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Utilization of D-Xylose by Wild-Type Strains of *Salmonella typhimurium*

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Enzyme studies of strains of *Salmonella typhimurium* representing biotypes that utilized D-xylose rapidly (xylose strong) or slowly (xylose weak) showed that they were different in the utilization of D-xylose because the xylose-weak strains were deficient in the transport of D-xylose. This observation is consistent with the idea that strains of the different xylose-weak biotypes, e.g. biotypes 17 to 32, were descended from strains of xylose-strong types, particularly from biotype 1.

Salmonella typhimurium is the most commonly isolated serotype of *Salmonella*. Its distribution is widespread, and the epidemiological investigation of outbreaks requires sensitive methods of differential typing, the most useful of which is phage typing (1).

Ancillary methods of differentiation used in conjunction with phage typing increase the sensitivity of strain discrimination (2). Duguid et al. (6) have described a biotyping scheme for *S. typhimurium* in which 32 primary biotypes were recognized by the combinations of positive and negative reactions in five primary tests. Subtypes, defined within the primary biotypes by 10 secondary tests, allowed each strain to be assigned a full biotype coding (6).

One of the five primary tests in the new biotyping scheme is the test with Bitter (3) D-xylose medium. In their biotyping survey of 2,030 wild-type strains of *S. typhimurium*, Duguid et al. recognized 935 strains in the seven known Bitter D-xylose-positive biotypes (primary biotypes 1, 2, 3, 4, 7, 9, and 12). These strains, called xylose strong (Xyl^+), utilized D-xylose as sole carbon source in minimal salts medium within 24 h at 37°C and fermented D-xylose in less than 10 h in peptone water, i.e., gave a strong acid response (6). On the other hand, in the 12 known Bitter D-xylose-negative biotypes (primary biotypes 17, 18, 19, 21, 23, 25, 26, 27, 29, 30, 31, and 32) there were 1,095 strains that did not utilize D-xylose as sole carbon and energy source. In peptone water medium containing D-xylose, however, most (1,083) of them fermented D-xylose in 10 to 24 h, i.e., were xylose weak (Xyl^w), and gave a late acid response (6). Only 12 did not ferment D-xylose in 24 h, i.e., were xylose negative (Xyl^-), and gave no acid response (6). Thus, with regard to D-xylose utilization there were three phenotypes, strong, weak, and negative, among wild-type strains of *S. typhimurium*.

The primary aim of this study was an examination of representative Bitter xylose-positive, xylose-strong strains and Bitter xylose-negative, xylose-weak strains to determine the basis for differences in the utilization of D-xylose by these two major biotype groups.

MATERIALS AND METHODS

Bacteria. *S. typhimurium* strains were the xylose-strong strains S844 (biotype 1a), S2594 (2h), and S2677 (2h) and the xylose-weak strains S3029 (biotype 17a), S2626 (17bf), S6631 (25b), S2595 (29bf), S3300 (32b), and S2291 (32by) from the collection of wild-type strains biotyped by Duguid et al. (6). *Klebsiella aerogenes dalC2*, a mutant constitutive for the production of D-arabitol dehydrogenase, was derived from *K. aerogenes* W70 (4).

Media. The culture media used were casein-salts, a mineral salts medium (17) supplemented with 0.5% casein hydrolysate (vitamin free, salt free, acid hydrolyzed; Nutritional Biochemicals, Cleveland, Ohio), and peptone water medium containing (per liter) 10 g of Difco peptone and 5 g of NaCl. When required, media were supplemented with D-xylose (0.5%).

Growth conditions. For the determination of enzymatic activities, bacteria were cultured (i) on a rotary shaker (New Brunswick Scientific Co. Inc., New Brunswick, N.J.) in flasks (125 ml) containing casein-salts (10 ml) with or without D-xylose at 37°C for 12 h, and, after dilution, cultured again with the same fresh medium (10 ml) for a further 90 min; (ii) in screw-capped tubes (13 by 1.5 cm) containing peptone water (5 ml) with or without D-xylose and incubated for 15 or 24 h statically at 37°C (6).

Cell-free extracts. Bacteria were harvested from cultures by centrifugation at 12,000 × *g* for 10 min in a refrigerated centrifuge (Ivan Sorvall, Norwalk, Conn.) and washed once in ice-cold buffer [10^{-2} M tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.5) containing 10^{-3} M mercaptoethanol and 10^{-4} M $MgCl_2$]. The bacteria, in polyethylene tubes, were placed in the cup of a 10-kilocycle magnetoconstrictive oscillator (Raytheon Co., S. Norwalk, Conn.) containing 25 ml of water and disrupted sonically for 15 min.

Unbroken cells and cell debris were removed by centrifugation of the suspension at 27,000 × *g* for 15 min (8).

Determination of enzymatic activities. The assay for D-xylose isomerase (EC 5.3.1.5) was similar to that described for L-fucose isomerase (EC 5.3.1.3.), except that D-xylose was substituted for L-fucose, D-arabitol dehydrogenase (EC 1.1.1.11) for ribitol dehydrogenase (EC 2.2.2.56), and $MgCl_2$ for $MnCl_2$ (16). D-Xylulokinase (EC 2.7.1.17) was determined by measuring ADP formation with the pyruvate kinase-lactic acid dehydrogenase system following the procedure of Wilson and Mortlock (17). In these coupled assays, absorbancy changes due to the oxidation of reduced nicotinamide adenine dinucleotide (NADH) were measured spectrophotometrically at 340 nm with a Gilford model 2000 recording spectrophotometer (Gilford Instruments, Oberlin, Ohio). A unit of enzyme activity catalyzed the oxidation of 1 μmol of NADH per min. Specific enzyme activities were defined as units of enzyme per milligram of protein.

Protein determination. The protein content of extracts was measured with the biuret reagent (7).

D-Xylose transport. D-Xylose accumulation by whole cells was determined using D-[*U*-^{14}C]xylose, modified from a method described for *Escherichia coli* (5). Cells were grown in peptone water medium to midlogarithmic phase and induced for 1 h with D-xylose (0,015%). The cultures were washed and suspended to approximately 5 × 10^8 bacteria per ml in peptone water containing chloramphenicol (100 μg/ml). The assay was started by the addition of D-[*U*-^{14}C]xylose (0.2 μmol/ml, 0.124 μCi). Samples (0.5 ml) were removed at intervals to 30 min, and the cells were collected on Millipore membrane filters (25-mm, 0.45-μm), which were washed and dried, then suspended in scintillation fluid and counted on a Tri-Carb counter (Packard).

D-Arabitol dehydrogenase. The method used to partially purify D-arabitol dehydrogenase from *K. aerogenes dalC2* followed an earlier report (10). D-Arabitol dehydrogenase was assayed in the presence of D-xylulose (18).

Isolation of mutants. From the xylose-weak strains S3029, S2626, and S6631 xylose-strong mutants were isolated spontaneously by following the procedure outlined for the isolation of D-arabinose-utilizing mutants of wild-type *S. typhimurium* (14). Two xylose-strong mutants, S3300.Xyl$^+$1 and Xyl$^+$2, derived from the xylose-weak strain S3300, were supplied by Ruth Barker.

Chemicals. D-Xylulose was prepared chemically by refluxing D-xylose with dry pyridine (11). D-[*U*-^{14}C]xylose was purchased from the Radiochemical Centre, Amersham, England. Scintillation fluid (NE233) was from Nuclear Enterprises, Edinburgh. D-Xylose, ATP, and lactic acid dehydrogenase-pyruvate kinase mixture were purchased from Sigma Chemical Co., St. Louis, Mo., and NADH came from Calbiochem, Los Angeles, Calif. Nutrient broth, nutrient agar, and peptone media were purchased from Difco Laboratories, Detroit, Mich. In the transport experiments, Oxoid peptone water was used.

RESULTS

Wild-type xylose-strong strains. The results of enzyme assays performed on crude cell-free extracts of the xylose-strong strains S844, S2594, and S2677 for the presence of the D-xylose catabolic enzymes, D-xylose isomerase and D-xylulokinase, are presented in Table 1. The cell-free extracts of wild-type Xyl$^+$ *S. typhimurium* strains grown in casein-salts medium, but not induced with D-xylose, failed to use D-xylose and were deficient in both enzymes. On the other hand, cell-free extracts of the same

TABLE 1. *D-Xylose isomerase and D-xylulokinase specific activities in cell-free extracts of wild-type S. typhimurium strains*

Strain no.	Biotype	Xylose phenotype	Growth medium[a]	Enzyme sp. act[b]	
				D-Xylose isomerase	D-Xylulokinase
S844	1a	Strong	CH	<0.001	0.007
			D-Xyl·CH	0.017–0.060	0.320–1.040
S2594	2h	Strong	CH	<0.001	<0.001–0.047
			D-Xyl·CH[c]	0.102	0.360
S2677	2h	Strong	CH	<0.001	0.005
			D-Xyl·CH[c]	0.065	0.180
S2626	17bf	Weak	D-Xyl·CH[c]	0.006	0.023
S2595	29 bf	Weak	D-Xyl·CH[c]	0.005–0.009	0.031–0.033
S2291	32by	Weak	CH	NT	0.004–0.011
			D-Xyl·CH	<0.001	0.054

[a] Cells were grown in casein hydrolysate-salts medium alone (CH) or with D-xylose (D-Xyl·CH).

[b] Specific enzyme activities derived from crude cell-free extracts of strains are expressed as micromoles of NADH oxidized per minute per milligram of protein. Activities represent high and low values obtained in separate assay. <, No enzyme activity was detected, but must be less than the indicated value, NT, Not tested.

[c] When counts were performed on the D-Xyl·CH-grown cultures of the Xyl$^+$ strains S2594 and S2677, the numbers of viable cells present (90 × 10^8 and 73 × 10^8 bacteria per ml, respectively) were four- to fivefold higher than the cultures of the Xyl^w strains S2626 and S2595 grown similarly (i.e., 17 × 10^8 and 22 × 10^8 bacteria per ml, respectively). The cultures of S2626 and S2595 did not grow on xylose minimal agar, i.e., were Xyl^w.

wild-type Xyl$^+$ strains grown in casein-salts medium in the presence of D-xylose showed levels of D-xylose isomerase (0.017 to 0.102) and D-xylulokinase (0.180 to 1.040) elevated significantly above the levels found in the uninduced cells grown in casein-salts alone (Table 1). These data are consistent with the idea that both enzymes are induced in wild-type Xyl$^+$ bacteria either by D-xylose or by an intermediate derived from it. Another 18 wild-type strains representing the seven known Bitter D-xylose-positive biotypes were shown to be capable of synthesizing both D-xylose catabolic enzymes in the presence of D-xylose.

Wild-type xylose-weak strains. The data in Table 1 show also that the cell-free extracts of three typical xylose-weak strains S2626, S2595, and S2291, grown in casein-salts medium supplemented with D-xylose, had low levels of D-xylulokinase (0.023 to 0.054); these levels, nevertheless, were 10- to 50-fold higher than those from Xyl^w bacteria grown in casein salts. In some extracts prepared from Xyl^w bacteria grown in casein-salts plus D-xylose, no D-xylose isomerase was detectable (<0.001) under the assay conditions employed, whereas in other extracts of similarly grown Xyl^w bacteria trace levels of D-xylose isomerase (0.005 to 0.009) were detected at the limit of sensitivity of our assay. Experiments with another 38 Xyl^w strains from the 12 known Bitter D-xylose-negative biotypes showed similar findings, i.e., low levels of D-xylulokinase and no, or trace, levels of D-xylose isomerase after growth in the presence of D-xylose.

The finding that the Xyl^w strains did not yield colonies when plated on xylose minimal agar confirmed that the low levels of D-xylose isomerase and D-xylulokinase in extracts of the Xyl^w bacteria were not due to the presence of mutant Xyl$^+$ bacteria in the population of Xyl^w bacteria (Table 1).

A question to be answered was why, despite their low levels of D-xylose isomerase and D-xylulokinase, the xylose-weak strains fermented D-xylose in peptone-grown cultures when incubation was extended beyond 10 h at 37°C. For example, when the Xyl^w strains S2595 and S2626 were grown in D-xylose-containing peptone water under aerobic and static conditions similar to those used in the biotyping scheme to score their xylose phenotype (6), they produced acid as early as 15 h. We examined, therefore, cell-free extracts of S2595 and S2626 prepared from such D-xylose-containing peptone water cultures and showed that, at 15 and 24 h, D-xylose isomerase (0.005 to 0.012) and D-xylulokinase (0.034 to 0.500) were both present.

We had detected slight increases in the activities of both D-xylose isomerase and D-xylulokinase in xylose-weak strains grown in casein-salts in the presence of D-xylose as compared to the same strains grown in casein-salts alone; one possible explanation for these findings is that small amounts of D-xylose had penetrated the cell. If the xylose-weak strains have a deficiency in D-xylose transport, they should respond to high concentrations of D-xylose in the growth medium. Attempts were made to force the induction of higher levels of these enzymes in the Xyl^w strain S3029 by increasing the concentrations of D-xylose in the growth medium. The bacteria were grown in aerated casein-salts medium without D-xylose for 12 h and, after dilution with an equal volume of fresh medium containing D-xylose, they were incubated for a further 6 h. The results of this experiment, in which we measured only D-xylulokinase (Table 2), demonstrated that the specific activity of the D-xylulokinase increased from <0.003 to 0.358 as the D-xylose concentration was raised from 0 to 2%. The latter value was as high as values obtained in cell-free extracts of Xyl$^+$ strains grown in casein-salts with 0.5% D-xylose.

Furthermore, when the xylose-weak strain S3029 was grown in aerated peptone water cultures containing up to 4% D-xylose, evidence of its growth stimulation, probably due to the increased entry of D-xylose into the bacteria under the conditions of this experiment, was obtained (Fig. 1).

D-Xylose transport. After growth in peptone water and induction with D-xylose (0.015%) for 1 h, Xyl$^+$ strains accumulated D-[*U*-^{14}C]xylose

TABLE 2. *D-Xylulokinase activities and D-[U-^{14}C]-xylose accumulation by the xylose-weak strain S3029 of S. typhimurium*

D-Xylose in medium (%)	Sp act of D-xylulokinase[a]	D-[*U*-^{14}C]xylose accumulated (in 30 min[b])
0	0.003	602
0.5	0.016	860
1.0	0.108–0.192	874
2.0	0.124–0.358	744

[a] Growth conditions were as described in the text. Specific activities for crude cell-free extracts of S3029, after growth in casein-salts medium with D-xylose at the concentrations shown, are expressed as micromoles of NADPH oxidized per minute per milligram of protein.

[b] D-Xylose accumulation by whole cells was determined as described in the text, except that the concentration of D-xylose as inducer was increased from 0.015 to 0.5, 1.0, and 2.0% and the time of induction was extended to 2 h. Results are expressed as counts per minute per milliliter of assay mixture (see Table 3).

effectively; see, for example, strains S844 and S2954 in Table 3. When grown in peptone water alone, D-xylose accumulation was poor and the D-xylose accumulated probably as a result of diffusion. When Xyl^w strains were grown in peptone water, with D-xylose as inducer or without, their accumulation of D-xylose was low and similar to the accumulation by xylose-strong strains without induction (Table 3). Results for the Xyl^w strains S2595, S3300, and S6631 are shown in Table 3. Even when the D-xylose used as inducer was increased to concentrations (up to 2%) at which appreciable levels of D-xylulokinase had been synthesized by the Xyl^w strain S3029, the uptake of D-xylose remained poor. At no concentration tested, up to 2%, did the levels of D-xylose transport with S3029 (Table 2) approach the high levels achieved by the Xyl$^+$ strains such as S844, S2594, or S3300.Xyl$^+$1 (Table 3). Thus, the xylose-weak strains appeared to be deficient in their ability to transport D-xylose.

Xyl$^+$ mutants derived from wild-type Xyl^w strains. Xyl$^+$ mutant bacteria with properties of wild-type Xyl$^+$ strains were derived from the wild-type Xyl^w strains S3029, S2626, S6631, and S3300 by prolonged culture in aerated liquid D-xylose minimal medium. Enzymatic analyses were performed on cell-free extracts of the Xyl$^+$ mutants and their four wild-type Xyl^w parent strains after growth in casein-salts medium, with or without D-xylose. This was done to determine the enzymatic basis of

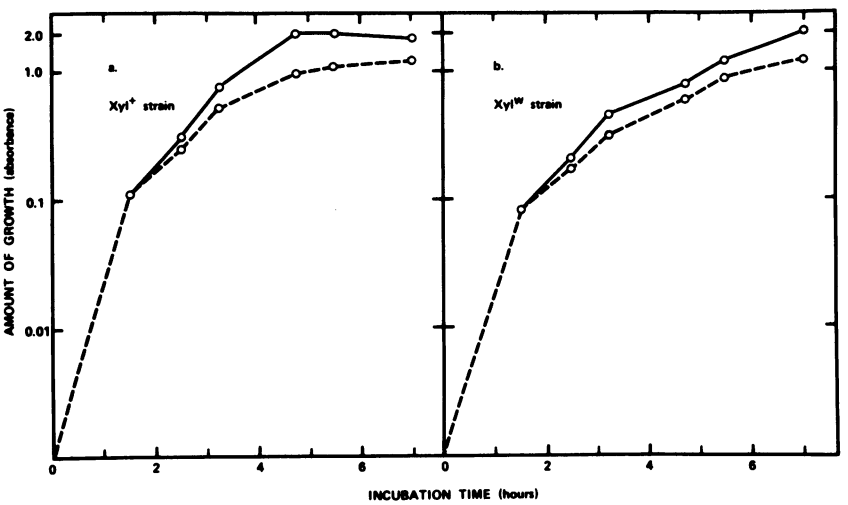

FIG. 1. *(a) Growth curve of Xyl$^+$ S. typhimurium S844. A culture of this strain, grown in peptone water and aerated with shaking, was divided after incubation for 1.5 h at 37°C into aliquots (50 ml), to which the following additions were made: distilled water (i.e., control)* (O-----O); *0.5%* (O——O). *(b) Growth curve of Xyl^w S. typhimurium S3029. This strain was processed as above, except that* D-*xylose* (O——O) *was added to the medium at a final concentration of 4%. Optical density measurements were made on a Spekker absorptiometer (Hilger Watts, London) with a red (no. 5) filter.*

TABLE 3. *Accumulation of D-[U-^{14}C]xylose by strains of S. typhimurium*[a]

Time of incubation (min)	Xylose-strong strains				Xylose-weak strains			
	S844		S2594 (XPW)	S3300·Xyl$^+$1[b] (XPW)	S2595		S3300 (XPW)	S6631 (XPW)
	PW	XPW			PW	XPW		
0	38	44	42	152	46	52	56	48
5	282	351	594	840	154	265	234	90
10	392	985	1,160	1,320	242	351	470	224
15	474	1,645	2,072	1,964	380	377	480	282
20	NT[c]	2,483	2,766	2,510	434	502	574	362
30	604	4,478	3,840	3,666	484	554	680	406

[a] Assay mixture contained, per milliliter, 5×10^8 bacteria, 100 μg of chloramphenicol, and 0.2 μmol of D-xylose (2.3×10^4 cpm/μmol). Activity is expressed as counts per minute per milliliter. Cells were grown in peptone water without induction (PW), or induced with 1 mM D-xylose (XPW) for 1 h.

[b] S3300·Xyl$^+$1, biotype 1b, is a xylose-strong mutant of the xylose-weak strain S3300.

[c] NT, Not tested.

the restored ability of the Xyl$^+$ mutants to ferment D-xylose promptly and use it as sole carbon source for growth. The results (Table 4) revealed that the mutations in the Xyl$^+$ mutants of the Xyl^w strains S3029, S6631, and S3300 were accompanied by gains in the ability of D-xylose to induce the synthesis of high levels of D-xylose isomerase and D-xylulokinase. These three Xyl$^+$ mutants, unlike their wild-type Xyl^w parents, also accumulated D-[^{14}C]xylose (see, for example, S3300.Xyl$^+$1 in Table 3). The mutation in the Xyl$^+$ mutant of strain S2626 was due apparently to constitutive synthesis of the enzymes which were formed at appreciable levels even when this mutant was cultured in casein-salts alone.

DISCUSSION

Our interest in D-xylose utilization by wild-type strains of *S. typhimurium* was influenced primarily by the knowledge that it had been a marker of such importance in the biotyping of *S. typhimurium* and the genealogy of its biotypes (6). Apart from L-rhamnose (6, 13), no other pentose or pentitol afforded such useful strain differentiation (13). Among wild-type strains, two major groups were recognized: (i) Xyl$^+$ strains using D-xylose as sole carbon source and fermenting it in peptone cultures in less than 10 h, and (ii) Xyl^w strains unable to use D-xylose as sole carbon source but fermenting it in peptone cultures in 10 to 24 h. Our enzymatic characterization of representative Xyl$^+$ and Xyl^w strains revealed differences between these two major groups of wild-type *S. typhimurium* strains that provided a probable explanation for their different responses to D-xylose.

The data presented in this paper indicate that D-xylose is metabolized by wild-type Xyl$^+$ strains of *S. typhimurium* by the action of an inducible D-xylose isomerase, converting D-xylose to D-xylulose, and D-xylulokinase, also inducible, converting D-xylulose to D-xylulose 5-phosphate, i.e., a mechanism similar to those known for other enterobacteria (5, 17).

In extracts of wild-type Xyl^w strains grown in the presence of D-xylose in aerated casein-salts medium, low levels of D-xylose isomerase and D-xylulokinase were found. When grown in D-xylose-containing peptone water in static cultures for up to 24 h, the Xyl^w strains synthesized levels of these enzymes that were probably sufficient to account for the weak fermentation of D-xylose and the late accumulation of acid products characteristic of Xyl^w strains. It may be that the relatively anaerobic conditions of static culture facilitate the entry of D-xylose into Xyl^w bacteria. The further observation that there were no Xyl$^+$ mutants in such late-fermented cultures of Xyl^w strains suggested that the low but detectable levels of enzymes present were products of intact structural genes for D-xylose isomerase and D-xylulokinase in Xyl^w strains.

If wild-type Xyl^w *S. typhimurium* strains are plated on D-xylose minimal medium they produce small colonies (diameter, 3 mm) after 7 to 10 days of incubation, although the bacteria retain their Xyl^w character when cultured to fresh D-xylose-containing media (D. C. Old, un-

TABLE 4. *D-Xylose isomerase and D-xylulokinase specific activities in cell-free extracts of wild-type, xylose-weak S. typhimurium strains and xylose-strong mutants derived from them*

Strain no.	Biotype	Xylose phenotype	Growth medium[a]	Enzyme sp act[b]	
				D-Xylose isomerase	D-Xylulokinase
S3029	17a	Weak	D-Xyl·CH	0.002–0.007	0.070
S3029·Xyl$^+$	1a	Strong	CH	NT	0.031
			D-Xyl·CH	0.028	0.186
S2626	17bf	Weak	D-Xyl·CH	0.002	0.060
S2626·Xyl$^+$	1bf	Strong	CH	0.021	0.218
			D-Xyl·CH	0.017	0.740
S6631	25b	Weak	D-Xyl·CH	NT	0.004–0.011
S6631·Xyl$^+$	9b	Strong	CH	0.002	0.050
			D-Xyl·CH	0.027	0.760
S3300	32b	Weak	D-Xyl·CH	<0.001–0.003	0.030–0.047
S3300·Xyl$^+$1	1b	Strong	D-Xyl·CH	0.031	0.620
S3300·Xyl$^+$2	16b	Strong	D-Xyl·CH	0.018	0.260

[a] Cells were grown in casein hydrolysate-salts medium alone (CH) or with D-xylose (D-Xyl·CH).

[b] Specific enzyme activities derived from crude cell-free extracts of strains are expressed as micromoles of NADH oxidized per minute per miligram of protein. Activities represent high and low values obtained in separate assays. <, No enzyme activity was detected, but must be less than the indicated value. NT, Not tested.

published data). This observation suggests that Xyl^w bacteria grow slowly in the presence of D-xylose in minimal medium by a gradual leakage of D-xylose into the cells. Further evidence that a barrier exists to the entry of D-xylose into Xyl^w cells was obtained from the findings that the Xyl^w strain S3029, grown in the presence of 2% D-xylose, contained high levels of D-xylulokinase (and presumably D-xylose isomerase) and that its growth was stimulated in the presence of high concentrations of D-xylose.

Such observations led us to believe that wild-type Xyl^w *S. typhimurium* strains might be deficient in a D-xylose transport system. This hypothesis was confirmed by experiments demonstrating the uptake of D-[^{14}C]xylose by Xyl$^+$ bacteria. Xyl^w bacteria were deficient in this ability. Furthermore, Xyl$^+$ mutants derived from wild-type Xyl^w bacteria synthesized high levels of D-xylose isomerase and D-xylulokinase in the presence of D-xylose and transported D-xylose. A deficiency in the ability to transport D-xylose would account for the ability of Xyl^w bacteria to use D-xylose only after prolonged incubation (either in peptone water or minimal medium), allowing leakage of D-xylose.

The genealogical tree previously presented (6) suggests the evolutionary routes whereby all known primary biotypes of *S. typhimurium* probably evolved from a presumed archetypal strain of biotype 1. Transduction studies (9) have indicated that the sites of the mutations in fimbriation and rhamnose utilization were homogeneous in all strains of the FIRN (i.e., *fim inl rha*) type from biotypes 29, 30, 31, and 32, and that these strains had a common ancestral origin. Speculation that FIRN strains of *S. typhimurium*, in turn, had descended from ancestral strains that were *fim*$^+$ *inl rha*$^+$ by successive mutations in fimbriation and rhamnose (9) received support from the observation that more than 90% of the strains in primary biotypes 25, 26, and 27 (*fim*$^+$ *inl rha*$^+$) and primary biotypes 29, 30, 31, and 32 (*fim inl rha*) showed conservation of the same temperature-sensitive inositol phenotype (12).

The most unlikely line of descent for strains of primary biotypes 25 to 32 would be from strains of the primary biotype 17 (*fim*$^+$ *inl*$^+$ *rha*$^+$), for all have the same Bitter xylose-negative phenotype (6). The finding that 42 wild-type strains from the 12 known Bitter xylose-negative primary biotypes, representing a total of 42 different phage type/biotype combinations, were similar when characterized enzymically is in line with that speculated line of descent. A more detailed presentation of the genealogical implications of the Bitter D-xylose phenotypes in *S. typhimurium* will be presented elsewhere.

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