Nutritional Variation in *Escherichia coli*

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Nutritional tests were carried out on 62 strains of *Escherichia coli* as part of a study on the genetic basis of natural nutritional variation. The ability of these strains to utilize 84 compounds as carbon, nitrogen and carbon plus nitrogen sources was tested using an auxanographic method. The tests revealed polymorphic characters which are suitable for genetic analysis. Very few of these strains grew on the amino acids classified as 'essential' for humans.

INTRODUCTION

Escherichia coli is normally found in the gut of warm-blooded animals, in some insects, in soil, in water near sewage outfalls and in other places contaminated by the faeces of man and other animals (Cooke, 1974; Cruikshank *et al.*, 1973).

Considerable variation occurs in *E. coli* and other members of the family *Enterobacteriaceae*. Many techniques have been used to detect genetically determined variation in *E. coli* and other bacteria. These allow criteria to be established for classifying and identifying strains in epidemiological, ecological and genetical research, e.g. biotyping (Crichton & Old, 1979, 1982), serotyping (Eveland *et al.*, 1971) and sequencing certain regions of the genome (Milkman & Crawford, 1983). The genetic basis of natural variation in *E. coli* and other members of the *Enterobacteriaceae* has been studied by H. P. Charles and co-workers. As a first stage in this investigation, characters were chosen for which *E. coli* K12 is negative and for which some wild strains are positive. Tests were then made to determine whether the genes coding for these characters are chromosomally located in *E. coli*. For ease of selection in genetic crosses, the characters of first choice were those concerned with the utilization of carbohydrates. So far, utilization of sucrose (Alaeddinoglu & Charles, 1979; Hill, 1980), L-sorbose (Woodward & Charles, 1982; Olukoya, 1984), and ribitol, D-arabitol and galactitol (Woodward & Charles, 1983) have been studied. Cloning of the genes encoding these characters was undertaken (Olukoya, 1984).

This report describes nutritional tests of 62 *E. coli* strains. The nutrition of the strains was examined to further our studies on polymorphism in *E. coli* and in particular to provide more polymorphic characters suitable for genetic analysis.

METHODS

Organisms. Except for laboratory strains (Table 1), these were isolated from the River Kennet, UK (Woodward & Charles, 1982). *E. coli* strains were identified on the basis of bacteriological and biochemical tests. Each isolate was Gram negative, methyl red positive, Voges-Proskauer negative, citrate negative, KCN negative, lactose positive, indole positive, H₂S negative and Eijkman positive.

Media. Minimal medium was Mineral Base E of Owens & Keddie (1969). Glucose is normally added to the medium at a concentration of 0.2% (w/v). Compounds to be tested as carbon and energy sources were substituted for glucose in minimal medium. Compounds to be tested as nitrogen sources were substituted for the nitrogen source in Mineral Base E.

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Table 1. *Laboratory strains of E. coli used*

Strain	Genotype	Source or reference
B	F ⁻ prototroph	Reiner (1975)
C	F ⁻ prototroph	
K12 strains		Departmental collection
K12 (λ)	F ⁻ prototroph	
KG 1673	Hfr <i>thi-1</i>	Woodward & Charles (1982)
AB1621	F ⁻ <i>ara lac tsx gal xyl mtl glpD thiA</i>	
P801	Hfr <i>lacY1 lacY40 xyl-7 mtl-2 ara-41</i>	B. Bachmann, <i>E. coli</i> Genetic Stock Center, Yale University
KL208	Hfr <i>relA1</i>	

Chemicals. All chemicals used were supplied by Sigma.

Growth on compounds as carbon, nitrogen and carbon plus nitrogen source. Fifty-five wild strains and seven laboratory strains (including K12, B and C) of *E. coli* were tested by an auxanographic method for the ability to use 84 compounds as carbon, nitrogen or carbon plus nitrogen sources. This is a convenient method when there are many compounds to be tested (Gutnick *et al.*, 1969; Parke & Ornston, 1984). Cultures were grown in nutrient broth to late exponential phase. The bacteria were washed and resuspended in minimal medium. After 4–6 h, bacteria were seeded into minimal agar cooled to 47 °C, to give about 2×10^6 bacteria ml⁻¹. Crystals, powder or single droplets of compounds to be tested were placed on the surface of solidified agar and growth was recorded after 7 d incubation at 37 °C. Four compounds were tested per plate. Since the compounds diffuse from the original point of deposition, a concentration gradient is established which permits growth even if certain concentrations are inhibitory. As a control, the same compounds were placed on similar plates lacking bacteria. Seeded plates with no substances spotted served as second controls. A zone of growth around the point of substrate application was scored as positive. Separate colonies around the point of substrate application showed that the strain mutated to use the substrate and such responses were scored as mutable. The absence of any visible response was scored as negative. When a growth response was for any reason uncertain, the strain and substance were retested.

RESULTS AND DISCUSSION

A summary of the results for growth on compounds as carbon, nitrogen and carbon plus nitrogen sources is shown in Tables 2 and 3. A table containing the individual results for the seven laboratory strains used has been deposited with the British Library Lending Division, Boston Spa, Yorkshire LS23 7BQM, UK, as Supplementary Publication no. SUP 28022 (5 pages). In comparison with organisms such as *Pseudomonas* which degrade a wide variety of substrates as carbon or nitrogen sources (Stanier *et al.*, 1966), *E. coli* is somewhat limited in the range of compounds it utilizes. Aromatic rings and large molecules were not degraded by the strains tested. About half of the naturally occurring amino acids were used as carbon sources. Some of the sugar and sugar derivatives were utilized. With few exceptions, most of the compounds that served as poor carbon sources were also poor nitrogen sources. The extent of nutritional diversity is illustrated by the fact that no two strains behaved in exactly the same way. No substances except glucose and glucosamine were used by every strain, and no strain used every substance.

Most of the utilizable substances were naturally occurring compounds. Few strains grew or mutated to grow on unnatural chemically synthesized compounds such as L-glucose and 3-O- β -D-galactopyranosyl-D-arabinose. Concerning the strains that utilize these compounds, questions arise about the route of degradation, the origins and functions of the genes involved and whether *E. coli* is exposed to similar substances in nature.

The choice of characters for genetic analysis is limited by the requirement that the character be easily selectable in transduction and conjugation experiments, and that *E. coli* K12 be naturally negative for it. It is also preferable that the character should be specified by one gene or cluster: if it depends on genes dispersed about the chromosome, its transfer and analysis would be difficult. In the present tests, many characters suitable for genetic analysis were discovered, e.g. the utilization of L-cysteine, palatinose, acetylsalicylic acid and 3-O- β -D-galactopyranosyl-D-arabinose as carbon sources.

An interesting finding that emerged from these tests was that no strain grew on four of the amino acids which are largely absent from intestinal mucins (Tables 2). Another interesting

Table 2. Percentages of strains of *E. coli* utilizing different compounds as carbon, nitrogen and carbon plus nitrogen sources

Strains were tested by the auxanographic technique as described in Methods. In a few cases results were confirmed by streaking on minimal agar containing 0.2% (w/v) of particular compounds as carbon source or on minimal agar with the nitrogen source omitted.

Compound	Carbon source	Nitrogen source		Carbon + nitrogen source
		Carbon source glucose	Carbon source lactate	
L-Alanine*	97	98	100	93
L-Arginine*	98	98	100	100
L-Asparagine	93	100	100	90
L-Aspartate*	89	100	100	97
L-Cysteine*	6.5	97	100	28
L-Cystine	2	16	85	3
L-Glutamate*	86	95	95	28
L-Glutamine	90	97	95	54
Glycine*	75	95	95	3
L-Histidine†	18	20	20	18
L-Homoserine	13	89	92	8
L-Isoleucine†	0	3	10	0
L-Leucine†	0	16	61	0
L-Lysine*†	2	8	75	0
L-Methionine†	0	8	47.5	0
L-Phenylalanine†	0	8	15	3
L-Proline*	93	39	95	65.5
L-Serine*	97	95	97	26
L-Threonine*†	72	8	13.5	8
L-Tryptophan†	20	8	79	46
L-Tyrosine	5	77	93	15
L-Valine†	0	20	18	3
L-Ornithine	6.5	39	97	2
Thiamin	0	0	0	0
D-Glucosamine	100	5	98	98
D-Mannosamine	6.5	0	80	0
Uridine	98	10	39	93
Adenosine	90	97	95	95
Cytidine	98	100	97	98
Guanosine	93	12	12	70
Thymidine	93	6.5	13	95
Inosine	93	8	11.5	90
Glycoprotein	0	2	0	0
N-Acetylneuraminic acid	77	87	79	82
Mucin (porcine)	13	100	98	0
Adenine	0	90	9	0
Guanine	0	37	8	0
Hypoxanthine	0	36	6.5	0
Thymine	0	34	8	0
Cytosine	0	36	12	0
Taurocholic acid	0	0	0	0
Glycocholic acid	0	0	0	0
N-Acetylgalactosamine	62	5	77	67
Taurine	0	0	6.5	0
Caffeine	3	0	0	0
N-Acetyl-D-glucosamine	100	5	100	98

* Found in gastrointestinal mucins (Clamp *et al.*, 1978).

† Essential for mammals (Bender, 1973; Crim & Munro, 1976). Arginine is essential in young growing animals but not in adults.

Table 3. Percentages of strains of *E. coli* utilizing different compounds as carbon sources

See Table 2 for details.			
Compound	%	Compound	%
3- <i>O</i> - β -D-Galactopyranosyl-D-arabinose	29.5*	D-Mannoheptulose	2
<i>N</i> -Acetyl- β -D-glucosamine naphthol	3	Uridine-5-diphosphoglucose	2
Muramic acid (2-amino-3- <i>O</i> -(1-carboxyethyl)-2-deoxy-D-glucose)	0	Acetylcholine chloride	0
Palatinose	11.5	<i>N</i> -Acetylneuramin lactose	0
Glucoheptonic acid (D-glycero-D-guloheptonic acid)	0	Neuraminic acid β -glycoside	0
D-Glucoheptose	2	D-Glucose	100
Acetylsalicylic acid	24	D-Galactose	98
D-Fucose	2	D-Ribose	90
L-Fucose	93	L-Arabinose	93
Vitamin A	0	D-Mannose	98
Vitamin C	10	Uracil	2
2-Deoxy-D-glucose	0	Salicin	8
6-Deoxy-D-glucose	0	<i>N</i> -Acetyl-D-mannosamine	74
2-Deoxy-D-galactose	0	Lactulose	93*
D-Arabinose	26	Caffeic acid	0
L-Mannose	52	D-Glucuronic acid	88.5
Glucosaminic acid (2-amino-2-deoxy-D-gluconic acid)	5*	Chlorogenic acid	0
L-Glucose	2	Starch (potato)	0
L-Fucosylamine	44	L-Sorbose	26

* Mutable response.

point is that the amino acids on which no strains, or few, grew are those which are the 'essential' amino acids in human nutrition.

Future work will concentrate on the genetic basis of some of the variable characters discovered in this study.

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