molecular mass of 72 megadaltons which specified tetracycline resistance and a plasmid of 5 megadaltons which coded for the ability to ferment sucrose. The small size of this sucrose plasmid clearly distinguishes it from others which have been reported.

INTRODUCTION

Salmonellae are normally described as being unable to ferment sucrose. In the few isolates which have been able to utilize this disaccharide, the property has been shown to be plasmid-specified (Le Minor *et al.*, 1973; Wohlhieter *et al.*, 1975). Plasmid-specified sucrose utilization has also been shown in *Escherichia coli* (Williams Smith & Parsell, 1975; Palchaudhuri *et al.*, 1977). This communication reports the isolation from a reptile host of *Salmonella arizonae* carrying a 5 megadalton (Mdal) plasmid encoding sucrose utilization.

METHODS

Thirty cultures of *S. arizonae* 47:r:253 (Ar 23:24-25) were obtained during 7 months from the faeces of a captive gila monster, *Heloderma suspectum*. All fermented sucrose (Scr⁺) (Edwards & Ewing, 1972), were capable of growth on Mueller-Hinton agar (Difco) containing tetracycline (Sigma) at 16 μ g ml⁻¹, and gave a negative *o*-nitrophenyl- β -D-galactosidase reaction (Le Minor & Ben Hamida, 1962; Lowe, 1962). Selected *S. arizonae* Scr⁺ Tc^R isolates were used as donors in conjugations with *E. coli* Cold Spring Harbor Laboratory strains CSH31 (F⁻ Trp⁻ Thi⁻), CSH7 (F⁻ Lac⁻ Trp⁻ Str^R Thi⁻) and CSH65 (F⁻ Leu⁻ Lac⁻ Nal^R Str^R Thi⁻). Equal volumes of donor (1×10^8 cells ml⁻¹) and recipient (2×10^8 cells ml⁻¹) in antibiotic assay medium D (BBL) were mixed and incubated at about 22 °C for 24 h. This temperature was used since the *S. arizonae* had been isolated from poikilothermic animals housed at room temperature. The selection medium was minimal agar (Curtiss, 1965) supplemented with appropriate amino acids. For selection and counterselection in CSH31 conjugations, tetracycline (8 μ g ml⁻¹) and lactose (0.2%, w/v) were used, and in CSH7 and CSH65 conjugations, sucrose (2%, w/v) and streptomycin (2 mg ml⁻¹) were used.

Plasmids were eliminated using novobiocin which was added in serial dilution to antibiotic assay medium D. A 25 μ l portion of a culture containing approximately 10^8 cells ml⁻¹ was inoculated into each 5 ml novobiocin dilution and incubated at 37 °C overnight. The tube displaying growth with the lowest novobiocin dilution was plated for single colonies which were then tested for retention of Scr⁺ and Tc^R phenotypes.

Plasmid DNA was isolated, subjected to electrophoresis and visualized according to the method of Meyers *et al.* (1976). The average molecular mass was determined by comparison with the relative mobilities of reference plasmid DNAs.

RESULTS AND DISCUSSION

The 30 cultures of *S. arizonae* 47:r:253 isolated from the faeces of the gila monster during 7 months were indistinguishable on the basis of colonial morphology or biochemical and serological properties. All 30 isolates differed from the majority of *S. arizonae* by their inability to produce a positive *o*-nitrophenyl- β -D-galactosidase reaction (Edwards & Ewing, 1972).

Three cultures (S_1 , S_2 , S_3) were selected at random and were conjugated with CSH31, CSH7 and CSH65. Transconjugants were obtained in the CSH31 conjugations using tetracycline selection at a frequency of approximately 10^{-6} transconjugants per recipient cell; all were tetracycline-resistant but none fermented sucrose. However, in conjugations with CSH7 or CSH65 using sucrose selection, transconjugants occurred at a frequency of about 10^{-5} per recipient cell and were all both tetracycline-resistant and sucrose-fermenting. These data suggested plasmid specification of sucrose fermentation and tetracycline resistance by separate plasmids, and that conjugal transfer of the plasmid specifying sucrose fermentation was dependent on the transfer of the plasmid specifying tetracycline resistance.

Further evidence for the specification of sucrose fermentation and tetracycline resistance by separate plasmids was provided by the different susceptibilities of the phenotypes to novobiocin elimination. The ability to ferment sucrose could not be eliminated from S_1 , S_2 , S_3 , or $S_2 \times$ CSH7 transconjugants using novobiocin. Tetracycline resistance, however, was eliminated from S_2 , and $S_2 \times$ CSH7 and $S_2 \times$ CSH31 transconjugants in approximately 5% of cells tested and from S_1 and S_3 in approximately 99% of cells tested. Elimination from S_1 , S_2 and S_3 required $171 \mu\text{g}$ novobiocin ml^{-1} and from transconjugants required $47 \mu\text{g}$ novobiocin ml^{-1} .

Agarose gel electrophoresis was used to investigate the plasmid complement in S_1 , S_2 and S_3 , in transconjugants, and in derivatives cured of tetracycline resistance. These experiments indicated that sucrose fermentation and tetracycline resistance were specified by plasmids having molecular masses of approximately 5 Mdal and 72 Mdal, respectively.

Isolates S_1 , S_2 and S_3 possessed both plasmids. Transconjugants which were only tetracycline-resistant carried the 72 Mdal plasmid but transconjugants which were tetracycline-resistant and fermented sucrose carried both plasmids. Derivatives from which tetracycline resistance had been eliminated but which retained the ability to ferment sucrose carried only the 5 Mdal plasmid.

The 5 Mdal plasmid responsible for sucrose fermentation in *S. arizonae* is small compared with other reported sucrose plasmids such as those of approximately 51 Mdal and 164 Mdal in *Salmonella typhimurium* and *Salmonella tennessee*, respectively (Wohlhieter *et al.*, 1975; Johnson *et al.*, 1976). In this latter case, the 164 Mdal plasmid also specified lactose utilization and was observed to segregate resulting in the isolation of a $\text{Lac}^+ \text{Scr}^-$ plasmid of approximately 148 Mdal. The Scr^+ phenotype was presumably specified by the eliminated 16 Mdal region. The sucrose plasmid of approximately 53 Mdal reported in *E. coli* (Palchaudhuri *et al.* 1977) is also much larger than that found in *S. arizonae*. None of the *S. arizonae* isolates utilized raffinose or lactose which were phenotypes previously found associated with plasmid-specified sucrose utilization (Williams Smith & Parsell, 1975).

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