

BACTERIAL DISSIMILATION OF L-FUCOSE AND L-RHAMNOSE

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ABSTRACT

EAGON, R. G. (University of Georgia, Athens). Bacterial dissimilation of L-fucose and L-rhamnose. *J. Bacteriol.* **82**:548-550. 1961.—Of 33 microorganisms screened for the ability to utilize the methylpentoses, L-fucose and L-rhamnose, 18 species utilized one or both methylpentoses. The majority of the bacterial species capable of utilizing these methylpentoses did so by isomerization of the methylpentoses to the corresponding ketoses followed by phosphorylation of the ketoses. However, the existence of a second pathway is suggested by results obtained from *Sarcina lutea*, *Bacillus megaterium*, *Gaffkya tetragena*, and *Rhizobium leguminosarum*.

The naturally occurring methylpentoses, L-fucose and L-rhamnose, are found in widely divergent forms of life. To date, however, the metabolism of these sugars has not been extensively studied. The initial step in the dissimilation of L-rhamnose by *Escherichia coli* has been reported to be the conversion of L-rhamnose to its corresponding ketose, L-rhamnulose (Wilson and Ajl, 1957a; Tecce and DiGirolamo, 1956). It has been indicated also that the L-rhamnulose so formed by *E. coli* was phosphorylated to yield L-rhamnulose-1-phosphate (Wilson and Ajl, 1957b). A similar mechanism for L-rhamnose dissimilation was noted for *Pasteurella pestis*, *Salmonella typhimurium*, and *Salmonella typhosa* (Englesberg, 1957; Englesberg and Baron, 1959). The dissimilation of L-fucose by *E. coli* likewise has been reported to involve the conversion of L-fucose to L-fuculose which in turn is phosphorylated to yield L-fuculose-1-phosphate (Green and Cohen, 1956).

This communication reports the results of a comparative study of 15 species of bacteria capable of dissimilating L-fucose or L-rhamnose. Utilizing cell-free extracts prepared from these bacteria, the following problems were investigated: (i) the conversion of L-fucose or L-rham-

nose to L-fuculose or L-rhamnulose; and (ii) the subsequent phosphorylation of L-fuculose or L-rhamnulose.

MATERIALS AND METHODS

Media. A basal mineral medium was used throughout and contained (per liter): KH_2PO_4 , 6.8 g; KNO_3 , 10.0 g; $(\text{NH}_4)_2\text{SO}_4$, 10.0 g; MgSO_4 , 1.23 g; ZnSO_4 , 0.14 g; MnSO_4 , 0.03 g; CaCl_2 , 1.83 g; and $\text{Fe}_2(\text{SO}_4)_3$, 0.2 g. It was neutralized with KOH, and L-fucose, L-rhamnose, or yeast extract were added as required.

Preparation of cell-free extracts. Crude enzyme preparations were prepared either by sonic oscillation of bacterial cells suspended in two volumes of 0.1 M tris(hydroxymethyl)aminomethane, pH 7.5 or by grinding with alumina and extracting with the same buffer system.

Assay procedure for isomerase activity. Isomerase activity was demonstrated by detection of the ketose formed by the cysteine-carbazole method of Dische and Borenfreund (1951) modified as follows: (i) a reaction mixture of 0.1 ml of 0.05 M L-fucose or L-rhamnose and 0.3 ml cell-free extract was incubated for 10 min at 37 C; (ii) the reaction was terminated by adding 6.0 ml of 75% H_2SO_4 ; (iii) 0.2 ml of 1.5% cysteine-HCl and 0.2 ml of 0.12% carbazole in ethanol were added; and (iv) the colored product was assayed in the Klett colorimeter with filter no. 54.

Assay procedure for kinase activity. Kinase activity was demonstrated by the technique of Hers (1956) modified as follows: (i) reaction mixtures of 0.5 ml extract, 0.1 ml of 0.05 M L-fuculose or L-rhamnulose, 0.1 ml of 0.05 M adenosine triphosphate, and 0.1 ml of 0.1 M MgCl_2 were incubated at 37 C for 10 min (L-fucose or L-rhamnose could be used instead if a 15-min preincubation period with the extract was allowed before the addition of adenosine triphosphate and MgCl_2); (ii) next, 2.0 ml of 5% ZnSO_4 and 2.2 ml of 0.3 N $\text{Ba}(\text{OH})_2$ were added; (iii) the assay tubes were held at room temperature for 15 min and then the precipitated phosphate

TABLE 1. *Microorganisms examined for ability to utilize the methylpentoses*

Microorganism	Rhamnose	Fucose
<i>Aerobacter aerogenes</i>	3+	3+
<i>Agrobacterium tumefaciens</i>	3+	3+
<i>Alcaligenes faecalis</i>	0	0
<i>Alcaligenes viscolactus</i>	0	0
<i>Bacillus laterosporus</i>	0	0
<i>Bacillus megaterium</i>	+	0
<i>Bacillus subtilis</i>	0	0
<i>Corynebacterium fascians</i>	+	+
<i>Corynebacterium flaccumfaciens</i>	3+	0
<i>Erwinia amylovora</i>	0	0
<i>Erwinia carotovora</i>	3+	0
<i>Escherichia coli</i>	3+	3+
<i>Gaffkya tetragena</i>	+	+
<i>Micrococcus luteus</i>	0	0
<i>Micrococcus roseus</i>	0	0
<i>Proteus vulgaris</i>	2+	0
<i>Pseudomonas aeruginosa</i>	0	0
<i>Pseudomonas fluorescens</i>	0	0
<i>Pseudomonas marginalis</i>	0	0
<i>Rhizobium leguminosarum</i>	+	+
<i>Saccharomyces ellipsoideus</i>	0	0
<i>Salmonella enteritidis</i>	2+	2+
<i>Sarcina lutea</i>	+	+
<i>Serratia marcescens</i>	0	3+
<i>Shigella boydii</i>	0	0
<i>Shigella dysenteriae</i>	2+	0
<i>Shigella sonnei</i>	0	2+
<i>Sporocytophaga congregata</i>	+	+
<i>Staphylococcus aureus</i>	0	0
<i>Xanthomonas carotae</i>	0	0
<i>Xanthomonas malvacearum</i>	0	0
<i>Xanthomonas papavericola</i>	0	0
<i>Xanthomonas phaseoli</i>	0	2+

Protocol: The microorganisms were inoculated into 15 ml medium in Ryan flasks and growth was estimated turbidimetrically with the Coleman Junior spectrophotometer. Media were prepared by adding 0.5% rhamnose or fucose to the basal mineral medium supplemented with 0.05% yeast extract. Growth recorded as + had a turbidity up to 0.25 optical density (OD) units more than the inoculated mineral salts-yeast extract control; 2+ was more turbid by 0.25 to 0.50 OD units; 3+ was more turbid by 0.50 to 0.75 OD units; and, 0 had a growth response not significantly greater than the control.

esters removed by centrifugation; and (iv) the supernatant was assayed for reducing sugars by the method of Nelson (1944).

L-Fuculose and L-rhamnulose were prepared

by refluxing L-fucose and L-rhamnose with pyridine and subsequent separation of the resulting isomers by paper chromatography according to the method of Wilson and Ajl (1957a).

RESULTS AND DISCUSSION

Nutritional studies. Thirty-three species of bacteria representing stock cultures on hand rather than a systematic selection were examined for their ability to utilize L-fucose and L-rhamnose. The results are indicated in Table 1. These

TABLE 2. *Rhamnose isomerase and rhamnulose kinase in extracts of rhamnose-grown bacteria*

Microorganism	Rhamnose isomerase	Rhamnulose kinase
<i>Aerobacter aerogenes</i>	25	48
<i>Agrobacter tumefaciens</i>	105	107
<i>Bacillus megaterium</i>	19	8
<i>Corynebacterium flaccumfaciens</i>	32	55
<i>Erwinia carotovora</i>	62	92
<i>Escherichia coli</i>	138	26
<i>Gaffkya tetragena</i>	30	14
<i>Proteus vulgaris</i>	106	120
<i>Rhizobium leguminosarum</i>	15	15
<i>Salmonella enteritidis</i>	118	35
<i>Sarcina lutea</i>	34	0
<i>Sporocytophaga congregata</i>	42	42

Results are expressed in Klett units after subtraction of enzyme and reagents blanks. See text for protocol. The enzymes were detected qualitatively but not quantitatively.

TABLE 3. *Fucose isomerase and fuculose kinase in extracts of fucose-grown bacteria*

Microorganism	Fucose isomerase	Fuculose kinase
<i>Aerobacter aerogenes</i>	92	47
<i>Agrobacter tumefaciens</i>	70	44
<i>Escherichia coli</i>	96	64
<i>Gaffkya tetragena</i>	18	24
<i>Rhizobium leguminosarum</i>	10	25
<i>Salmonella enteritidis</i>	25	60
<i>Sarcina lutea</i>	10	0
<i>Serratia marcescens</i>	25	26
<i>Sporocytophaga congregata</i>	34	21
<i>Xanthomonas phaseoli</i>	100	81

See Table 2 and text for protocol.

data show that 18 of the 33 species examined were able to utilize either L-fucose or L-rhamnose or, in 9 cases, both methylpentoses. Furthermore, the data indicated that ability to utilize one of the methylpentoses did not lend ability to utilize the other. Thus, different enzyme systems for their utilization were involved and neither of these methylpentoses was an inducer of enzymes for utilization of the other. It was also apparent that ability to utilize the methylpentoses was a property of the individual bacterial species rather than a property possessed by the genus as a whole.

Isomerases and kinases. Cell-free extracts were prepared from those bacteria that were capable of utilizing either of the methylpentoses except the pathogens, *Shigella dysenteriae* and *Shigella sonnei*, and except *Corynebacterium fascians*. These extracts were assayed for the presence of L-fucose and L-rhamnose isomerases and L-fuculose and L-rhamnulose kinases, and the results are presented in Tables 2 and 3. These data indicate that the methylpentoses were converted to the corresponding ketoses followed by phosphorylation of the ketoses by the majority of bacteria. However, the results obtained with *Sarcina lutea* in particular, as well as with *Bacillus megaterium*, *Gaffkya tetragena*, and *Rhizobium leguminosarum*, suggest the existence of another pathway. In the case of *S. lutea*, neither L-fuculose kinase nor L-rhamnulose kinase could be detected, whereas the low values obtained for the isomerase assays suggest either the absence of the isomerase or a new product of different characteristics. The same conclusions can be reached for *B. megaterium*, *G. tetragena*, and *R. leguminosarum*.

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