JOURNAL OF BACTERIOLOGY, Oct. 1969, p. 215-219
Vol. 100, No. 1

Printed in U.S.A.

Compounds Which Serve as the Sole Source of Carbon or Nitrogen for *Salmonella typhimurium* LT-2

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Received for publication 7 August 1969

About 600 compounds were screened as possible carbon or nitrogen sources for *Salmonella typhimurium* LT-2. About 100 utilizable compounds were found.

In an attempt to provide new genetic markers in the chromosome of *Salmonella typhimurium*, a search for compounds which can serve as a sole carbon source or as a sole nitrogen source for this organism was undertaken. About 100 utilizable compounds were found. Mapping of the genes concerned with the utilization of these compounds should add considerable detail to the known genetic map (5) on which 200 genes are already localized.

These compounds will also be useful in the investigation of strains carrying deletion mutations if a gene involved in the utilization of one of these compounds is deleted. For example, certain strains, in which part of the leucine operon is deleted, cannot utilize arabinose as a sole source of carbon because the deletion extends into the nearby arabinose operon (2), while a strain with a leucine deletion extending in the other direction cannot utilize a number of other carbon sources (Calvo et al., *unpublished data*). In contrast, we have determined that *his-520*, in which a large deletion extends through the histidine operon and lipopolysaccharide (rfb) regions, grows on all the listed sources of carbon and nitrogen.

The list of compounds should also be useful for taxonomic studies comparing related enteric bacteria. Such studies often make use of smaller lists of compounds (1, 4).

MATERIALS AND METHODS

Compounds were tested for their ability to support the growth of *S. typhimurium* strain LT-2 by a simple auxanographic technique. Minimal salts-agar was prepared by dissolving the following in a liter of distilled water: K_2SO_4, 1.0 g; K_2HPO_4, 13.5 g; KH_2PO_4, 4.7 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; agar, 15 g. For testing possible carbon sources, plates containing 30 ml of sterile minimal agar were overlayed with 2 ml of soft agar (0.6% in 0.5% NaCl solution) containing 0.1 ml of an overnight nutrient broth culture of bacteria and 0.1 ml of 0.1 M NH_4Cl. For testing possible nitrogen sources, the 0.1 M NH_4Cl was replaced by 0.2 ml of 50% glucose. When testing the amino acids as nitrogen sources, an additional set of plates was used containing 0.1% citrate as a sole carbon source.

Approximately 2 mg of each compound was placed on the surface of the solidified agar, and growth of the bacteria in the agar was recorded after incubation for 2 days at 37 C. Because the compound diffuses from the original point of deposition, a concentration gradient is established which allows the organism to grow even if certain concentrations are inhibitory. For example, compounds such as lactic acid, tested without prior neutralization, resulted in an inner zone of inhibition surrounded by a region of profuse growth. As controls, compounds were placed on similar plates lacking bacteria. No more than four compounds were tested on a single plate, and those compounds which gave positive results were each retested on an individual plate.

All chemicals were obtained from commercial sources; their purity was not determined, although several independent batches were tried whenever a positive response was obtained. This auxanographic technique was found to be simple, sensitive, and reliable.

RESULTS AND DISCUSSION

We have found about 100 utilizable carbon (Table 1) and nitrogen (Table 2) sources out of some 600 compounds that were screened. We first tested all of the natural compounds that seemed likely to be utilizable; this yielded most of our 100 compounds. Further screening yielded

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TABLE 1. *Compounds which serve as sole carbon sources for Salmonella typhimurium LT-2*

Acetic acid	L-Fucose	Guanosine	Oleic acid
N-acetyl-D-glucosamine	Fumaric acid	Inosine	Oxaloacetic acid
N-acetyl-D-mannosamine	Galactaric acid (mucic acid)	*meso*-Inositol	3-Phosphoglyceric acid
Adenosine[a]	Galactitol (dulcitol)	DL-Isocitric acid	L-Proline[a]
L-Alanine[a]	D-Galactose	α-Ketoglutaric acid	Propionic acid
L-Arabinose	D-Glucaric acid (saccharic acid)	L-Lactic acid	Pyruvic acid
DL-Citramalic acid	D-Gluconolactone	Lauric acid	L-Rhamnose
Citric acid	D-Gluconic acid	L-Lyxose	D-Ribose
L-Cysteine[a]	D-Glucosamine	L-Malic acid	D-Serine[a]
Cytidine	D-Glucose	D-Maltose	L-Serine[a]
2-Deoxyadenosine	D-Glucose-6-phosphate	D-Mannitol	D-Sorbitol
2-Deoxycytidine	D-Glucuronic acid	D-Mannose	Succinic acid
2-Deoxyguanosine	D-Glucuronolactone	D-Mannosamine	*meso*-Tartaric acid[b]
2-Deoxy-D-ribose	DL-glyceric acid	Melibiose	Thymidine
Deoxyuridine	Glycerol	L-Methionyl-L-alanine[a]	D-Trehalose
Dihydroxyacetone	α-Glycerophosphate	6-Methylaminopurine riboside	Tricarballylic acid
D-Erythrose[b]	Glycolic acid	α-Methyl-D-galactoside	Tridecanoic acid
D-Fructose		Myristic acid	Uridine
D-Fructose-6-phosphate			D-Xylose

[a] Can serve as the sole source of both carbon and nitrogen.
[b] The bacteria will mutate to use this compound.

TABLE 2. *Compounds which serve as sole nitrogen sources for Salmonella typhimurium*

Adenosine[a]	Deoxycytidine
L-Alanine[a]	D-Glutamic acid
Ammonium chloride	L-Glutamic acid
L-Arginine	L-Glutamine
D-Asparagine	Glutathione (reduced)
L-Asparagine	Glycine
D-Aspartic acid	L-Homoserine
L-Aspartic acid	DL-β-Hydroxy glutamic acid
L-Cysteine[a]	L-Methionyl-L-alanine[a]
L-Cystine	L-Proline[a]
Cytidine	D-Serine[a]
Cytosine	L-Serine[a]
Deoxyadenosine	

[a] Can serve as the sole source of both carbon and nitrogen.

few new utilizable compounds; therefore, we feel that the list is probably fairly exhaustive in its present form. Table 3 lists the compounds which are not utilizable as sole carbon or nitrogen sources.

The utilization by *Escherichia coli* of a number of carbon sources related to the Krebs cycle has recently been reviewed, with particular emphasis on regulation (3). The pattern of the utilization of fatty acids is of some interest, as there are detailed studies of this in *E. coli* (7). The pattern is fairly similar; the C_4 to C_{10} fatty acids are not utilized in either of the two organisms.

In comparison with organisms such as *Pseudomonas* (6), *S. typhimurium* is somewhat limited in the range of compounds that can be utilized as a sole carbon source for growth. Thus, phosphorylated compounds are, in general, poorly utilized. Few of the fatty acids are utilized, amides and ester linkages are generally not hydrolyzed, aromatic rings are not degraded to useful carbon sources, few of the naturally occurring amino acids serve as carbon sources, large molecules are not degraded, and few of the wide variety of available sugars and sugar derivatives are utilized. Some known metabolic intermediates on the list of nonutilizable compounds are presumably not utilized because they do not get into the cells, e.g., *cis*-aconitic acid and ribose-5-phosphate, 6-phosphogluconate, 3-phosphoglycerate, and glucose-1-phosphate.

As noted in a footnote to Table 1, several compounds in the table only support the growth of mutant derivatives of the parent strain. It is likely that by using more bacteria in the inoculum or by employing mutagens, other mutants would be identified which utilized some of the compounds in Table 3. Further study of such clones might provide information on interesting pathways or uptake systems in *Salmonella*.

It is of interest to make a rough estimate of the percentage of the *Salmonella* genome used for the capacity to utilize these various carbon and nitrogen sources. An estimate is made as follows. There are about 4,000 genes in a *Salmonella* (or *E. coli*) if one takes the average size gene to be about 1,000 bases in length. If one allows a permease and about two enzymes for the utilization of each of the compounds involved, that would account for 300 genes. In addition, there may be some utilizable compounds that we have not tested; we allow 100 genes for this. Thus, approximately

TABLE 3. *Compounds which do not serve either as a sole carbon or a sole nitrogen source for Salmonella typhimurium*[a]

Acetamide
Acetone oxime
N-acetyl-D-alanine
N-acetyl-L-alanine
Acetyl-D-alloisoleucine
N-acetyl-*S*-benzyl-DL-cysteine
Acetyl-dihydro-phenylalanine ethyl ester
N-acetyl-galactosamine
N-acetyl-glycine
N-acetyl-L-leucine
ε-Acetyl-L-lysine
N-acetyl-D-methionine
N-acetyl-DL-methionine
1-Acetyl-3-methyl urea
N-acetyl-D-phenylalanine
Acetyl-DL-phenylalanyl glycine
Acetylene dicarboxylic acid
Acetyl-DL-proline
Acetyl-DL-tyrosine
cis-Aconitic acid
Adenine
Adenosine-3,5-cyclic phosphate
Adenosine-5-phosphate
Adipamide
Adipic acid
Adonitol
Agmatine sulfate
β-Alanine
D-Alanine
DL-α-Alanine ethyl ester
DL-Alanylglycylglycine
Allantoin
Allantolactone
D-Allose
D-Altrose
DL-α-Aminoadipic acid
p-Aminobenzoic acid
α-Aminobutyric acid
DL-β-Aminobutyric acid
ε-Amino-*n*-carpoic acid
ω-Aminocaprylic acid
1-Aminocyclopentane carboxylic acid
α-Aminodecanoic acid
Aminoguanidine sulfate
Aminoguanidine bicarbonate
α-Aminolauric acid
δ-Aminolevulinic acid
2-Amino-2-methyl-1,3-propanediol
DL-2-Amino phenylacetic acid
2-Aminopurine
2-Aminopyridine
δ-Amino-*n*-valeric acid
Amygdalin
Aniline
L-Anserine
Anthranilic acid
Arabinic acid
D-Arabinose
D-Arabitol
L-Arabitol
D-Araboascorbic acid
D-Arabonate, calcium
Arcaine sulfate
D-Arginine
DL-Arterenol
L-Ascorbic acid
L-Aspartic diethyl ester
Azelaic acid
L-Azetidine-2-carboxylic acid
Barbital, sodium
Barbituric acid
Benzamide
Benzidine
Benzilic acid
Benzoic acid
Benzoin
Benzoin acetate
DL-*N*-benzoyl-2-alanine
Benzoyl glycine
S-benzyl cysteine
Benzyl glycinate
Betaine·HCl
Bicarbonate, sodium
Bicine
Biotin
Biuret
Brucine
Bufotenine mono oxalate hydrate
n-Butyl-DL-malate
n-Butyramide
n-Butyric acid
D-Butyroin
Caffeine
n-Caproic acid
Carbamyl glutamic acid
Carbamyl phosphate
N-carbobenzoxy glucosamine
Casein
Catechol
D-Cellobiose
Cellotriose
Celtrobiose
L-α-Chloro-β-phenyl-propionyl glycine
Cholesterol
Cholic acid
Choline chloride
D-Chondrosamine (galactosamine)
Cinnamic acid (*trans*)
Citraconate, sodium
L-Citrulline
Creatine
Creatinine
Crotonic acid
Cyanate, potassium
DL(+)-Cystathionine, *allo*
Cysteic acid
3-Deoxyglucose
2-Deoxy-D-glucose
α,β-Diacetyl-diamino-DL-propionic acid
3,5-Diamino benzoic acid
2,4-Diaminobutyric acid
4,5-Diamino-2,6-dihydroxypyrimidine
α,ε-Diaminopimelic acid
2,6-Diaminopurine
L-Dihydroorotic acid
Dihydrourocanic acid
Dihydroxynorephedrine·HCl
Dihydroxyphenylalanine (DOPA)
β,β-Dimethyl acrylic acid
5,5-Dimethyl-1,3-cyclohexane dione
3,3-Dimethyl glutaric acid
3,5-Dinitro salicylic acid
Diphenylamine
Dipicolinic acid
Egg albumin
d-Epinephrine
Erythritol
erythro-L-*galacto*-octitol
D-*erythro*-L-*gluco*-octitol
D-*erythro*-*talo*-octitol
D-*erythro*-L-*talo*-octose
Esculin
Ethanolamine
Ethanolamine phosphate
L-Ethionine
Ethyl acetate
Ethyl anthranilate
Ethyl lactate
Ethyl levulinate
N-ethylmaleimide
Ethylamine·HCl
Ethylene glycol
Ethyl itaconate
5-Fluorouracil deoxyriboside
Folic acid
Formate, sodium
Formimino glycine
Formyl alanine
Formyl glycine
Formyl-D-phenylalanine
Formyl-L-phenylalanine
D-Fructose-1,6-diphosphate
D-Fucose
2-Furan pyruvic acid oxime
β-D-Galactose-1-phosphate
α-D-Galacturonic acid
Gelatin
Gentiobiose
d-Glucoascorbic acid
Glucoheptonic acid
D-*gluco*-Heptulose
L-*gluco*-Heptulose
α-D-Glucose pentaacetate
β-D-Glucose-1-phosphate
L-Glutamic acid dimethyl ester
L-Glutamic acid-α-ethyl ester
Glutaric acid
DL-Glyceraldehyde
D-*glycero*-D-*allo*-heptose
D-*glycero*-D-*galacto*-heptose
D-*glycero*-L-*galacto*-heptose
D-*glycero*-D-*gluco*-heptitol
D-*glycero*-D-*gluco*-heptose
D-*glycero*-L-*gluco*-heptose
L-*glycero*-D-*gulo*-heptitol
D-*glycero*-D-*gulo*-heptose
D-*glycero*-D-*ido*-heptose
β-Glycerophosphoric acid
Glycine amide
Glycine ethyl ester
Glycine methyl ester
Glycocyamine
Glycogen
Glycyl-D-methionine
Glycylglycine
Glyoxylic acid
Guanidine
α-Guanidobutyric acid
Guanido-DL-phenylalanine
Guanidopipecolic acid
β-Guanidopropionic acid
Guanine
D-Gulonolactone
L-Gulonolactone
Hemin

TABLE 3—*Continued*

n-Heptylic acid
1-Hexadecanol
Hexamethylenetetramine
Hippuric acid
Histamine diphosphate
L-Histidine
L-Homocitrulline
L-Homocysteic acid
Hydrazine sulfate
p-Hydrazinobenzoic acid
Hydrocinnamic acid
trans-β-Hydromuconic acid
p-Hydroxybenzoic acid
β-Hydroxybutyric acid
D(−)-β-Hydroxybutyric acid
DL-Hydroxylysine
Hydroxyproline
Hydroxy-D-proline, *allo*
Hydroxypyruvic acid
8-Hydroxyquinoline
Hypoxanthine
L-Iduronic acid·barium
Imidazole
Indole
Indole-3-acetic acid
3-(2-Amino-ethyl) indole hydrochloride
Inosine-5-phosphate
Inulin
3-Iodopropionic acid
Isatin
Isobutyric acid
Isoglutamine
DL-Isoleucamine·HCl
D-Isoleucine, *allo*
L-Isoleucine
L-Isoleucine benzyl ester
L-Isoleucine ethyl ester
L-Isoleucine methyl ester
L-Isoleucyl-L-isoleucine
DL-*N*-isopropyl arterenol
Itaconic acid
5-Keto-D-gluconate
β-Ketoglutaric acid
Kojic acid
D-Lactic acid
Lactose
Lecithin
L-Leucinamide
D-Leucinamide
Leucinamine
D-Leucine
L-Leucine
L-Leucine ethyl ester
L-Leucine methyl ester
D-Leucyl-L-isoleucine
Levulinic acid
L-Lysine
L-Lysine methyl ester
D-Lyxonic acid
D-Lyxose
Maleic acid
D-Malic acid
Malonamide
Malonic acid
DL-Mandelic acid
L-Mannitol
D-Mannoheptulose
D-Mannuronic acid
D-Melezitose
Menthol glucuronide
Mercaptosuccinic acid
meso-Allitol
meso-glycero-gulo-Heptitol
L(+) *meso* Lanthionine
D-Methionine
L-Methionine
DL-Methionine sulfone
DL-Methionine sulfoxide
α-Methyl-*N*-acetyl mannosamine furanoside
Methyl amine·HCl
Methyl albumin
S-methyl-L-cysteine
β-Methyl-D-galactoside
α-Methyl-D-glucoside
β-Methyl-D-glucoside
DL-α-Methyl glutamic acid
Methyl itaconate
α-Methyl-D-mannoside
Methyl-α-L-rhamnoside
DL-α-Methyl serine
DL-*O*-methyl serine
Methyl-β-thiogalactoside
α-Methyl-D-xyloside
β-Methyl-D-xyloside
Mevalonic acid
2,3-Monoacetone-D-xylulofuranose
trans trans Muconic acid
α-Naphthol
β-Naphthyl alanine
Nicotinamide
Nicotinamide adenine dinucleotide
Nicotinamide adenine dinucleotide phosphate
Nicotinic acid
Nitrate, sodium
Nitrite, sodium
p-Nitrobenzoic acid
p-Nitrobenzoyl-L-glutamic acid
Nitroguanidophenylalanine
β-Nitroguanidopropionic acid
4-Nitroimidazole
Norleucamine·HCl
DL-Norleucine
DL-Normetanephrine
Norvalamine·HCl
D-Norvaline
Nucleinate, sodium
n-Octanoic acid
Orcinol
DL-Ornithine·HCl
Orotic acid
Oxalic acid
Palmitic acid
D-Pantothenic acid
D-Pantoyl lactone
Perseitol
Phenethyl-β-D-galactoside
Phenol
D-Phenylalanine
L-Phenylalanine
Phenylalanine methyl ester
Phenylalanyl phenylalanine
L-Phenylephrine
DL-*C*-phenyl glycine
DL-α-Phenyllactic acid
6-Phosphogluconic acid
Phosphoribosyl pyrophosphate
DL-*O*-phosphoserine
DL-*O*-phosphothreonine
Phthalic acid
Phthalimide
α-Picolinic acid
Picric acid
2,6-Piperidine dicarboxylic acid
Piperonal
Polygalacturonic acid
Prolamine·HCl
D-Proline
L-Proline methyl ester
Propionamide
Protocatechuic acid
Putrescine
4(*p*-Nitrobenzyl) pyridine
Pyridoxine
Pyrocatechol
Pyruvoyl glycine
DL-Pyruvoyl phenylalanine
Quercetin
D-Raffinose
Resorcinol
D-Rhamnose
Riboflavin
D-Ribonolactone
D-Ribose-5-phosphate
Salicin
Salicylaldehyde
Salicylamide
Salicylic acid
Sedoheptulose anhydride
Semicarbazide·HCl
DL-Serine ethyl ester
Serotonin
Sorbic acid
D-Sorbose
L-Sorbose
Spermidine
Spermine
Stearamide
Stearanilide
Stearic acid
Succinamide
Succinimide
Sucrose
Sulfanilate, sodium
D-Tagaturonic acid
D-Taloheptulose
D-Talose
Tannic acid
D-Tartaric acid
L-Tartaric acid
Taurine
Taurocholic acid
2,4,5,6-Tetra-amino-pyrimidine sulfate
Tetrahydroxysuccinic acid
Tetramethylammonium bromide
Thiamine
Thioglycolate, sodium
Thiosemicarbazide
Thiourea
Threamine
D-*threo*-L-*gulo*-Octose
D-Threonine
L-Threonine
L-Threonine, *allo*
Thymine
2,4,5-Triamino-6-hydroxy pyrimidine sulfate
Tripalmitin
Tristearin
Tryptamine
D-Tryptophan
L-Tryptophan
L-Tryptophan ethyl ester
D-Tyrosine
L-Tyrosine
L-Tyrosine-*O*-sulfate
Uracil
Urea
Uric acid
Valeric acid
D-Valine
L-Valine
DL-Valine ethyl ester
Vanillic acid
Vanillin
Vanillyl alcohol
Xanthine
Xanthosine
Xanthurenic acid
Xylitol
L-Xylose

[a] Additional compounds *not* serving as carbon sources are listed in Table 2, and additional compounds *not* serving as nitrogen sources are listed in Table 1.

10% (400/4,000) of the genome is responsible for the capacity to utilize these compounds.

ACKNOWLEDGMENTS

We thank Anne Liggett for technical assistance and V. Ginsburg for samples of many carbohydrates.

This research was supported by Atomic Energy Commission contract AT(1101) 34, no. 156, to B.N.A., by Public Health Service grant 5R01 AM 12092-02 to B.N.A., and by National Science Foundation grant GB-3557 to J.M.C.

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