Assimilation of oxalate, acetate, and CO_2 by *Oxalobacter formigenes*[1]

N.A. Cornick and M.J. Allison

Abstract: *Oxalobacter formigenes* is the only well-documented oxalate-degrading bacterium isolated from the gastrointestinal tract of animals. The production of ATP by *Oxalobacter formigenes* is centered around oxalate metabolism and oxalate is required for growth. A small amount of acetate (0.5 mM) is also required. Oxalate is decarboxylated to formate plus CO_2 in nearly equimolar amounts. Experiments were conducted to determine which potential carbon sources (oxalate, acetate, formate, CO_2) were assimilated by *Oxalobacter formigenes* and which metabolic pathways were operative in carbon assimilation. Measurements of the specific activities of total cell carbon after growth with different ^{14}C-labeled precursors indicated that at least 54% of the total cell carbon was derived from oxalate and at least 7% was derived from acetate. Carbonate was also assimilated, but formate was not a significant source of cell carbon. Labeling patterns in amino acids from cells grown in [^{14}C]oxalate or $^{14}CO_3$ were different; however, in both cases ^{14}C was widely distributed into most cellular amino acids. Carbon from [^{14}C]acetate was less widely distributed and detected mainly in those amino acids known to be derived from α-ketoglutarate, oxaloacetate, and pyruvate. Cell-free extracts contained citrate synthase, isocitrate dehydrogenase, and malate dehydrogenase activities. The labeling observed in amino acids derived from acetate is in agreement with the function of these enzymes in biosynthesis and indicates that the majority of acetate carbon entered into amino acid biosynthesis via well-known pathways.

Key words: biosynthesis, carbon assimilation, metabolism.

Résumé : L'*Oxalobacter formigenes* est la seule bactérie bien connue pour dégrader l'oxalate à avoir été isolée du tractus digestif animal. La production d'ATP par l'*Oxalobacter formigenes* est centrée autour du métabolisme de l'oxalate et ce composé est nécessaire à sa croissance. Une petite quantité d'acétate (0,5 mM) est également nécessaire. L'oxalate est décarboxylé en formate plus CO_2 en quantités presqu'équimolaires. Des essais expérimentaux ont été faits pour vérifier quelles sources de carbone (oxalate, acétate, formate, CO_2) étaient potentiellement assimilées par l'*Oxalobacter formigenes* et quels sentiers métabolique étaient actifs dans l'assimilation du carbone. Des mesures d'activité spécifique du carbone cellulaire total après croissance en présence de divers précurseurs marqués au ^{14}C ont indiqué qu'au moins 54% du carbone cellulaire total dérivait de l'oxalate et 7% de l'acétate. Le carbonate était aussi assimilé, mais le formate n'était pas une source significative de carbone cellulaire. Les profils de marquage des acides aminés provenant de cellules cultivées en présence de [^{14}C]oxalate ou de $^{14}CO_3$ étaient différents, mais dans les deux cas le ^{14}C était largement distribué dans la plupart des acides aminés de la cellule. Le carbone issu de l'[^{14}C]acétate avait une distribution plus restreinte et il se retrouvait surtout dans les acides aminés dérivés de l'α-cétoglutarate, l'oxaloacétate et du pyruvate. Des extraits acellulaires affichaient des activités enzymatiques dont la citrate synthase, l'isocitrate déhydrogénase et la malate déhydrogénase. Le marquage observé dans les acides aminés dérivés de l'acétate concorde avec le rôle de ces enzymes dans la biosynthèse et indique que la majorité du carbone provenant de l'acétate entre dans la biosynthèse des acides aminés via des sentiers bien connus.

Mots clés : biosynthèse, assimilation du carbone, métabolisme.
[Traduit par la rédaction]

Introduction

Most oxalate-degrading bacteria utilize a variety of substrates in addition to oxalate (Allison et al. 1995). The majority of these bacteria are aerobic and decarboxylate oxalate to formate plus CO_2. Three anaerobic bacteria have been described that require oxalate as a substrate. These are *Oxalobacter formigenes* (Allison et al. 1985), *Oxalobacter vibrioformis*,

Received March 26, 1996. Revision received July 8, 1996. Accepted July 11, 1996.

N.A. Cornick.[2] Enteric Diseases and Food Safety Research Unit, National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture and Department of Microbiology, Immunology and Preventative Medicine, Iowa State University, Ames, IA 50011, U.S.A.
M.J. Allison.[3] National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 70, Ames, IA 50010, U.S.A.

1 A preliminary report of this work was presented at the Annual Meeting of the American Society for Microbiology, 1994.
2 Present address: Veterinary Medical Research Institute, Iowa State University, Ames, IA 50011, U.S.A.
3 Author to whom all correspondence should be addressed (e-mail: mallison@iastate.edu).

and *Oxalophagus oxalicus (Clostridium oxalicum)* (Collins et al. 1994; Dehning and Schink 1989), and all differ from aerobic oxalate-degrading bacteria in that formate is produced as an end product and does not appear to be oxidized.

Oxalobacter formigenes has been isolated from the rumen of cattle and sheep (Dawson et al. 1980); from the large bowel contents of pigs (Allison et al. 1985), humans (Allison et al. 1986), and rats (Daniel et al. 1987); and from anoxic sediment (Smith et al. 1985). *Oxalobacter vibrioformis* and *Oxalophagus oxalicus (Clostridium oxalicum)* were isolated from anoxic sediment (Dehning and Schink 1989). All three of the anaerobic species require small amounts of acetate (0.5 mM) for growth. This absolute requirement for acetate suggests that acetate is needed as a source of cell carbon and that oxalate is not reduced to acetate by these organisms. Information regarding the incorporation of oxalate and acetate into cell constituents by *Oxalobacter vibrioformis* and *Oxalophagus oxalicus (Clostridium oxalicum)* is not available (Dehning and Schink 1989). Aerobic oxalate-degrading bacteria do not require acetate and assimilate carbon from oxalate by first reducing it to 3-phosphoglycerate (3P-glycerate), which then serves as an important precursor molecule for biosynthetic pathways (Allison et al. 1995). Previous data from our laboratory suggests that *Oxalobacter formigenes* also reduced oxalate to 3P-glycerate via the glycerate pathway (Cornick and Allison 1996).

When *Oxalobacter formigenes* is grown on defined medium, there are four potential sources of carbon for cell synthesis: oxalate, acetate, CO_2, and formate. We grew *Oxalobacter formigenes* in the presence of each of these ^{14}C-labeled compounds to determine the relative contributions of each precursor to cell biomass. In addition, we determined which amino acids were labeled with ^{14}C from each carbon source and assayed cell-free lysates for key enzymes of the Krebs cycle, the reductive C_4 dicarboxylic acid pathway, and the acetyl-CoA (Wood) pathway.

Materials and methods

Culture media

Oxalobacter formigenes (ATCC 35274) was grown in medium E or F for all of the experiments. Medium E contained the following (in g/L): 2-*N*-morpholinoethanesulfonic acid (MES), 0.49; NaMES, 0.54; K_2HPO_4, 0.25; $(NH_4SO_4)_2$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.025; trace metals solution (Pfennig and Lippert 1966), 20mL; cysteine-HCl $\cdot H_2O$, 0.5; resazurin, 0.001. The pH was adjusted to 6.0 and the medium was boiled and cooled under N_2. Sterile, anaerobic sodium oxalate and sodium acetate were added to a final concentration of 100 and 0.5 mM, respectively. Medium F was the same as medium E, except the MES buffer was replaced with Na_2CO_3 (4 g/L). The pH was adjusted to 6.8 and the medium was boiled and cooled under CO_2.

Growth of bacteria in ^{14}C compounds

The growth of the bacteria was followed by measuring the absorbance (A_{600}) using a Spectronic 70 (Bausch and Lomb, Rochester, N.Y.) with 18-mm cuvettes. Bacteria were grown in either medium E or F to early log phase (A_{600}, 0.1–0.25). The cells were collected by centrifugation, washed once, and resuspended in 10 mL of media containing either [^{14}C]oxalate (0.25 mCi/mmol), [1-^{14}C]acetate (18.6 mCi/mmol), [2-^{14}C] acetate (20 mCi/mmol), [^{14}C]formate (43 mCi/mmol), or $Na^{14}CO_3$ (56 mCi/mmol) (1 Ci = 37 GBq). All steps were carried out under an atmosphere of N_2 or CO_2. The bacteria were grown for another 0.5 generation (4.5 h) and then collected by centrifugation. Cells were washed twice and fractionated using the methods of Roberts et al. (1963). The cold 5% trichloroacetic acid soluble fraction was designated as the intermediate fraction, the ethanol–ether soluble fraction was designated as the lipid fraction, the hot trichloroacetic acid soluble fraction was designated as the nucleic acid fraction, and the remaining pellet was considered cell protein. The cell protein was hydrolyzed in 6 N HCl at 105°C for 20 h under N_2 and the HCl was removed under vacuum.

High-performance liquid chromatography

Amino acids in the cell protein fraction were analyzed as the dabsylated derivatives using HPLC with a diode array spectrophotometer (436 nm) (System Gold, Beckman Instruments, San Ramon, Calif.) (Allen 1989; Anderson et al. 1993). Radioactivity in the column eluate was measured using a flow-through scintillation counter (Radiomatic Instruments, Meriden, Conn.). Scintillation fluid (FloScint IV, Radiomatic) was added in a 2:1 ratio with the mobile phase from the HPLC. Samples containing 10 000 – 40 000 cpm were injected onto the column. Norleucine was used as an internal standard and individual amino acids were used as external standards. Two pairs of amino acids could not be adequately separated using this method. Valine was not distinguished from proline and arginine was not distinguished from isoleucine.

Radioactivity measurements

Samples (10 μL) from major cell fractions were added to 10 mL of scintillation fluid (Ecoscint, National Diagnostics, Atlanta, Ga.) and counted using a LS-7800 liquid scintillation counter (Beckman Instruments). Efficiency was monitored by H number and [^{14}C]toluene was used as the internal standard. The percentage of isotope incorporated was calculated from measurement of radioactivity in washed cells from a known volume of medium.

$Na^{14}CO_3$ (56 mCi/mmol) was purchased from ICN (Irvine, Calif.). [1-^{14}C]Acetic acid (18.6 mCi/mmol) was purchased from Sigma (St. Louis, Mo.). Sodium [2-^{14}C]acetate (50 mCi/mmol and 22.5 mCi/mmol) was purchased from Schwartz/Mann (Orangeburg, N.Y.) and CalBiochem (San Diego, Calif.), respectively. [^{14}C]Oxalic acid (3.4 mCi/mmol) and sodium [^{14}C]formate (43 mCi/mmol) were purchased from New England Nuclear (Boston, Mass.).

Enzyme assays

Oxalobacter formigenes cells were broken using a French pressure cell and cell-free extracts were prepared under N_2 as previously described (Cornick and Allison 1996). Assays were done under an aerobic atmosphere unless otherwise specified. Data from positive assays are reported as means of triplicate determinations from each of two cell-free extracts. Pyruvate synthase (EC 1.2.7.1) and 2-oxoglutarate synthesis (EC 1.2.7.3) were assayed in the reverse direction under a CO_2–H_2–N_2 atmosphere (5:10:85) using methyl viologen as the electron acceptor (Odom and Peck 1981) and by measuring the production of CO_2. Assays for CO_2 production were performed under N_2 atmosphere and stopped in a 70°C water bath. The mixture was acidified by injecting HCl (500 μL, 4 N) through the rubber stopper. The headspace gas was analyzed by gas chromatography (Cornick et al. 1994). Pyruvate dehydrogenase (EC 1.2.4.1) and α-ketoglutarate dehydrogenase complex were assayed by following the reduction of NAD using a spectrophotometer (Gilford Response II, Ciba Corning, Oberlin, Ohio) (Reed and Mukherjee 1969). Malate dehydrogenase (EC 1.1.1.37) and isocitrate dehydrogenase (EC 1.1.1.41) were assayed spectrophotometrically using previously described methods (Reeves et al. 1971). Citrate synthase (EC 4.1.3.7) and malate synthase (EC 4.1.3.2) were assayed spectrophotometrically by following the loss of absorbance at 232 nm of the thioester bond of acetyl-CoA (Reeves et al. 1971; Stadtman 1957). Carbon monoxide dehydrogenase activity was measured under N_2 atmosphere using

Table 1. Specific activity of cell carbon of *Oxalobacter formigenes* grown in [^{14}C]oxalate or [1-^{14}C]acetate.

^{14}C substrate	Expt.	Dry weight (mg/mL)	Specific activity (dpm/mg C)		
			Substrate	Cell carbon	Incorporation (%)
Oxalate	A	0.034	2.5×10^5	1.3×10^5	52
	B	0.044	5.4×10^4	3.1×10^4	57
Acetate	C	0.023	3.3×10^6	2.0×10^5	6
	D	0.049	1.3×10^6	1.3×10^5	10

Note: For experiments A and C, bacteria were grown in medium F containing either [^{14}C]oxalate (2.9 μCi/mmol) or [1-^{14}C]acetate (36.8 μCi/mmol). For experiments B and D, bacteria were grown in medium E containing either [^{14}C]oxalate (0.62 μCi/mmol) or [1-^{14}C]acetate (14.6 μCi/mmol). The inoculum size for all of the experiments was 0.6%.

Table 2. Relative distribution of ^{14}C in fractions of *Oxalobacter formigenes* cells after growth in labeled compounds.

	^{14}C-labeled substrates			
	Oxalate	CO_3	[1-^{14}C]Acetate	[2-^{14}C]Acetate
% of ^{14}C incorporated	0.7	1.3	14.8	9
% recovery in fractions[a]	70	102	60	67
% of ^{14}C recovered in:				
Intermediates	29	9	15	16
Lipids	17	21	48	48
Nucleic acids	29	34	8	9
Proteins	27	36	28	27

Note: Mean values of two experiments are given. Bacteria were grown in medium E for experiments with $^{14}CO_3$ or in medium F for experiments with [^{14}C]oxalate and [^{14}C]acetate.

[a]Cells were fractionated using the method of Roberts et al. (1963).

methyl viologen dye as the electron acceptor (Diekert and Thauer 1978). The assay mixture contained approximately 40 μmol CO. Protein was measured using a modification of the Lowry assay (Peterson 1977). Bovine serum albumin was the standard.

Determination of the specific activity of cellular carbon

Bacteria were grown in medium E or F with [^{14}C]oxalate or [1-^{14}C]acetate to the end of logarithmic growth. Cells were harvested by centrifugation and washed. The cell pellets were frozen in an acetone – dry ice bath and lyophilized (Freezemobile, Vitris Instruments, Mt. Prospect, Ill.). The cells were burned in a combustion oven (Biological Material Oxidizer, RJ Harvey Instrument Co., Hillsdale, N.J.) at 900°C. The resulting $^{14}CO_2$ was trapped in 15 mL of 0.25 N NaOH (prepared from CO_2-free water). Water free of carbon dioxide was prepared by boiling and cooling distilled water under N_2. The water was stored in a stoppered bottle connected to a trap filled with soda lime. Mannitol (25 mg) was burned between each sample to eliminate any carryover of $^{14}CO_2$ between samples. The $^{14}CO_3$ was precipitated with 2 mL of 1 M $BaCl_2$ (CO_2-free). The $Ba^{14}CO_3$ was collected by filtration, washed with distilled water and ethanol, and dried at 125°C. The $Ba^{14}CO_3$ was ground to a powder and weighed amounts (duplicate samples) were suspended in scintillation fluid (Ecoscint) containing fumed silica (Cab-o-sil, Packard Instruments, Downers Grove, Ill.) (Hash 1972; Turner 1969). Counting efficiency was determined from measurements of $Ba^{14}CO_3$ with a known specific activity. The contribution of each precursor to total cell carbon was calculated by dividing the specific activity of the ^{14}C obtained as $Ba^{14}CO_3$ by the initial specific activity of the ^{14}C of the precursor.

Results and discussion

Sources of cell carbon

Measurements of specific activities of carbon in cells from cultures grown with either [^{14}C]oxalate or [^{14}C]acetate indicated that at least 54% of the cell carbon was derived from oxalate and at least 7% of the cell carbon was derived from acetate (Table 1). Owing to the dilution of the specific activity of CO_3 by the decarboxylation of oxalate during growth, we were unable to estimate the contribution of CO_3 to cell carbon. For these experiments oxalate and acetate were added to the media as carbon sources. Formate and CO_2 were also produced by the decarboxylation of oxalate and available as sources of carbon, and cysteine (0.5 g/L) was added to the media as a reducing agent. Since previous experiments have demonstrated that sodium sulfide can replace cysteine as a reducing agent (Allison et al. 1985), cysteine is not a required source of carbon for *Oxalobacter formigenes*.

Distribution of label in cell fractions

Cells of *Oxalobacter formigenes* exposed to ^{14}C-labeled substrates for 0.5 generations incorporated approximately 1% of the available carbon from oxalate (100 mM) and carbonate, and 12% of the available carbon from acetate (0.5 mM) into cell biomass (Table 2). This is consistent with previous data which indicated that the primary function of oxalate metabolism is to

Table 3. Relative specific activity of amino acids labeled by [^{14}C]oxalate, [^{14}C]acetate, and $^{14}CO_3$.

	Labeled substrates			
Amino acid	[^{14}C]Oxalate	$^{14}CO_3$	[1-^{14}C]-Acetate	[2-^{14}C]-Acetate
Glutamate	9	65	30	16
Proline/valine	16	42	64	73
Arginine/isoleucine	66	100	81	37
Aspartate	48	—[a]	19	8
Methionine	—	58	—	—
Threonine	9	8	—	—
Lysine	50	38	—	16
Leucine	16	2	77	100
Alanine	11	—	10	15
Serine	17	16	—	—
Glycine	7	6	—	—
Phenylalanine	100	2	—	—

Note: Mean values of two experiments are given. Based on cpm/nmol of amino acid. The amino acid with the highest specific activity was set at 100 and the specific activities of the other amino acids are expressed relative to that value. For [1-^{14}C]acetate, arginine/isoleucine had the highest specific activity in one experiment and leucine had the highest specific activity in the second experiment.

[a]No ^{14}C detected.

Table 4. Enzymatic activities in cell-free extracts of *Oxalobacter formigenes*.

Enzyme	Assay measurement	Specific activity[a]
Citrate synthase	Thioester bond reduction	23
Isocitrate dehydrogenase	NAD reduction	9
α-Ketoglutarate dehydrogenase	NAD reduction	NA(<1)[b]
Malate dehydrogenase	NAD reduction	2
Pyruvate dehydrogenase	NAD reduction	NA(<1)
Malate synthase	Thioester bond reduction	NA(<1)
Pyruvate synthase reverse reaction	CO_2 production	NA(<1)
	Methyl viologen reduction	NA(<10)
2-Oxoglutarate synthase reverse reaction	CO_2 production	NA(<1)
	Methyl viologen reduction	NA(<10)
Carbon monoxide dehydrogenase	Methyl viologen reduction	NA(<20)

[a]nmol/(min · mg protein), average of triplicate assays from each of two cell-free extracts.

[b]NA, no activity detected (limit of detection).

provide energy for the cell and that 99% of the oxalate utilized was decarboxylated to formate and CO_2 (Cornick 1995; Dawson et al. 1980). Although only 1% of the carbon from oxalate was assimilated into biomass, the majority of cell carbon (54%) was derived from oxalate. Carbon from oxalate, acetate, and CO_3 was distributed into all of the major cell fractions of *Oxalobacter formigenes* (Table 2). Radioactive carbon from acetate was incorporated primarily into the lipid and protein fractions. The distribution of radioactivity from [1-^{14}C]- and [2-^{14}C]-labeled acetate in cell fractions was similar, with twice as much label recovered from the lipid fraction as from the protein fraction.

When *Escherichia coli* was grown in glucose and [U-^{14}C]acetate, 43% of the carbon from acetate that was incorporated was detected in the lipid fraction and another 43% was detected in the protein fraction (Roberts et al. 1963). *Methanosarcina barkeri* (Weimer and Zeikus 1978) and *Chlorobium thiosulfatum* (Hoare and Gibson 1964) also incorporated a greater percentage of carbon from acetate into cell protein (50–70%) than did *Oxalobacter formigenes*. In both of the latter organisms, acetate was the only source of C_2 compound available to build larger carbon molecules. This is in contrast to *Oxalobacter formigenes*, which utilizes both acetate and oxalate. When cells of *Oxalobacter formigenes* were grown in [^{14}C]formate, only 0.08% of the label was incorporated into cell biomass versus 1.3% of the label from $^{14}CO_3$. Since the specific activities of [^{14}C]formate and $^{14}CO_3$ would be diluted to the same extent by the decarboxylation of oxalate during growth, the calculated contribution of formate carbon to cell biomass is clearly much less than the contribution of carbon from CO_3.

Relative specific activity of amino acids

^{14}C-labeled carbon from oxalate was incorporated into amino acids expected to be derived from α-ketoglutarate (glutamate, arginine, proline), oxaloacetate (aspartate, threonine, methionine, lysine, isoleucine), pyruvate (alanine, valine, leucine), and 3P-glycerate (serine, glycine) and into the aromatic amino acids (phenylalanine) (Table 3). This agrees with previous enzymatic data which indicated that oxalate was reduced to 3P-glycerate before entering central metabolic pathways (Cornick and Allison 1996). Carbon from $^{14}CO_3$ was also detected in amino acids expected to be derived from α-ketoglutarate, oxaloacetate, pyruvate, and 3P-glycerate and in the aromatic amino acids. Tryptophan would be destroyed during the acid hydrolysis step. The apparent lack of ^{14}C incorporation into histidine and tyrosine probably reflects our limited ability to measure ^{14}C in these two amino acids, owing to their relatively low concentrations in the protein hydrolysates studied (data not shown).

Label from acetate was incorporated into the amino acids expected to be derived from pyruvate, oxaloacetate, and α-ketoglutarate. When the relative specific activities of glutamate, aspartate, and alanine from cells grown with [1-^{14}C]- or [2-^{14}C]-labeled acetate were compared, the specific activity of aspartate was approximately half the specific activity of glutamate. Alanine derived from [2-^{14}C]acetate had approximately the same relative specific activity as glutamate, but alanine derived from [1-^{14}C]acetate had only one third of the relative specific activity of glutamate. Label from [2-^{14}C]acetate was also incorporated into lysine, but label from [1-^{14}C]acetate was not (Table 3).

Methanobacterium thermoautotrophicum assimilates acetate by the reductive C_4 dicarboxylic acid pathway via pyruvate synthase and 2-oxoglutarate synthase; therefore, the specific

activities of glutamate, aspartate, and alanine from cells grown in [U-^{14}C]acetate are all similar (Fuchs et al. 1978). *Desulfovibrio vulgaris* (Badziong et al. 1979), *Methanosarcina barkeri* (Weimer and Zeikus 1979), and *Thermoproteus neutrophilus* (Schafer et al. 1989) assimilate acetate using a horseshoe type of tricarboxylic acid (TCA) pathway. Acetyl-CoA is condensed with oxaloacetate via citrate synthase in the oxidative direction of the TCA pathway and acetate is also carboxylated by pyruvate synthase in the reductive direction. When acetate is incorporated by this pathway, the specific activity of glutamate is double that of both alanine and aspartate. The specific activities of alanine, aspartate, and glutamate did not change when *Methanosarcina barkeri* was grown with either [1-^{14}C]- or [2-^{14}C]-acetate (Weimer and Zeikus 1979). This is in contrast to our data from *Oxalobacter formigenes* in which the specific activity of alanine derived from [1-^{14}C]- and [2-^{14}C]-acetate-grown cells was different. Our results suggest that acetate was not always incorporated as a C_2 unit into amino acids.

Enzymatic activity

We detected citrate synthase, isocitrate dehydrogenase, and malate dehydrogenase activities in cell-free extracts of *Oxalobacter formigenes* (Table 4). The presence of citrate synthase and isocitrate dehydrogenase activities suggests that the reactions of the TCA pathway operate in the oxidative direction as far as α-ketoglutarate. The lack of α-ketoglutarate dehydrogenase activity was not surprising, since this enzyme is not expected to be produced by strictly anaerobic bacteria. We assayed cell-free extracts of *Oxalobacter formigenes* for malate synthase, because it catalyzes the condensation of glyoxylate and acetyl-CoA to form malate. This reaction functions in an anaplerotic role in many aerobic bacteria to replace C_4 units siphoned off into biosynthetic pathways (Gottschalk 1979). Although oxalate is reduced to glyoxylate by *Oxalobacter formigenes* (Cornick and Allison 1996) and other oxalate-degrading bacteria (Quayle et al. 1961; Zaitsev et al. 1993), malate synthase activity has not been detected in any of these organisms. Our failure to detect pyruvate synthase, 2-oxoglutarate synthase, and carbon monoxide dehydrogenase activities implies that the reductive C_4 dicarboxylic acid pathway and the acetyl-CoA (Wood) pathway do not function in *Oxalobacter formigenes*.

Overall, our results indicate that *Oxalobacter formigenes* utilizes oxalate, acetate, and CO_2 as sources of carbon for cell synthesis. The reductive carboxylation of acetate by pyruvate synthase is a common reaction utilized by anaerobic bacteria to assimilate acetate. The incorporation of carbon from [^{14}C]acetate into amino acids derived from pyruvate (alanine, valine) would be compatible with the function of pyruvate synthase; however, we were unable to detect this activity in cell-free extracts. The lack of incorporation of $^{14}CO_3$ into alanine (Table 3) and the apparent differences in the relative specific activities of alanine derived from [1-^{14}C]- and [2-^{14}]-acetate suggest that *Oxalobacter formigenes* may incorporate acetate into pyruvate by a different mechanism. Subsequent data obtained by growing cells with [^{13}C]acetate supports this and indicates that 40% of the acetate incorporated into amino acids enters as single carbons (Cornick 1995; Cornick et al. 1996).

Acknowledgements

We thank Dr. Henry Stahr for the use of the combustion oven and Herbert Cook for his technical assistance.

References

Allen, G. 1989. Sequencing of proteins and peptides. *In* Laboratory techniques in biochemistry and molecular biology. *Edited by* R.H. Burdon and P.H. van Knippenberg. Vol. 9. Elsevier, New York. pp. 52–53.

Allison, M.J., Dawson, K.A., Mayberry, W.R., and Foss, J.G. 1985. *Oxalobacter formigenes* gen.nov., sp.nov.: oxalate-degrading anaerobes that inhabit the gastrointestinal tract. Arch. Microbiol. **141**: 1–7.

Allison, M.J., Cook, H.M., Milne, D.B., Gallagher, S., and Clayman, R.V. 1986. Oxalate degradation by gastrointestinal bacteria from humans. J. Nutr. **116**: 455–460.

Allison, M.J., Daniel, S.L., and Cornick, N.A. 1995. Oxalate-degrading bacteria. *In* Calcium oxalate in biological systems. *Edited by* S.R. Khan. CRC Press, Boca Raton, Fla. pp. 131–168.

Anderson, R.C., Rasmussen, M.A., and Allison, M.J. 1993. Metabolism of the plant toxins nitropropionic acid and nitropropanol by ruminal microorganisms. Appl. Environ. Microbiol. **59**: 3056–3061.

Badziong, W., Ditter, B., and Thauer, R.K. 1979. Acetate and carbon dioxide assimilation by *Desulfovibrio vulgaris* (Marburg), growing on hydrogen and sulfate as sole energy source. Arch. Microbiol. **123**: 301–305.

Collins, M.D., Lawson, P.A., Willems, A., Cordoba, J.J., Fernandez-Garayzabal, J., Garcia, P., Cai, J., Hippe, H., and Farrow, J.A.E. 1994. The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. Int. J. Syst. Bacteriol. **44**: 812–826.

Cornick, N.A. 1995. Biosynthetic pathways in *O. formigenes*. Ph.D. dissertation, Iowa State University, Ames.

Cornick, N.A., and Allison, M.J. 1996. Anabolic incorporation of oxalate by *Oxalobacter formigenes*. Appl. Environ. Microbiol. **62**: 3011–3013.

Cornick, N.A., Jensen, N.S., Stahl, D.A., Hartman, P.A., and Allison, M.J. 1994. *Lachnospira pectinoschiza* sp.nov., an anaerobic pectinophile from the pig intestine. Int. J. Syst. Bacteriol. **44**: 87–93.

Cornick, N.A., Allison, M.J., Yan, B., and Bank, S. 1996. Biosynthesis of amino acids by *Oxalobacter formigenes*: analysis using ^{13}C-NMR. Can. J. Microbiol. **42**. In press.

Daniel, S.L., Hartman, P.A., and Allison, M.J. 1987. Microbial degradation of oxalate in the gastrointestinal tracts of rats. Appl. Environ. Microbiol. **53**: 1793–1797.

Dawson, K.A., Allison, M.J., and Hartman, P.A. 1980. Isolation and some characteristics of anaerobic oxalate-degrading bacteria from the rumen. Appl. Environ. Microbiol. **40**: 833–839.

Dehning, I., and Schink, B. 1989. Two new species of anaerobic oxalate-fermenting bacteria, *Oxalobacter vibrioformis* sp.nov. and *Clostridium oxalicum* sp.nov., from sediment samples. Arch. Microbiol. **153**: 79–84.

Diekert, G.B., and Thauer, R.K. 1978. Carbon monoxide oxidation by *Clostridium thermoaceticum* and *Clostridium formicoaceticum*. J. Bacteriol. **136**: 597–606.

Fuchs, G., Stupperich, E., and Thauer, R.K. 1978. Acetate assimilation and the synthesis of alanine, aspartate, and glutamate in *Methanobacterium thermoautrophicum*. Arch. Microbiol. **117**: 61–66.

Gottschalk, G. (*Editor*). 1979. Bacterial metabolism. Springer-Verlag, New York.

Hash, J.H. 1972. Liquid scintillation counting in microbiology. *In* Methods in microbiology. *Edited by* J.R. Norris and D.W. Ribbons. Academic Press, New York. pp. 129–132.

Hoare, D.S., and Gibson, J. 1964. Photoassimilation of acetate and the biosynthesis of amino acids by *Chlorobium thiosulphatophilum*. Biochem. J. **91**: 546–559.

Odom, J.M., and Peck, H.D. 1981. Localization of dehydrogenases, reductases and electron transfer components in the sulfate-reducing bacterium *Desulfovibrio gigas*. J. Bacteriol. **147**: 161–169.

Peterson, G.L. 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem. **83**: 346–356.

Pfennig, N., and Lippert, K.D. 1966. Über das vitamin B_{12} bedürfins phototroper Schwefelbacterien. Arch. Mikrobiol. **55**: 245–256.

Quayle, J.R., Keech, D.B., and Taylor, G.A. 1961. Carbon assimilation by *Pseudomonas oxalaticus* (OX1). 4. Metabolism of oxalate in cell-free extracts of the organism grown on oxalate. Biochem. J. **78**: 225–236.

Reed, L.J., and Mukherjee, B.B. 1969. α-Ketoglutarate dehydrogenase complex from *Escherichia coli*. Methods Enzymol. **13**: 55–61.

Reeves, H.C., Rabin, R., Wegener, W.S., and Ajl, S.J. 1971. Assays of enzymes of the tricarboxylic acid and glyoxylate cycles. *In* Methods in microbiology. *Edited by* J.R. Norris, and D.W. Ribbons. Academic Press, New York. pp. 462–462.

Roberts, R., Abelson, P.H., Cowie, D.B., Bolton, E.T., and Britten, R.J. (*Editor*). 1963. Studies of biosynthesis in *Escherichia coli*. Carnegie Institute, Washington, D.C.

Schafer, S., Paalme, T., Vilu, R., and Fuchs, G. 1989. ^{13}C-NMR study of acetate assimilation in *Thermoproteus neutrophilus*. Eur. J. Biochem. **186**: 695–700.

Smith, R.L., Strohmaier, F.E., and Oremland, R.S. 1985. Isolation of anaerobic oxalate-degrading bacteria from freshwater lake sediments. Arch. Microbiol. **141**: 8–13.

Stadtman, E.R. 1957. Preparation and assay of acyl coenzyme A and other thiol esters; use of hydroxylamine. Methods Enzymol. **3**: 931–941.

Turner, J.C. 1969. Gel suspension counting of barium carbonate-^{14}C. Int. J. Appl. Radiat. Isot. **20**: 761–766.

Weimer, P.J., and Zeikus, J.G. 1978. Acetate metabolism in *Methanosarcina barkeri*. Arch. Microbiol. **119**: 175–182.

Weimer, P.J., and Zeikus, J.G. 1979. Acetate assimilation pathway of *Methanosarcina barkeri*. J. Microbiol. **137**: 332–339.

Zaitsev, G.M., Govorukhina, N.I., Laskovneva, O.V., and Trotsenko, Y.A. 1993. Properties of the new oxalotrophic bacterium *Bacillus oxalophilus*. Microbiology, **62**: 378–382.