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Salmonella crossness – a new serotype containing a new somatic (O) antigen, 67

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SUMMARY

A new salmonella subgenus I serotype is described. Strain C.S. 1608/73, serotype 67:r:1, 2, was isolated from activated sewage sludge and was assigned the name *S. crossness*. The serotype is biochemically atypical in its ability to utilize sucrose and this ability appears to be controlled by a transferable plasmid.

INTRODUCTION

Salmonellas identified by their somatic and flagellar antigens are described in the Kauffmann–White Scheme (Kauffmann, 1972) to which an annual supplement is published (Le Minor, Rohde & Rowe, 1975). In 1972, the scheme contained about 1600 serotypes.

Salmonellas may be grouped into four subgenera according to their biochemical reactions (Kauffmann, 1972). Names are now given only to new serotypes in subgenus I: in the remaining subgenera new serotypes are described by their antigenic structure. Most new serotypes possess known 'O' antigens but have combinations of flagellar (H) antigens not previously found in association with their particular O antigen. New O antigens are rarely found. *S. crossness* contains a somatic (O) antigen not previously described.

Salmonellas rarely utilize sucrose and Edwards & Ewing (1972) reported that out of 371 strains, only 2 (0·5 %) utilized this substrate. Subsequent studies (Le Minor *et al.* 1973, Smith & Parsell, 1975, Wohlhieter *et al.* 1975) showed that in some strains it was determined by a transferable plasmid.

MATERIALS AND METHODS

Source of strain

Strain C.S. 1608/73 was isolated from activated sludge received from Crossness Sewage Treatment Works on 22 March 1973. This works treats the crude sewage from a population of approximately 1·6 millions living in London south of the

Thames. The sample contained 600 salmonellas per 100 ml. *S. agona*, *S. bredeney* and *S. oranienburg* were also isolated.

Methods

Basic biochemical tests were carried out by the methods described by Cowan & Steel (1974): organic acid utilization tests by the method described by Kauffmann (1966).

Serological analysis was made using single factor sera produced at the Salmonella and Shigella Reference Laboratory, Colindale. All sera used had been checked against all known salmonella antigens and had been absorbed as required to ensure specificity (Kauffmann, 1972).

The method of Smith & Parsell (1975) was used to determine whether the ability of *S. crossness* to utilize sucrose (Sac^+) was transferable. The mixed culture of donor and recipient strains was incubated at 37° C without shaking for 24 hr. The recipient strains which were sucrose negative (Sac^-) were: *Escherichia coli* K 12 strain J53 *nal*^{r} *met pro* and *S. typhimurium* strain SL 692 *str*^{r} *met trp*. *S. crossness* was sensitive to streptomycin and nalidixic acid and grew on the minimal medium (Smith & Parsell, 1975) requiring no additional growth factors.

RESULTS

Biochemical reactions

Strain C.S. 1608/73 gave the biochemical reactions of the family Enterobacteriaceae and of *Salmonella* subgenus I, but proved atypical in that it fermented sucrose promptly. For this reason hydrogen sulphide production was not detected by triple sugar iron agar but was readily detected by lead acetate papers (Bulmash & Fulton, 1964; Bulmash, Fulton & Jiron, 1965).

Investigation of the utilization of sucrose

The ability of *S. crossness* to utilize sucrose was transferred during mixed culture to *E. coli* J53 and *S. typhimurium* SL 692. After 24 hr. at 37° C. about 10^{-2} bacteria of the former strain and about 10^{-4} bacteria of the latter strain acquired this ability. In turn the Sac^+ derivatives of the recipients were able to transfer the Sac^+ determinant in mixed culture. The sucrose-utilizing ability was not acquired when the recipients were grown in a sterile filtrate of a broth culture of *S. crossness*.

Serological reactions

The strain showed no reaction on slide and tube agglutination with antisera to salmonella somatic (O) antigens 1–66. Using the method and immunization schedule of Kauffmann (1972), an O antiserum was prepared in rabbits with a vaccine made from C.S. 1608/73. To the antiserum 0·01 % merthiolate was added as preservative.

The antiserum was tested against salmonella 'O' antigens 1–66, and against the α antigen (Stamp & Stone, 1944). The titre against the homologous strain was 1/5120. Somatic antigens 1–66 were negative at a titre of 1/40.

Absorption of the antiserum with a suspension of the homologous strain removed all agglutinins completely.

When received the strain was monophasic and was agglutinated by diagnostic dilutions of phase 1 antiserum r (*S. rubislaw*). Following incubation in a semi-solid medium containing antiserum r (*S. rubislaw*) a second phase was obtained which was agglutinated in diagnostic dilutions of phase 2 flagellar antiserum 1, 2 (*S. newport* var. *puerto-rico*). Tests with monospecific phase 2 flagellar antisera 2 (*S. paratyphi* B), 5 (*S. thompson*), 6 (*S. london*) and 7 (*S. bredeney*) showed that the strain possessed the factors, 1, 2.

CONCLUSION

S. crossness possessed a new somatic (O) antigen which has been designated 67 in succession to 66, the last new somatic antigen described (*S. brookfield*, Le Minor, Rohde & Rowe, 1973). The complete antigenic formula of *S. crossness* is 67:r:1, 2. *S. crossness* atypically utilizes sucrose; this ability is transferable and it seems likely to be plasmid-determined.

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