The Utilization of Pentoses and Pentitols by Different Biotypes of *Salmonella typhimurium*

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INTRODUCTION

Biotyping is useful for differentiating types in *Salmonella typhimurium*. Duguid *et al.* (1975) used a two-tier scheme to classify 2030 *S. typhimurium* strains from different countries and sources into 19 of 32 potential primary biotypes and into 144 full biotypes. One advantage of their system is that it allows the incorporation of further tests at the secondary level. Thus, new tests and substrates may be introduced and new biotypes may be described by further sub-type designations.

D-Xylose and L-rhamnose proved useful in the differentiation of biotypes of *S. typhimurium* at both the primary and secondary levels (Duguid *et al.*, 1975). The primary aim of this investigation, therefore, was to establish whether representatives of the series biotyped by Duguid *et al.* (1975) could be further discriminated into new sub-types by their reactions with the pentitols and pentoses other than D-xylose or L-rhamnose.

METHODS

Bacteria. The 120 strains of *S. typhimurium* examined were among those studied by Duguid *et al.* (1975) and represented 18 of the 19 known primary biotypes. Their single biotype 23 strain was not examined. The number of strains chosen from each biotype reflected approximately the size of the primary biotype groups of the larger series. The strains, isolated from 27 sources and 31 different countries, represented 66 different phage-types, 57 different full biotypes and 119 different phage-type/biotype combinations. Stock cultures were maintained on Dorset's egg medium (Cruickshank *et al.*, 1975).

Media and culture conditions. The basic minimal salts medium of Davis & Mingioli (1950) was used. For auxotrophic strains, growth factors were added to the basal medium: amino acids were added at $10\text{ }\mu\text{g ml}^{-1}$, nicotinamide at $0.6\text{ }\mu\text{g ml}^{-1}$ and other growth factors at the concentrations recommended by Meynell & Meynell (1965). Solutions of the carbohydrates, sterilized by filtration, were added to sterile basal medium to a final concentration of 0.3% (w/v), and then dispensed in approx. 20 ml portions in sterile plastic Petri dishes. The carbohydrates tested were: D-arabinose, D-ribose, D-xylose and L-rhamnose from BDH, and D-lyxose, L-arabinose, L-fucose, L-lyxose, L-xylose, D-arabitol, L-arabitol, ribitol (adonitol) and xylitol from Sigma; they were used without further purification. Bacteria from 24 h cultures on nutrient agar (Oxoid) were emulsified in saline (0.85% w/v) to give approx. 10^8 bacteria ml^{-1} and were then inoculated on to the plates using a 12-pronged stainless-steel inoculator. The plates were incubated at 37 °C and the presence or absence of growth was noted at 24 and 48 h, and after 4 and 7 days; 48 h was chosen as the time for definitive reading.

Peptone water (Oxoid; 1.5% w/v), containing bromocresol purple ($20\text{ }\mu\text{g ml}^{-1}$) and the test carbohydrate (0.5% w/v), was dispensed in 5 ml portions into 6 ml bottles and inoculated with approx. 5×10^7 bacteria. The bottles were incubated at 37 °C and examined for acid production (colour change from purple to yellow) at intervals during the first 48 h and then daily for 90 days. Strains were judged to be strong, weak or non-fermenters by the criteria of Duguid *et al.* (1975).

Phage-types. This information was supplied by Professor E. S. Anderson, Enteric Reference Laboratory, Central Public Health Laboratory, London.

Table 1. *Ability of 120 wild-type Salmonella typhimurium strains from 18 primary biotypes to utilize pentoses and pentitols as carbon source*

The primary biotypes (and number of strains in each biotype) tested were: 1(9), 2(5), 3(8), 4(1), 7(4), 9(4), 12(1), 17(20), 18(2), 19(5), 21(1), 25(14), 26(13), 27(3), 29(3), 30(2), 31(18) and 32(7).

Sugars not utilized by any strain of any biotype:	D-arabinose, D-lyxose, L-lyxose, L-xylose D-arabitol, L-arabitol, ribitol, xylitol
Sugars utilized by all strains of all biotypes:	L-arabinose, D-ribose, L-fucose
Sugars utilized by strains of some biotypes:	D-xylose by strains of biotypes 1, 2, 3, 4, 7, and 12 L-rhamnose by strains of biotypes 1, 2, 3, 4, 17, 18, 19, 25 and 26

RESULTS AND DISCUSSION

Among the 2030 wild-type strains of *S. typhimurium* examined by Duguid *et al.* (1975) three distinct phenotypes – strong fermenters, weak fermenters and non-fermenters – found on the basis of utilization of D-xylose and L-rhamnose. The strong fermenters (Xyl⁺, 935 strains; Rha⁺, 1677 strains) fermented D-xylose or L-rhamnose in less than 10 h in peptone water and utilized these substrates as sole carbon sources in Bitter medium (Bitter, Weigmann & Habs, 1926) in 24 h and on minimal agar in 48 h. Weak fermenters (Xyl^w, 1083 strains; Rha^w, 54 strains) fermented D-xylose or L-rhamnose in peptone water in 10 to 24 h but not in Bitter's medium or on minimal agar. Non-fermenters (Xyl⁻, 12 strains; Rha⁻, 299 strains) did not utilize these substrates in 48 h in any of the media tested, but from cultures of every strain 'late' mutational fermentation was always observed after incubation for 2 to 40 days or more.

My results (Table 1) agree with those of Duguid *et al.* (1975) for strains from biotypes 1 to 12 and strains from biotypes 1, 2, 3, 4, 9, 17, 18, 19, 25 and 26 which used, respectively, D-xylose and L-rhamnose as sole carbon source. Furthermore, it was confirmed that strains from biotypes 17 to 32 and strains from biotypes 12 and 27 fermented, respectively, D-xylose and L-rhamnose in 10 to 24 h in peptone water, although they were unable to use them as sole carbon source. Of the other substrates, only D-ribose, L-arabinose and L-fucose were utilized as sole carbon sources and they had no differentiating value because they were used and fermented promptly by all strains regardless of biotype.

Although unable to utilize D-arabinose or L-lyxose as a sole carbon source, all strains, regardless of biotype, fermented D-arabinose in 2 to 3 days and L-lyxose in 18 to 24 h. From late-fermenting cultures, mutants were isolated that used these carbohydrates as sole carbon source, and fermented L-lyxose in 6 h or D-arabinose in 18 to 24 h.

No other carbohydrate was used as sole carbon source by any wild-type strain, no continued incubation of the peptone water cultures for up to 90 days yield late (mutant) fermenters.

Other enterobacteria are more versatile in the use of these same substrates. *Klebsiella pneumoniae* strains, for example, possess inducible enzymes for the degradation of D-ribose, D-xylose, L-arabinose, D-arabitol and ribitol and readily produce mutants for growth on less common pentoses and pentitols such as D-arabinose, L-lyxose, L-xylose, L-arabitol and xylitol, converting them to intermediates in the degradative pathways of the native substrates (Mortlock & Wood, 1964; Mortlock, 1976). However *S. typhimurium* strains like *Escherichia coli* (Le Blanc, 1970), are refractive to the pentoses and pentitols.

There is little information on the utilization of the pentoses and pentitols by *S. typhimurium* and the results reported here are useful for they confirm and expand the results of Guttridge *et al.* (1969) who examined the single phage-type *S. typhimurium* strain LT-2 (Lille: 1948) which belongs to biotype 1dfj in the scheme of Duguid *et al.* (1975). My results

indicate that no extra biotype information would be gained by adding any other pentose or pentitol to the biotyping scheme of Duguid *et al.* (1975).

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