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Microbial Metabolism of Amino Alcohols

METABOLISM OF ETHANOLAMINE AND 1-AMINOPROPAN-2-OL IN SPECIES OF *ERWINIA* AND THE ROLES OF AMINO ALCOHOL KINASE AND AMINO ALCOHOL *O*-PHOSPHATE PHOSPHO-LYASE IN ALDEHYDE FORMATION

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1. Growth of *Erwinia carotovora* N.C.P.P.B. 1280 on media containing 1-aminopropan-2-ol compounds or ethanolamine as the sole N source resulted in the excretion of propionaldehyde or acetaldehyde respectively. The inclusion of $(NH_4)_2SO_4$ in media prevented aldehyde formation. 2. Growth, microrespirometric and enzymic evidence implicated amino alcohol *O*-phosphates as aldehyde precursors. An inducibly formed ATP–amino alcohol phosphotransferase was partially purified and found to be markedly stimulated by ADP, unaffected by NH_4^+ ions and more active with ethanolamine than with 1-aminopropan-2-ol compounds. Amino alcohol *O*-phosphates were deaminated by an inducible phospho-lyase to give the corresponding aldehydes. This enzyme, separated from the kinase during purification, was more active with ethanolamine *O*-phosphate than with 1-aminopropan-2-ol *O*-phosphates. Activity of the phospho-lyase was unaffected by a number of possible effectors, including NH_4^+ ions, but its formation was repressed by the addition of $(NH_4)_2SO_4$ to growth media. 3. *E. carotovora* was unable to grow with ethanolamine or 1-aminopropan-2-ol compounds as sources of C, the production of aldehydes during utilization as N sources being attributable to the inability of the microbe to synthesize aldehyde dehydrogenase. 4. Of seven additional strains of *Erwinia* examined similar results were obtained only with *Erwinia ananas* (N.C.P.P.B. 441) and *Erwinia milletiae* (N.C.P.P.B. 955).

Interest in the metabolism of 2-amino alcohols arose from studies of the origin of the D-1-aminopropan-2-ol moiety of vitamin B_{12}, and the possible role of 1-aminopropan-2-ol–NAD^+ oxidoreductases in its biosynthesis (Turner, 1966, 1967; Higgins *et al.*, 1968; Pickard *et al.*, 1968; Lowe & Turner, 1968). It was observed later (Jones & Turner, 1971) that the plant pathogen *Erwinia carotovora*, growing with either 1-aminopropan-2-ol or ethanolamine as N source, produced large amounts of volatile aldehydes.

The aldehydes were shown to be propionaldehyde and acetaldehyde respectively, but the route of their formation was not clear. Acetaldehyde formation from ethanolamine had been observed with *Proteus morganii* (Miyaki *et al.*, 1959*a*,*b*) and *Clostridium* sp. (Bradbeer, 1965*a*,*b*; Kaplan & Stadtman, 1968*a*,*b*). In both cases, however, the ethanolamine deaminase system was inactive with 1-aminopropan-2-ol as a substrate (Miyaki *et al.*, 1959*a*; Kaplan & Stadtman, 1968*a*) and the aldehydic carbon of the product was derived from the carbinol carbon of ethanolamine (Miyaki *et al.*, 1959*b*; Kaplan & Stadtman, 1968*b*). The production of propionaldehyde rather than acetone from 1-aminopropan-2-ol suggested a different mechanism in *Erwinia carotovora*. Although ethanolamine was known to be metabolized via glycolaldehyde by an oxidase in an *Arthrobacter* sp. which was slightly active with 1-aminopropan-2-ol (Narrod & Jakoby, 1964, 1966) this did not seem a likely route to aliphatic aldehydes.

Evidence is presented here that the formation of aldehydes from 1-amino-2-alcohols proceeds via their 2-*O*-phosphate esters (Jones & Turner, 1971) as shown in Scheme 1. The ATP–amino alcohol phosphotransferase and amino alcohol *O*-phosphate phospho-lyase enzymes responsible (referred to as 'kinase' and 'phospho-lyase' respectively), previously discovered in a species of pseudomonad (Jones & Turner, 1973), have been found in several species of *Erwinia*. Whereas the enzymes play a role in the assimilation of carbon by pseudomonads (Jones & Turner, 1973) similar to that envisaged for the incorporation of ethanolamine into fatty acids via acetate in mammals (Sprinson & Coulon, 1954; Fleshood & Pitot, 1970*b*), the pathway from amino

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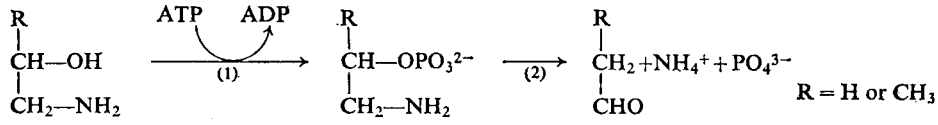

Scheme 1. *Pathway of amino alcohol metabolism in E. carotovora*

Key to enzymes: (1) ATP–amino alcohol phosphotransferase (kinase); (2) amino alcohol *O*-phosphate phospho-lyase (deaminating).

alcohols to aldehydes in *Erwinia* species appears to be primarily a deamination process necessary for N assimilation.

Methods

Micro-organisms and media

Erwinia ananas, *E. carotovora* and *Erwinia milletiae* were obtained from the National Collection of Plant Pathogenic Bacteria, Ministry of Agriculture, Fisheries and Food, Hatching Green, Harpenden, Herts., U.K., and bore the N.C.P.P.B. numbers 441, 1280 and 955 respectively. (Other species obtained from the same source are described in Table 6.)

Micro-organisms were maintained on nutrient agar by standard procedures. Cultivation was usually on simple synthetic media containing (per litre) 7.0g of K_2HPO_4, 3.0g of KH_2PO_4, 0.1g of $MgSO_4,7H_2O$, 1.0g of Na_2SO_4, 3g of C source and 1.0g of N source unless otherwise stated. When $(NH_4)_2SO_4$ was the N source this replaced Na_2SO_4. Media were adjusted to pH7 before sterilization by autoclaving. Liquid cultures were incubated at 30–32°C in conical flasks on a rotary shaker (170 oscillations/min), usually in 2-litre flasks containing 1 litre of medium. The length of incubation depended on the inoculum and medium composition.

Growth in liquid cultures was usually followed turbidimetrically as E_{540}^{1cm}. An extinction value of 1.0 corresponded to 0.54mg dry wt. of organisms/ml. In some cases growth was measured automatically in a Recording Biophotometer (Jouan, Paris 6, France). Cultures were harvested during exponential growth unless otherwise stated.

Detection, isolation and characterization of volatile aldehydes formed during growth

Detection, and quantitative and semi-quantitative assays of aldehyde production, were done as described by Jones & Turner (1973). Aldehydes were trapped as their 2,4-dinitrophenylhydrazones and characterized by t.l.c., u.v.- and visible-light spectrophotometry, mass spectrometry and m.p. analysis, also as previously described (Jones & Turner, 1973).

Incorporation of radioactivity from DL-1-*amino*[3-^{14}C]-*propan-2-ol and from* [2-^{14}C]*ethanol-2-amine during bacterial growth*

Radioactivity in microbial material, culture filtrates and aldehydes was measured as described by Jones & Turner (1971). Microbial material was fractionated by the extraction procedure of Roberts *et al.* (1957). Radioactive fractions were examined for phospholipids by t.l.c. on silica gel in chloroform–methanol–acetic acid–water (25:15:4:2, by vol.) (Skipski *et al.*, 1964). Reference compounds used as markers were phosphatidylcholine, phosphatidylserine, cephalin, phosphatidylinositol, sphingomyelin, phosphatidic acid, ethanolamine *O*-phosphate and ethanolamine. Dried plates were scanned for radioactivity on a Pannax scanner and sprayed with either phosphomolybdic acid (10%, w/v, in ethanol) or ninhydrin [0.25% in acetone–lutidine (9:1, v/v)], or were treated with I_2 vapour.

O_2 uptake by washed suspensions

This was measured manometrically with conventional equipment and procedures (Umbreit *et al.*, 1964) as described for a pseudomonad by Jones & Turner (1973).

Enzyme assays

Micro-organisms were disrupted and cell-free extracts prepared as previously described for *Escherichia coli* (Turner, 1966). Enzymes were assayed at 37°C.

Amino alcohol O-phosphate phospho-lyase (deaminating). Activity was assayed either enzymically with alcohol dehydrogenase added to measure aldehyde formation, or colorimetrically with *N*-methylbenzothiazolone hydrazone as described by Jones & Turner (1973).

ATP–amino alcohol phosphotransferase (amino alcohol kinase). Activity was usually assayed colorimetrically by direct linkage with the phospho-lyase either present in crude extracts or added back after partial purification by ion-exchange chromatography.

A modification of the method described by Jones & Turner (1973) was used, where the concentration of the amino alcohol was 2mM, that of ATP was 5mM, and ADP at 7mM was also present in reaction mixtures. A further modification of this assay, suitable for the purified enzyme, involved the separate incubation of kinase reaction components, heat treatment of the reaction mixture followed by a second incubation with phospho-lyase, and finally the colorimetric assay of aldehyde. Thus incubation mixtures contained 50μmol of Tris–HCl buffer, pH 7.5, 5μmol of ATP, 7μmol of ADP, 5μmol of $MgSO_4$, 2μmol of amino alcohol and enzyme preparation in a volume of 1ml. After incubation for 15min reactions were stopped by heating at 100°C for 10min, and the mixtures were cooled and centrifuged. To 0.5ml of supernatant was added 0.5ml of phospho-lyase preparation in Tris–HCl buffer (containing 0.4μmol of pyridoxal phosphate) and the mixture was incubated for 90min. The aldehyde formed was measured colorimetrically. In some cases the formation of aldehyde by the directly coupled system was measured enzymically (Jones & Turner, 1973).

Aldehyde dehydrogenase. Searches for enzyme activity used a modification of the methods described by King & Cheldelin (1956) and Jakoby (1958) as described by Jones & Turner (1973). A variety of buffers, pH values, cofactor and substrate concentrations were tested.

Other enzymes. Standard methods were used to assay ethanolamine deaminase (Kaplan & Stadtman, 1968*a*), L-threonine dehydratase (Datta, 1966), L- and D-1-aminopropan-2-ol dehydrogenases (Pickard *et al.* 1968; Lowe & Turner, 1970) and alcohol dehydrogenase (Vallee & Hoch, 1955).

Assay of protein

Protein was measured by a modified biuret procedure as described previously (Turner, 1966).

Chromatographic separation of kinase and phospho-lyase enzymes

Crude extracts were chromatographed on DEAE-cellulose or Biogel P-300 by standard methods as previously described for extracts of pseudomonads (Jones & Turner, 1973; Pickard *et al.*, 1968). Phospho-lyase activities were detected in eluate samples by colorimetric assay of aldehyde formation. The phospho-lyase was used to locate the kinase by the direct linkage of activities as described above. Results are shown in Fig. 2.

Chemicals

Radioactive DL-1-amino[3-^{14}C]propan-2-ol was a generous gift from Pfizer Ltd. (Sandwich, Kent, U.K.). Cobamide coenzyme was a generous gift from Glaxo Laboratories (Greenford, Middx., U.K.). Optically active amino alcohols and their *O*-phosphates were prepared as described previously (Jones & Turner, 1973). All other chemicals were obtained from commercial sources. Tris–HCl buffers were prepared from the specially purified Trizma base (Sigma Chemical Co., St. Louis, Mo., U.S.A.).

Results

Growth and aldehyde production by Erwinia species

E. carotovora (N.C.P.P.B. 1280) was capable of good growth on a variety of C and energy sources with $(NH_4)_2SO_4$, ethanolamine or DL-1-aminopropan-2-ol as sole source of N. The final growth response was similar with each N source when the concentration of this was growth-limiting (below 100mg of N/l of medium containing 5g of glycerol), which suggested that both D- and L-1-aminopropan-2-ol were deaminated. A variety of 2-amino alcohols was tested for their ability to act as N sources for growth on glycerol or malate. Most were either poor sources or inactive (Table 1). In contrast to *Pseudomonas* sp. P6 (Jones & Turner, 1973) *E. carotovora* grew rapidly with L- or DL-1-aminopropan-2-ol, but only slowly with D-1-aminopropan-2-ol as sole N source (see Fig. 1) although the eventual cell density was similar in all cases. Also in contrast to the pseudomonad, *E. carotovora* was incapable of growth on either ethanolamine or DL-1-aminopropan-2-ol as sole C and N source or on propionaldehyde or propionate with $(NH_4)_2SO_4$. Studies of the C and N nutrition of the micro-organism (Table 1) suggested that it was closely related to the *Erwinia* species studied by Grula *et al.* (1968) except that *E. carotovora* grew well on glycerol but only poorly on succinate.

With either ethanolamine or DL-1-aminopropan-2-ol, but not with $(NH_4)_2SO_4$, substantial amounts of volatile aldehydes accumulated (see the Methods section) during growth on any utilizable carbon source (Table 1). These were trapped as their 2,4-dinitrophenylhydrazone derivatives and examined by a variety of chromatographic and physicochemical techniques (Table 2). Their identities were established to be acetaldehyde and propionaldehyde respectively. Each stereoisomer of 1-aminopropan-2-ol served as a precursor of propionaldehyde, but aldehyde production closely paralleled growth and was slow during growth on D-1-aminopropan-2-ol. The inclusion of $(NH_4)_2SO_4$ in media containing ethanolamine or 1-aminopropan-2-ol prevented aldehyde formation. The addition of $(NH_4)_2SO_4$ to cultures actively growing with these amino alcohols rapidly suppressed aldehyde formation.

Studies with DL-1-amino[3-^{14}C]propan-2-ol showed that amino alcohol utilization and propionaldehyde formation occurred stoicheiometrically and

Table 1. *Growth of E. carotovora on various sources of C and N*

Compounds were added as sole sources of C (3 g/l), or N (1 g/l), or C and N (3 g/l), to the basal salts medium as described in the Methods section. Growth was measured over a period of 96 h during aerobic incubation in shake flasks as described in the Methods section.

	Compounds supporting rapid growth	Compounds supporting slow growth	Compounds not supporting growth
(a)	Sources of C†		
	Citrate	Fumarate	Acetate
	D-Glucose*	L-Glutamate	Glycollate
	Glycerol	α-Oxoglutarate*	Glyoxylate
	DL-Malate	DL-Lactate	Oxalate
	Oxaloacetate*	Succinate	DL-Propane-1,2-diol
	Pyruvate		Propionaldehyde*
	Sucrose*		Propionate
(b)	Sources of N‡		
	DL-1-Aminopropan-2-ol	Aminoacetone*	Allylamine*
	L-1-Aminopropan-2-ol	DL-4-Amino-3-hydroxybutyrate	DL-2-Aminobutan-1-ol
	$(NH_4)_2SO_4$	D-1-Aminopropan-2-ol	3-Aminopropan-1-ol
	L-Aspartate	DL-1-Aminopropan-2-ol *O*-phosphate	DL-5-Hydroxylysine
	Ethanolamine	L-Lysine	DL-Phenylserine
	L-Glutamate	L-Threonine	Propylamine*
(c)	Sources of C and N		
	L-Alanine	L-Glutamate	Aminoacetone*
	L-Aspartate	L-Serine	DL-1-Aminopropan-2-ol
			L-1-Aminopropan-2-ol
			D-1-Aminopropan-2-ol
			Ethanolamine
			L-Threonine

* Sterilized by membrane filtration.
† N sources used were $(NH_4)_2SO_4$, ethanolamine or DL-1-aminopropan-2-ol.
‡ The C source was DL-malate.

that both processes closely paralleled growth. Little or no incorporation of radioactivity into micro-organisms occurred. These results contrasted with those obtained with *Pseudomonas* sp. P6 (see Table 3). When radioactive propionaldehyde was trapped as its 2,4-dinitrophenylhydrazone, specific-radioactivity measurements showed no dilution by propionaldehyde from metabolites of the C substrate for growth. All radioactivity disappeared from the medium when the N of DL-1-aminopropan-2-ol limited growth, confirming that amino alcohol deamination was non-stereospecific. Experiments with [2-^{14}C]ethanol-2-amine showed that appreciable radioactivity was assimilated by *E. carotovora*. In one case 58% of the radioactivity utilized from the medium was detected in the washed bacteria and 39% as acetaldehyde trapped as its 2,4-dinitrophenylhydrazone. The analysis of radioactivity in subcellular fractions of the micro-organism (Roberts *et al.*, 1957) showed that 84% of the label from ethanolamine was in the alcohol-soluble and alcohol–ether-soluble fractions, both of which contain lipids. Analysis of the material by t.l.c. on silica gel, with a variety of markers (see the Methods section), showed the presence of only one radioactive band, which co-chromatographed with phosphatidylethanolamine. Ethanolamine is also known to occur in the lipopolysaccharide fraction of Gram-negative bacteria. The addition of NH_4^+ to cultures growing with [2-^{14}C]-ethanolamine as N source decreased the incorporation of label into bacterial material.

Examination of possible routes for aldehyde formation

No ethanolamine deaminase activity (Kaplan & Stadtman, 1968*a*) towards ethanolamine itself or 1-aminopropan-2-ol could be detected in extracts of *E. carotovora* grown with either amino alcohol as N source. A variety of assay conditions in the presence of cobamide coenzyme were tested.

Aminoacetone was considered as a possible precursor of propionaldehyde. The aminoketone served as a poor source of N for growth on glycerol, but crop sizes were only a fraction of those observed with

equivalent amounts of DL-1-aminopropan-2-ol when used at limiting N concentrations. Although some evidence for methylglyoxal accumulation by cultures growing with aminoacetone was obtained, no propionaldehyde was detected. The addition of iodoacetate (5mM) or arsenite (1mM) to cultures growing with DL-1-aminopropan-2-ol did not result in aminoacetone accumulation. This contrasted with results obtained in analogous experiments with *Pseudomonas* sp. N.C.I.B. 8858 (Higgins *et al.*, 1968). Neither DL-1-aminopropan-2-ol nor aminoacetone stimulated O_2 uptake by washed suspensions of *E. carotovora* grown with these compounds as N sources, although glycerol (the C source) and tricarboxylic acid-cycle intermediates were rapidly oxidized. Extracts of *E. carotovora* exhibited only low dehydrogenase activities towards DL-, D- or L-1-aminopropan-2-ol (less than 4nmol of aminoacetone formed/min per mg of protein), irrespective of the N source for growth. Substrate-specificity studies suggested that low 1-aminopropan-2-ol dehydrogenase activities were due to a broadly specific secondary alcohol dehydrogenase similar to that found in *Escherichia coli* and a *Pseudomonas* sp. (Lowe & Turner, 1968, 1970). Thus the deamination of 1-aminopropan-2-ol was concluded to be independent of its initial oxidation to aminoacetone.

Other possible precursors of propionaldehyde were considered by similar approaches. *E. carotovora* was incapable of growth with allylamine or *n*-propylamine as N sources, or on propane-1,2-diol with $(NH_4)_2SO_4$. Extracts of the microbe grown on glycerol with amino alcohols exhibited no activities analogous to that of the pyridoxal phosphate-dependent L-threonine dehydratase (Datta, 1966). Although small amounts of this enzyme were found with the amino acid as substrate, it appeared to be present constitutively, and the effects of isoleucine, valine and AMP on activity indicated a biosynthetic function. Thus deamination by any of a number of possible

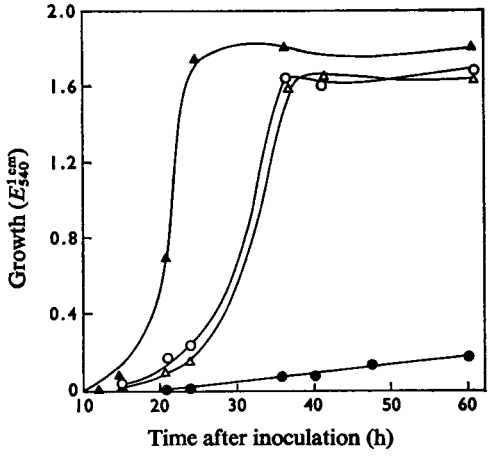

Fig. 1. *Growth of Erwinia carotovora on* DL-1-*aminopropan-2-ol and its stereoisomers as N sources*

Media contained 3g of glycerol/l and 1g of $(NH_4)_2SO_4$ (▲), DL-1-aminopropan-2-ol (△), the D-isomer (●) or the L-isomer (○)/l as supplements to the basal mineral salts media described in the Methods section. Growth was measured as E_{540}.

Table 2. *Characterization of the aldehydes produced during growth of E. carotovora on* DL-1-*aminopropan-2-ol or ethanolamine*

E. carotovora was grown in the outer well of a 250ml conical flask on 100ml of mineral salts medium containing glycerol and either DL-1-aminopropan-2-ol or ethanolamine. The centre well contained 5ml of 0.1% (w/v) 2,4-dinitrophenylhydrazine in 2M-HCl to trap the aldehydes produced. Crystallized derivatives of these and authentic carbonyl compounds were prepared and subjected to the analytical techniques as referred to in the Methods section. The chromatographic solvents used were: I, light petroleum (b.p. 60–80°C)–diethyl ether (3:2, v/v); II, benzene–tetrahydrofuran (93:7, v/v).

2,4-Dinitrophenylhydrazone	Melting point (°C)	Molecular ion (M^+)	Chromatographic R_F		Spectroscopic $\lambda_{max.}$ (nm)	
			Solvent I	Solvent II	No addition	KOH (1%, w/v) added
Propionaldehyde	155–156	238	0.37	0.59	359	520
Acetaldehyde	144–147	224	0.27	0.53	358	520
Acetone	131–132	224	0.32	0.54	351	532
Lactaldehyde (bis derivative)	306–309	474	0	0.32	370	565
Product of growth on DL-1-aminopropan-2-ol	153–155	238	0.37	0.59	358	520
Product of growth on ethanolamine	144–146	224	0.28	0.54	358	520

Table 3. *Distribution of radioactivity after the growth of E. carotovora and Pseudomonas sp. P6 on either* DL-1-*amino*-[3-^{14}C]*propan-2-ol or* [2-^{14}C]*ethanolamine*

E. carotovora was grown on mineral salts medium containing glycerol (3 g/l) plus DL-1-amino[3-^{14}C]propan-2-ol (2.6 μCi/nmol, 0.15 g/l) or [2-^{14}C]ethanol-2-amine (2.0 μCi/nmol, 0.15 g/l) in the outer well of a 250 ml conical flask as described previously (Jones & Turner, 1971). *Pseudomonas* sp. P6, which grows only slowly on ethanolamine and produces little or no acetaldehyde, was similarly grown in the presence of DL-1-amino-[3-^{14}C]propan-2-ol (0.13 μCi/nmol, 3.0 g/l) alone. Radioactivity in bacteria and products was measured as previously described (Jones & Turner, 1971). The aldehyde concentration for specific-radioactivity measurements (values given in parentheses as μCi/nmol) was determined colorimetrically by using *N*-methylbenzothiazolone hydrazone (Jones & Turner, 1973).

	Radioactivity (% of total after growth as indicated)		
	E. carotovora on glycerol plus N source shown		
Fraction	DL-1-Amino[3-^{14}C]propan-2-ol	[2-^{14}C]Ethanolamine	*Pseudomonas* sp. P6 on DL-1-Amino[3-^{14}C]propan-2-ol
Bacterial material	2.9	11.5	23.8
Spent medium	86.3	79.9	9.0
Propionaldehyde	9.2 (2.8)	—	2.2
Acetaldehyde	—	7.7 (2.1)	—

Table 4. *Effect of growth conditions on ATP–amino alcohol phosphotransferase and amino alcohol O-phosphate phospho-lyase activities of Erwinia species*

Bacteria were grown as described in the Methods section. Growth media contained 3 g of glycerol and 1 g of each or both N sources/l, as indicated. Phosphotransferase (kinase) activities of crude extracts were measured by incubating 50 μmol of Tris–HCl buffer, pH 7.5, 2 μmol of amino alcohol, 5 μmol of ATP, 10 μmol of $MgSO_4$, 7 μmol of ADP, extract (approx. 1 mg of protein), 1–2 units of kinase-free phospho-lyase preparation and 0.4 μmol of pyridoxal phosphate in a total volume of 1 ml for 20 min. Aldehyde formation was measured colorimetrically by using *N*-methylbenzothiazolone hydrazone as described previously (Jones & Turner, 1973). Phospho-lyase activity was measured by incubating 100 μmol of Tris–HCl buffer, pH 7.8, 10 μmol of amino alcohol *O*-phosphate, 0.4 μmol of pyridoxal phosphate and extract (approx. 1 mg of protein) in a total volume of 1 ml for 15 min at 37°C. Aldehyde formation was measured colorimetrically as described above. N.D., Not determined. The results in parentheses were obtained in a separate experiment.

	Enzyme activities (nmol/min per mg of protein at 37°C) with the substrates shown			
	Kinase		Phospho-lyase	
Micro-organisms and growth media	DL-1-Amino-propan-2-ol	Ethanolamine	DL-1-Aminopropan-2-ol-*O*-phosphate	Ethanolamine *O*-phosphate
E. carotovora				
Glycerol+aminopropanol	110	132	97	220
Glycerol+ethanolamine	140	162	78 (59)	170 (132)
Glycerol+aminopropanol +$(NH_4)_2SO_4$	N.D.	103	0	0
Glycerol+ethanolamine +$(NH_4)_2SO_4$	N.D.	136	0	0
E. ananas				
Glycerol+aminopropanol	N.D.	N.D.	30	84
Glycerol+ethanolamine	100 (110)	90 (100)	42	140
E. milletiae				
Glycerol+aminopropanol	N.D.	N.D.	62	156
Glycerol+ethanolamine	N.D.	50 (60)	28 (44)	62 (102)

mechanisms did not appear to be the initial step in propionaldehyde formation.

Reports of a phospho-lyase in mammalian tissues catalysing the formation of acetaldehyde from ethanolamine *O*-phosphate (Fleshood & Pitot, 1969, 1970*a*) prompted a consideration of 1-aminopropan-2-*O*-phosphate as the immediate precursor of propionaldehyde in *E. carotovora*. High phospholyase activities were found in extracts of the organism grown with either ethanolamine or 1-aminopropan-2-ol as N source. Both amino alcohol *O*-phosphates were active as substrates (Table 4). Although low kinase activity towards amino alcohols was detectable in some crude extracts, it was not until the activating effect of ADP was discovered that the enzyme was recognized to be metabolically significant. The effect of growth conditions on amino alcohol kinase activity in extracts of *E. carotovora* is shown in Table 4. It was concluded that the coupled activity of the amino alcohol kinase and the phospho-lyase enzymes was responsible for the formation of both propionaldehyde and acetaldehyde by intact growing micro-organisms.

ATP–amino alcohol phosphotransferase of E. carotovora

Activity of the kinase, purified about 5-fold by ion-exchange chromatography (Fig. 2), was assayed by coupling with the phospho-lyase enzyme and measuring the aldehyde formed either colorimetrically or enzymically. In each case ADP was an essential component of reaction mixtures (see the Methods section). The enzyme had optimum activity at pH 7.6 in Tris–HCl buffer, and was slightly more active with ethanolamine (100%) than with DL- (95%) and L-1-aminopropan-2-ol (83%). L-Serine at 20mM caused 40% inhibition of activity with either substrate. Additional properties of the partially purified enzyme are given in Table 5. The K_m values for ATP were virtually the same with either amino alcohol substrate.

Amino alcohol O-phosphate phospho-lyase of E. carotovora

The enzyme, purified about 15-fold, had optimum activity at pH 7.8 in Tris–HCl buffer and was considerably more active with ethanolamine *O*-phosphate (100%) than with DL-1-aminopropan-2-ol *O*-phosphate (44%). The same relative activities were found after heat treatment of crude extracts, exclusion chromatography on Biogel P-300 and ion-exchange chromatography. Activities toward both substrates were inhibited by substrate analogues in a similar manner. With substrate and inhibitor both at 10mM, the inhibition was greatest with DL-threonine *O*-phosphate (90%), less with DL-choline *O*-phosphate (30%) and only slight with DL-β-glycerophosphate (15%). DL-Serine *O*-phosphate was not inhibitory. Relative activities with the phosphates of the D- and L-isomers of 1-aminopropan-2-ol were 35% and 60% respectively. Anion-exchange chromatography on

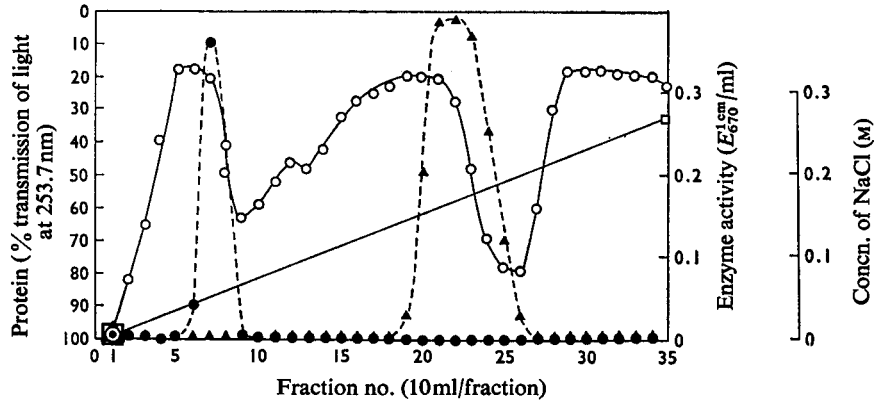

Fig. 2. *Partial purification of kinase and phospho-lyase enzymes of E. carotovora by ion-exchange chromatography*

A column of Whatman DE-52 DEAE-cellulose (25cm × 1.5cm diam.) was equilibrated with 0.03M-Tris–HCl buffer, pH 7.5, containing 0.01M-$MgSO_4$. A sample of crude extract prepared in Tris–HCl–$MgSO_4$ mixture (volume 3ml) was applied to the top of the column. Protein was eluted by downward flow with an increasing gradient of NaCl (——), over the range 0–0.3M in a total volume of 500ml, and fractions of approx. 10ml were collected automatically. Transmission of u.v. light at 253.7nm by the eluate (○) was recorded automatically. Phospho-lyase (▲) and kinase (●) activities in eluted fractions were assayed as described in the Methods section.

Table 5. *Some kinetic properties of the kinase and phospho-lyase enzymes of Erwinia species*

Bacteria were grown on mineral salts plus glycerol and aminopropanol medium. Enzyme activities were measured as described for Table 4. The enzymes of *E. carotovora* were preparations purified by ion-exchange chromatography, as described in the Methods section. Crude extracts of the other bacteria were used.

	Michaelis constants (mM) for the substrates shown					
	Kinase			Phospho-lyase		
Micro-organism	DL-1-Amino-propan-2-ol	Ethanol-amine	ATP	DL-1-Amino-propan-2-ol *O*-phosphate	Ethanol-amine *O*-phosphate	Pyridoxal phosphate
E. carotovora	0.25	0.15	1.1	0.25	1.0–2.0*	0.008
E. ananas	0.22†	0.16†	1.4	0.32	0.36	0.006
E. milletiae	0.35	0.36	1.0	0.32	0.46	0.006

* Some substrate inhibition occurred above 5mM.
† Marked substrate inhibition occurred above 1mM.

Table 6. *Growth and aldehyde production by species of Erwinia in the presence of amino alcohols as N sources*

Sterile medium (50ml) containing glycerol (3g/l) and either DL-1-aminopropan-2-ol or ethanolamine (each 1g/l) in a 250ml conical flask, with a centre well containing 5ml of 0.1% 2,4-dinitrophenylhydrazine in 2M-HCl, was inoculated with the respective microbe. Growth and aldehyde production were scored visually on a scale of nil (–) to excellent (+++).

Micro-organism	N.C.P.P.B. catalogue no.	Growth on either amino alcohol	Acetaldehyde production	Propionaldehyde production
Erwinia ananas	441	+++	++	+++
Erwinia carotovora	1280	+++	++	++
Erwinia milletiae	955	+++	–	–
Erwinia atroseptica	549	+	+	+
Erwinia nimipressuralis	440	+	–	–
Erwinia aroideae	1274	–	–	–
Erwinia salicis	447	–	–	–

DEAE-cellulose (Fig. 2) showed complete coincidence of elution patterns for activity with all substrates. Additional properties of the enzyme are given in Table 5. The K_m values for pyridoxal phosphate were independent of the substrate used.

Inability of E. carotovora to oxidize aldehydes

In addition to *E. carotovora* being unable to grow on propionaldehyde, propionate or acetate as C sources (Table 1), propionaldehyde and propionate did not stimulate O_2 uptake by suspensions of the organism grown on a variety of media. After growth on media containing either amino alcohol as N source, no aldehyde dehydrogenase activity (either acylating or non-acylating) could be detected towards acetaldehyde or propionaldehyde in crude extracts (see the Methods section). The inability of *E. carotovora* to oxidize aldehydes contrasts with the situation in two species of pseudomonad studied (Jones & Turner, 1973).

Regulation of aldehyde formation from amino alcohols

Growth of *E. carotovora* on glycerol with either DL-1-aminopropan-2-ol or ethanolamine led to the formation of both the kinase and phospho-lyase enzymes (Table 4). Neither enzyme was formed after growth on glycerol with $(NH_4)_2SO_4$ or on nutrient broth. Whereas the inclusion of $(NH_4)_2SO_4$ in media containing amino alcohols had no effect on kinase synthesis, the formation of phospho-lyase was completely repressed. This accounted for the suppression of aldehyde formation by the addition of $(NH_4)_2SO_4$ during growth with amino alcohols as initial N sources. The non-utilizable NH_4^+ analogues methylammonium chloride and 2-aminoisobutyric acid inhibited growth with either DL-1-aminopropan-2-ol

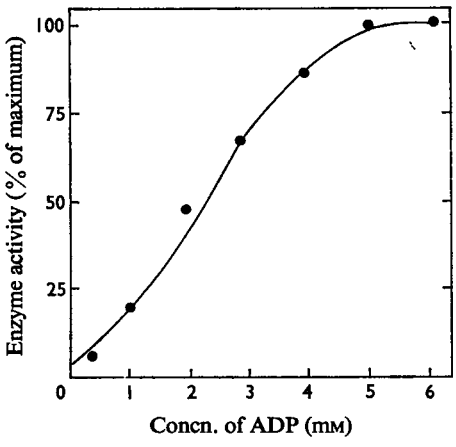

Fig. 3. *Effect of ADP on amino alcohol kinase activity of E. carotovora*

The enzyme was purified by ion-exchange chromatography and its activity assayed colorimetrically, as described in the Methods section, except that the concentration of ADP was varied as shown. Ethanolamine was used as the substrate.

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or ethanolamine, and partly repressed phospho-lyase formation by inhibited cultures. Neither kinase nor phospho-lyase activities were inhibited by NH_4^+ (up to 100mM). Whereas activity of the phospho-lyase was unaffected by nucleoside phosphates, the kinase was markedly activated by ADP (Fig. 3).

Amino alcohol metabolism by other Erwinia species

Of the eight strains of *Erwinia* examined, only two other than *E. carotovora* were capable of good growth on glycerol plus DL-1-aminopropan-2-ol medium (Table 6). Whereas *E. ananas* formed significant amounts of aldehyde during such growth, none could be detected during the growth of *E. milletiae*. Enzyme assays showed that the formation of both amino alcohol kinase and the *O*-phosphate phospho-lyase were induced by growth on media containing either amino alcohol as the N source. Phospho-lyase formation was repressed by the addition of $(NH_4)_2SO_4$ to such media and no activity was present after growth on nutrient broth. Neither *E. ananas* nor *E. milletiae* possessed aldehyde dehydrogenase enzymes. Extracts of the latter micro-organism exhibited high alcohol dehydrogenase activity, formed constitutively. Some properties of the enzymes responsible for aldehyde formation by these species are given in Table 5.

Discussion

All the experimental evidence described above was consistent with the metabolism of ethanolamine and 1-aminopropan-2-ol via their *O*-phosphate esters to acetaldehyde and propionaldehyde respectively. The two enzymes concerned were identified as a kinase and phospho-lyase analogous to those found previously in a pseudomonad (Jones & Turner, 1973). In contrast to the kinase of *Pseudomonas* sp. P6, the enzyme of *E. carotovora* exhibited an obligatory requirement for ADP. The phospho-lyase catalysed the deamination step required to permit growth on amino alcohols as N sources. The same reaction was responsible for aldehyde formation and provided an explanation for the close correlation between growth and aldehyde production observed with growing cultures. The repression of phospho-lyase formation by $(NH_4)_2SO_4$ in media also explained why aldehyde formation did not occur during growth in this case.

In contrast to *Pseudomonas* sp. P6, no aldehyde dehydrogenase activity could be detected in extracts of *E. carotovora*, *E. ananas* or *E. milletiae* grown with amino alcohols as N sources. No aldehyde dehydrogenase could be detected in *Erwinia amylovora* by Haq & Dawes (1971). Although the copious production of aldehydes by *E. carotovora* in contrast with *Pseudomonas* sp. P6 was thus explicable, *E. milletiae* did not excrete detectable amounts. This was possibly due to their reduction to the corresponding alcohols rather than oxidation to carboxylic acids. Ethanol formation from acetaldehyde by *E. amylovora* has been described (Haq & Dawes, 1971). In view of doubts about the classification of the genus *Erwinia* it is noteworthy that no other members of the Enterobacteriaceae family have been found to possess inducible amino alcohol kinase or *O*-phosphate phospho-lyase enzymes. Apart from a number of pseudomonads, only some bacteria belonging to the *Achromobacter* and *Flavobacter* genera are similar in this respect (A. Faulkner & J. M. Turner, unpublished work).

The metabolic role of the enzymes responsible for the conversion of amino alcohols into aldehydes by species of *Erwinia* is apparently deamination. The activating effect of ADP on the kinase of *E. carotovora* is consistent with a catabolic role, but in contrast to the case of some pseudomonads (Jones & Turner, 1973) the carbon skeleton cannot be degraded further than the aldehyde. In addition the biosynthesis of the phospho-lyase is regulated by NH_4^+ in growth media. It thus appears that in species of *Erwinia* the pathway is involved in N metabolism, possibly the catabolism of exogenous ethanolamine. Not only is the synthesis of phospho-lyase by *Pseudomonas* sp. P6 unaffected by NH_4^+, but both the kinase and phospho-lyase enzymes are markedly more active with 1-aminopropan-2-ols than with ethanolamine. This suggests that although analogous, the enzymes may play different metabolic roles in different organisms.

It is noteworthy that whereas ethanolamine

degradation to acetaldehyde and ammonia by species of *Proteus* (Miyaki *et al.*, 1959*a*,*b*) and *Clostridium* (Bradbeer, 1965*a*,*b*) occurs by the well-known cobamide coenzyme-dependent deaminase mechanism, the same overall reaction is brought about by a novel two-enzyme system in species of *Erwinia* and *Pseudomonas*.

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