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Fermentation Mechanism of Fucose and Rhamnose in *Salmonella typhimurium* and *Klebsiella pneumoniae*

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An equimolar amount of 1,2-propanediol was detected in the medium when *Salmonella typhimurium* or *Klebsiella pneumoniae* fermented L-fucose or L-rhamnose. These metabolic conditions induced a propanediol oxidoreductase that converted the lactaldehyde formed in the dissimilation of either sugar into the diol. The enzyme was further identified by cross-reaction with antibodies against *Escherichia coli* propanediol oxidoreductase. This indicates that L-fucose and L-rhamnose fermentation takes place in these species by 1,2-propanediol production and excretion.

Salmonella typhimurium and *Klebsiella pneumoniae* are able to grow aerobically on L-fucose (17, 18) or L-rhamnose (13, 16) as a sole source of carbon and energy. For the dissimilation of fucose, a pathway consisting of a fucose isomerase, a fuculose kinase, and a fuculose 1-phosphate aldolase has been described in *Klebsiella aerogenes* (18). A parallel pathway formed by a set of similar, but rhamnose-specific, enzymes, as shown for *Escherichia coli* (4, 8–10, 19, 20), is presumably responsible for the rhamnose metabolism in these cells. On the other hand, although no description of the fucose pathway has been made so far for *S. typhimurium*, a description of the rhamnose pathway has been published recently (1).

In the well-known pathway in *E. coli* the aldolase splits the sugar phosphate into dihydroxyacetone phosphate and L-lactaldehyde, which aerobically are dissimilated by general cellular metabolism. Anaerobically there is a need for the regeneration of oxidized NAD, and that is accomplished by the reduction of L-lactaldehyde to L-1,2-propanediol, which is excreted (6). In this paper the 1,2-propanediol fermentation system is analyzed in *S. typhimurium* and *K. pneumoniae*.

Cells of *S. typhimurium* LT2 and *K. pneumoniae* C3 were grown on fucose or rhamnose with generation times in the range of those described for *E. coli* K-12 (3). For aerobic conditions, cells were routinely grown in 2-liter flasks that contained 200 ml of minimal medium (2) plus 10 mM sugar; for anaerobic growth, cells were grown in 150-ml flasks filled to the top with minimal medium plus 20 mM sugar and gently stirred by a magnet. *S. typhimurium* presented under these conditions a generation time on fucose of 94 min aerobically and 180 min anaerobically. On rhamnose the doubling times were 110 and 190 min, respectively. *K. pneumoniae* presented similar doubling times on fucose, 80 min aerobically and 180 min anaerobically, whereas on rhamnose the doubling time was similar aerobically, 140 min, but somewhat longer anaerobically, 300 min.

Both species were found to excrete 1,2-propanediol when grown anaerobically on fucose or rhamnose. The medium was inoculated and grown under the indicated conditions to the end of the logarithmic phase. After the cells were removed by centrifugation, the 1,2-propanediol excreted was identified by paper chromatography in a solvent system consisting of propanol-ammonia (60:40) and with the α-1,2-diol specific staining of metaperiodate and benzidine (5). A spot with the same R_f of a 1,2-propanediol standard was detected under fucose or rhamnose fermentation conditions (data not shown).

The concentration of propanediol, estimated by the 1,2-propanediol-specific colorimetric method described by Jones and Riddick (11), was close to 20 mM, the concentration of sugar when the cells grew anaerobically (Table 1). This is consistent with a stoichiometry of one lactaldehyde per molecule of sugar corresponding to the aldolase-catalyzed reaction. Propanediol was virtually undetectable when the cells grew aerobically on fucose, rhamnose, or other sugars such as glucose (Table 1).

Fermentation of fucose and rhamnose through 1,2-propanediol production and excretion was in agreement with the presence of propanediol oxidoreductase in *S. typhimurium* and *K. pneumoniae* grown anaerobically on either methylpentose. After growth to late exponential phase, cells were collected by centrifugation and suspended in four times its weight of 10 mM Tris-hydrochloride buffer (pH 7.3). The suspended cells were sonically disrupted in a 60-W MSE sonicator for periods of 30 s/ml of cell suspension while chilled in a −10°C bath. Extracts were clarified at 100.000 × *g* for 30 min at 4°C. Propanediol oxidoreductase activity was subsequently determined in each extract by measuring at 25°C the NADH disappearance in an assay mixture (1 ml) that consisted of 2.5 mM L-lactaldehyde, 100 mM sodium phosphate buffer (pH 7.0), and 0.125 mM NADH. The three-carbon substrate was omitted from the blank mixture. All reactions were started by the addition of the enzyme. One unit of enzyme activity was defined as the amount of enzyme that transforms 1 μmol of substrate per min. Concentration of the protein in cell extracts was determined by the method of Lowry et al. (12).

Propanediol oxidoreductase was induced in *S. typhimurium* by anaerobiosis in fucose or rhamnose, but not in glucose or casein acid hydrolysate (Table 2). The presence of oxygen prevented the expression of the enzyme activity. We point out that the levels of activity were in this case in the same range of those determined for *E. coli* (2, 15). *K. pneumoniae* displayed also a pattern of induction of propanediol oxidoreductase with the same requirements of *S. typhimurium* or *E. coli*, although the level of activity was clearly lower. Differences in activities between the three species might be due to assay conditions, which were optimal for the *E. coli* enzyme. In a screening of the assay

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TABLE 1. Excretion of 1,2-propanediol as fermentation product of fucose or rhamnose by *S. typhimurium* and *K. pneumoniae*

Carbon source	Concn (mM)	Growth conditions	Concn of 1,2-propanediol excreted (mM)	
			S. typhimurium	*K. pneumoniae*
Fucose	10	Aerobic	0.1	0.2
Fucose	20	Anaerobic	18.0	19.6
Rhamnose	10	Aerobic	0.1	0.4
Rhamnose	20	Anaerobic	19.2	20.0
Glucose	20	Anaerobic	0.1	0.2

conditions no difference was found among the three enzymes, although the possibility for the existence of a special requirement for the *K. pneumoniae* enzyme is still open. Alternatively, species differences in expression and structure of propanediol oxidoreductase may obviously account for the activity differences.

The existence of propanediol oxidoreductase in *S. typhimurium* and *K. pneumoniae* was further confirmed by the presence of a protein in the extracts of cells grown under inducing conditions that cross-reacts with antibodies against the *E. coli* propanediol oxidoreductase raised in New Zealand White rabbits as indicated elsewhere (3). No cross-reaction was found with extracts of cells grown under noninducing conditions such as aerobic or anaerobic growth on glucose or glycerol. The results of the immunodiffusion experiments (Fig. 1), performed by the method of Ouchterlony (14), showed the formation of precipitin lines with extracts not only of *E. coli* but also of both *S. typhimurium* and *K. pneumoniae*. The precipitin line produced with the *S. typhimurium* enzyme intersected the *E. coli* band, forming a spur. This indicates that the antiserum reacts with a smaller number of antigenic determinants on the *S. typhimurium* propanediol oxidoreductase than on the *E. coli* enzyme. In contrast the precipitin lines produced with the *K. pneumoniae* and *E. coli* extracts fused, indicating a high degree of antigenic homology between these two enzymes (7, 21).

The excretion of 1,2-propanediol by anaerobic growth of *S. typhimurium* or *K. pneumoniae* in fucose or rhamnose is consistent with the reduction of lactaldehyde to propanediol as the fermentative mechanism for these two sugars. The concomitant induction of propanediol oxidoreductase, the key enzyme in this conversion, corroborates these fermentation pathways already described in *E. coli*. The high activity detected under aerobic conditions on fucose for *S. typhimurium*, a phenomenon also observed in *E. coli*, may reflect further similarities with this species where the control exercised by fucose on propanediol oxidoreductase is clearly different from that exercised by rhamnose (3). Experiments

TABLE 2. Activities of propanediol oxidoreductase in crude extracts of *S. typhimurium* and *K. pneumoniae* grown on different media

Carbon source	Growth conditions	Sp act (U/mg of protein)	
		S. typhimurium	*K. pneumoniae*
Fucose	Aerobic	0.14	0.04
Fucose	Anaerobic	0.45	0.15
Rhamnose	Aerobic	0.04	0.03
Rhamnose	Anaerobic	0.43	0.13
Glucose	Anaerobic	0.04	0.03
CAA[a]	Anaerobic	0.08	0.05

[a] Casein acid hydrolysate plus 1 mM pyruvate.

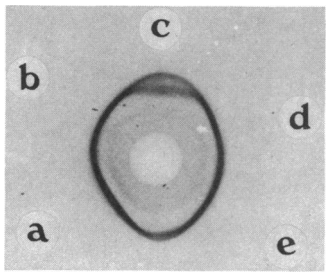

FIG. 1. Immunodiffusion of various propanediol oxidoreductase preparations. Wells in the Ouchterlony immunodiffusion plates contained 25 μl of extract (30 μg of protein) of cells of *E. coli* (b and e), *K. pneumoniae* (a and d), or *S. typhimurium* (c) grown anaerobically on fucose. The center well contained 25 μl of antiserum.

to study these similarities in the regulatory aspects and apparent differences with *K. pneumoniae* are in progress.

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