Results and Discussion

The 1 : 2 stoichiometry observed for the oxidation of amino-acids by CAT can be represented as:

$$R''CH(NH_2)COOH + 2\,RNClNa \longrightarrow 2\,RNH_2 + R''CN + CO_2 + NaCl$$

where $R = p.CH_3C_6H_4SO_2$ and R'' is $(CH_3)_2CH$ for valine, $(CH_3)_2CHCH_2$ for leucine and $C_6H_5CH_2$ for phenyl alanine.

Paper chromatography[2] was used to identify the sulphonamide ($R_f = 0{\cdot}905$). Benzyl alcohol saturated with water was used as the solvent with 0·5% vanillin in 1% HCl solution in ethanol as the spray reagent. The nitriles formed, namely, 2-methyl propionitrile, 2-methyl butyronitrile and phenyl acetonitrile were detected by their colour reactions[5] with hydroxylamine and ferric chloride.

The rate of oxidation of the amino-acids by CAT is highly retarded in sulphuric and perchloric acid media. The same trend was observed in the oxidation of unsaturated alcohols by CAT[6,7]. This could probably be attributed to the combined specific inhibitory effect of H^+, SO_4^{--} and H^+, ClO_4^- ions. In contrast, presence of hydrochloric acid accelerated the reaction between CAT and unsaturated alcohols, while a retardation effect is noticed in the present case. It is likely that protonated HOCl is not the species[8] responsible for the oxidation of amino-acids.

The presence of foreign ions such as Ba^{2+}, Zn^{2+}, NO_3^-, $SO_4^=$ and PO_4^{3-} and sodium chloride (up to 0·2 mole) had no effect on the rate or stoichiometry of oxidation of amino-acids by CAT. Further, the stoichiometry is unaffected by a reversal of the order of addition of oxidant and the amino-acid.

The rapid rate of oxidation at pH 4–6 can probably be attributed to the rapid disproportionation of monochloramine-T present in acidified CAT solutions to dichloramine-T and *p*-toluene sulphonamide in this pH range, as suggested by Higuchi *et al.*[9]. It is also to be noted that the isoelectric points of these amino acids lie within this pH range.

1. Gowda, N. M. M., Murthy, A. S. A. and Mahadevappa D. S., *Curr. Sci.*, 1975, **44**, 5.
2. Mahadevappa, D. S. and Gowda, N. M. M., *Talanta*, 1975, **22**, 771.
3. Vogel, A. I., *Quantitative Organic Analysis*, Longmann and Greens, London, 1958, Part 3, p. 708.
4. Morris, J. C., Salazar, J. A. and Wineman, M. A., *J. Am. Chem. Soc.*, 1948, **70**, 2036.
5. Soloway, S. and Lipschietz, A., *Anal. Chem.*, 1952, **24**, 898.
6. Mahadevappa, D. S. and Naidu, H. M. K., *Talanta*, 1973, **20**, 349; *Indian J. Chem.*, 1975, **13**, 976.
7. Naidu, H. M. K. and Mahadevappa, D. S., *Curr. Sci.*, 1976, **45**, 216.
8. Mahadevappa, D. S. and Naidu, H. M. K., *Australian J. Chem.*, 1974, 26, 1203; 1975, **28**, 899.
9. Higuchi, T., Ikeda, K. and Hussain, A., *J. Chem. Soc.*, *B*, 1967, p. 546.

ISOLATION AND CHARACTERIZATION OF SOME NEW FORMATE UTILIZING BACTERIA

T. S. CHANDRA and Y. I. SHETHNA
Microbiology and Cell Biology Laboratory, Indian Institute of Science, Bangalore 560012, *India*

Abstract

Five isolates degrading and assimilating formate were isolated from chicken dung. Characterization indicated two different types. One of these belonged to the genus *Alcaligenes* and assimilated formate autotrophically. The other four isolates were identical, belonged to the genus *Protaminobacter* and assimilated formate heterotrophically by the serine pathway.

Introduction

FORMATE, the simplest of organic compounds is oxidized by a number of bacteria, plants and animals[1–2]. However, only a few bacteria utilize it as a sole carbon source and its assimilation involves two different pathways. Organisms like *Pseudomonas* AM1 assimilate it by the serine pathway[3], while *Ps. oxalaticus*, *Bacterium formoxidans*, *Hydrogenomonas eutropha* Z-1 and *Thiobacillus novellus* (see discussion) assimilate it autotrophically. The fact that only a few cases of autotrophic growth on formate have been reported indicates that this may be a rare type of metabolism as considerations of economy may favour the selection of organisms such as *Ps.* AM1 which conserve the reduction level of formate[4]. The only other C_1 organic compound on which autotrophic growth has been recorded is on methanol by *Micrococcus denitrificans*[5]. As these C_1 compounds are at the borderline between organic and inorganic, a wider study of autotrophic growth on such compounds in a number of genera can be expected to throw light on the biochemical basis of facultative autotrophy.

We report here the isolation and characterization of an autotrophic formate-utilizing bacterium *Alcaligenes* FOR$_1$ and heterotrophic *Protaminobacter* FOR$_2$ from chicken dung by enrichment culture with formate

as sole carbon source. These isolates differ physiologically from other formate utilizers already known.

Methods

Isolation and Identification.—The media, isolation and identification procedures were the same as described by Chandra and Shethna[6], except that 0·2% (w/v) of sodium formate was substituted for oxalate.

The substrate assimilation and generation time determinations were also done essentially as reported in the above paper.

Preparation of extracts.—The cells were grown as described in Table III, washed and stored below 0° C until use[4]. Extracts in 0·05 M-potassium phosphate buffer pH 7·0 were obtained either by grinding with glass powder or by disrupting in a Raytheon Model DF 101 sonicator for 10 min at 0° C and centrifuged in Sorvall RC2-B at 27000 × g for 15 min. The supernatant was used for the enzyme assays.

Enzyme assays.—All spectrophotometric assays were carried out in a Pye-Unicam spectrophotometer. Formate dehydrogenase (EC 1.2.1.2), hydroxypyruvate reductase (EC 1.1.1.29) and ribulose diphosphate carboxylase (EC 4.1.1.39) were assayed by standard procedures[7,3,8]. Protein was determined by the method of Lowry *et al*[9]. All the cofactors and fine chemicals were obtained from Sigma Chemical Co., U.S.A.

Results and Discussion

Enrichment yielded several cultures many of which were pink pigmented. Five colourless colonies were studied for the cellular, colony and biochemical characteristics (Table I). Four were identical in their cultural and biochemical properties and were designated as *Protaminobacter* FOR_2 and one was designated *Alcaligenes* FOR_1. The growth on C_1 and C_2 compounds, uptake of oxygen and enzyme studies were determined with one isolate from each type mentioned above.

All the five isolates were negative for the methyl red, Voges-Proskauer, H_2S production, indole formation, cellulose degradation, alginate utilization, and hydrolysis of arginine, casein or gelatin. No growth was observed with 0·2% formate or 0·2% formate *plus* 0·2% acetate, malate or succinate anaerobically. Acetate, malate and succinate supported growth aerobically as the sole carbon source. All were positive for the oxidase, catalase and urea hydrolysis tests. Characteristics in which the isolates varied are shown in Table I.

Even with a heavy inoculum from nutrient agar slants *A*.FOR_1 was noted to alkalise formate only after a 4–5 day period, but subsequent transfers in formate medium led to a more rapid growth. The generation time on 0·3% and 0·5% formate was 13–14 hours with an increasing lag period of 14 and 18 hours respectively as noted even in *Ps. oxalaticus*[10]. Growth on 0·3% formate could not be stimulated by biotin (150 µg/100 ml). One per cent formate completely inhibited growth.

A·FOR_1 assimilated only formate and none of the other C_1 compounds (Table II). *Pr*·FOR_2 assimilated a number of C_1 compounds in addition to formate. The stimulation of growth of *A*·FOR_1 and *Pr*·FOR_2 by yeast extract on oxalate and a few C_1 compounds was also tested as yeast extract was found to stimu-

TABLE I

Description of the isolates

	Alcaligenes FOR_1	*Protaminobacter* FOR_2
Cellular morphology	0·75–1·5 µm, Gram negative coccobacilli, non-motile non-flagellated.	0·75–1·5 µm, Gram negative coccobacilli. Few cells with a central unstained area. Non-motile non-flagellated.
Colonies on formate, gelatin, starch and nutrient agar in 3–4 days	2–3 mm, whitish, translucent, convex, entire-edged smooth, glistening.	2–3 mm, creamish white, opaque, convex, entire-edged, smooth, glistening mucoid.
Growth in nutrient broth (72 hr)	Turbid	Highly turbid.
Tests:		
Action on milk	Acidic	Alkaline
Nitrate reduction*	+	+
Citrate utilization	+	−
Starch hydrolysis	−	+
Acid formation in Hugh and Leifsons' basal medium[26] *with*:**		
Glucose	+	−
Sucrose	−	−
Lactose	+	−
Glycerol	−	+
Fructose	−	+
Arabinose	+	+
Xylose	+	−
Galactose	+	+
Maltose	−	−
Mannitol	−	+

− = No growth or negative reaction;
+ = Growth or positive reaction.
* Nitrate is reduced beyond nitrite in *Pr*.FOR_2.
** No gas was produced with any of the sugars. No acid was produced with glucose, sucrose or lactose anaerobically. *A*.FOR_1 formed acid from lactose aerobically only after 2–4 days.

late growth of *T. novellus* on methanol and formamide (Chandra and Shethna, unpublished observation). However, no increase in growth in presence of yeast extract and C_1 compound compared to controls with yeast extract alone or C_1 substrate alcre was noted.

TABLE II

Assimilotion of C_1, C_2 and other compounds.

The absorbance was measured at 650 nm in a Bausch and Lomb Spectronic-20 colorimeter after 5–7 days of incubation on a rotary shaker. The turbidity has been recorded without subtracting the control values

Substrates (0·2% w/v)	*Alcaligenes* FOR_1		*Protaminobacter* FOR_2	
	Without yeast extract	With 0·02% yeast extract	Without yeast extract	With 0·02% yeast extract
None	0·05	0·10	0·04	0·09
Formate	0·23	—	0·23	—
Oxalate	0·03	0·11	0·02	0·08
Methanol	0·05	0·11	0·02	0·10
Ethanol	0·04	0·12	0·18	—
Methylamine	0·05	0·09	0·32	—
Formamide	0·06	0·11	0·29	—
Ethylamine	0·06	—	0·04	—
Formaldehyde	0·04	—	0·04	—
Trimethylamine	—	—	0·18	—
Ethanolamine	—	—	0·04	—
Butylamine	—	—	0·03	—
H_2–CO_2–air*	No growth	—	No growth	—

— = Not tested.
* Tested as described by Chandra and Shethna[6].

The biochemical characteristics of *Pr.* FOR_2 indicate that it is a facultative methylotroph closely resembling *Ps. aminovorans*[13] and bacterium 5 B1[16]. On account of its non-flagellation and nutritional characteristics, it has been identified as belonging to the genus *Protaminobacter*. Although it is non-pigmented, it differs from the 4 varieties of *Pr. alboflavus* mentioned in Bergey's Manual[17] in a few nutritional characteristics.

A. FOR_1 possesses cellular, biochemical and nutritional characteristics that agree with the description of the *Achromobacter* genus in Bergey's Manual[17]. However, its properties like facultative autotrophic nature (discussed later), non-flagellation, and acid production from sugars aerobically agree even better with the emended description of the genus *Alcaligenes* by Henrie *et al.*[18]. In view of the uncertain status of the genus *Achromobacter* pointed out by the above authors, we have identified this isolate as belonging to the genus *Alcaligenes*. Its closest resemblance is with *Bacterium formoxidans*[19] but differs from it in the production of acid from milk and sugars, and in not utilizing ethyl alcohol. It is different from *Bacterium formicum*[20] which is known to grow on formate *plus* succinate or malate anaerobically.

The oxidation of formate (Table III) was completely inhibited by 1 mM NaH_2PO_2, an inhibitor of formate dehydrogenase[11] in the case of *A.* FOR_1 similar to the observation with *Ps. oxalaticus*[12]. Only partial inhibition was noted with *Pr.* FOR_2 as in *Ps.* AM1[13]. The oxidation of inorganic substrates, hydrogen and thiosulfate was noted with formate grown *H. eutropha* Z–1[14] and *T. novellus* (Chandra and Shethna, unpublished observation) respectively. However, *A.* FOR_1 and *Pr.* FOR_2 did not oxidize thiosulfate or nitrite.

TABLE III

Oxidative properties of A. FOR_1 and Pr. FOR_2.

Cells were grown in 0·3% formate for 3-4 days with addition of 0·3% formate further after 2 days. The pH was maintained at 7·2 by the addition of 2N HCl. The assay system was the same as described by Chandra and Shethna[6]

Organism	QO_2–μ liters of O_2 uptake/hr/mg dry wt with 30 μ moles formate	% inhibition of QO_2* with 1mM NaH_2PO_2
Alcaligenes FOR_1	38–42 (2)	100 (2)
Protaminobacter FOR_2	31–47 (2)	100 (2)

* Cells were preincubated with NaH_2PO_2 for 15 min. In *Pr.* FOR_2 100% inhibition was observed only in the first 20 min after which period there was only 73% inhibition. In *A.* FOR_1 there was 100% inhibition even after 20 min.
The number in parenthesis indicates the number of determinations.

The specific activity of NAD-linked formate dehydrogenase in *A.* FOR_1 and *Pr.* FOR_2 was 0·260 and 0·182 units/mg protein respectively. The specific activity of hydroxypyruvate reductase in *A.* FOR_1 was low with a value of 0·037 units/mg protein with NADH and 0·02 with NADPH as electron donors. This observation ruled out the heterotrophic serine pathway as the major route of formate carbon assimilation in this culture. Ribulose diphosphate carboxylase the key enzyme of the alternative autotrophic pathway of formate carbon assimilation was investigated in

A. FOR_1 extract and was detected at a specific activity of 10 m. moles of phosphoglyceric acid formed/min/mg protein. It is reported[27] that in some autotrophic bacteria natural macromolecular inhibitors of ribulose diphosphate carboxylase occur giving rise to low activities of this enzyme. It is possible that this could be the reason for the low activity of ribulose diphosphate carboxylase in *A.* FOR_1 and needs further investigation. It is therefore tentatively concluded that *A.* FOR_1 grows autotrophically on formate. In *Pr.* FOR_2 the specific activity of hydroxypyruvate reductase with NADH as electron donor was high (0·40 units/mg protien) indicating the heterotrophic serine pathway as the major metabolic route of formate carbon assimilation as in *Ps.* AM1 and AM2[15]. In view of the good activity of this enzyme the presence of ribulose diphosphate carboxylase was not further examined.

Relatively few attempts have been made to isolate formate utilizing bacteria[19,21,22]. The autotrophic pathway of formate assimilation has been conclusively demonstrated only in 4 non-photosynthetic organisms, *viz.*, *Ps. oxalaticus*[8], *H. eutropha* Z-1[14], *B. formoxidans*[19] and *T. novellus*[23] by radioactive and/or enzymic studies. Evidence in *Nitrobacter winogradskyi* and *Thiobacillus* A2 is lacking although the authors have reported that they utilize formate[24-25]. Of the four organisms mentioned, *B. formoxidans* is the only autotroph obtained from soil directly by enrichment with formate. Our results indicate that *A.* FOR_1 obtained by similar procedure is one of the few organisms that can assimilate formate autotrophically and *Pr.* FOR_2 is a heterotroph like most facultative methylotrophs.

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1. Quayle, J. R., *Adv. Microb. Physiol.*, 1972, **7**, 119.
2. Chandra, T. S. and Shethna, Y. I., *Ant. van Leeuwenhoek*, 1975, **41**, 465.
3. Large, P. J. and Quayle, J. R., *Biochem. J.*, 1963, **87**, 386.
4. Storm, T., Ferenci, T. and Quayle, J. R., *Ibid.*, 1974, **144**, 465.
5. Cox, R. B. and Quayle, J. R., *Ibid.*, 1975, **150**, 569.
6. Chandra, T. S. and Shethna, Y. I., *Ant. van Leeuwenhoek.*, 1975, **41**, 101.
7. Johnson, P. A., Jones-Mortimer, M. C. and Quayle, J. R., *Biocheim. Biophys. Acta*, 1964, **89**, 351.
8. Blackmore, M. A. and Quayle, J. R., *Biochem. J.*, 1968, **107**, 705.
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
10. Dijkhuizen, L. and Harder, W., *Ant. van Leeuwenhoek.*, 1975, **41**, 135.
11. Takamiya, A., *J. Biochem.* (Tokyo), 1953, **40**, 415.
12. Quayle, J. R. and Keech, D. B., *Biochem. J.*, 1959, **72**, 623.
13. Peel, D. and Quayle, J. R., *Ibid.*, 1961, **81**, 465.
14. Namsaraev, B. B., Nozhevnikova, A. N. and Zavarzin, G. A., *Mikrobiologiya* (Transl.), 1971, **40**, 675.
15. Blackmore, M. A. and Quayle, J. R., *Biochem. J.*, 1970, **118**, 53.
16. Colby, J. and Zatman, L. J., *Ibid.*, 1973, **132**, 101.
17. *Bergey's Manual of Determinative Bacteriology*, By Breed, R. S., Murray, E. G. D. and Smith, N. R. The Williams and Wilkins Company, Seventh Edition, 1957.
18. Henrie, Margaret, S., Holding, A. J. and Shewan, J. M., *Int. J. Syst. Bact.*, 1974, **24**, 534.
19. Sorokin, Yu. I., *Mikrobiologiya* (Transl.), 1961, **30**, 337.
20. Shaposhnikov, V. N. and Loriya, Zh. K., *Dokl. Acad. Nauk., SSSR*, 1967, **175**, 721.
21. Bhat, J. V., *J. Ind. Inst. Sci.*, 1956, **38**, 43.
22. Pavri, Khorshed M. and Bhat, J. V., *Proc. Ind. Acad. Sci.*, 1953, **38 B**, 171.
23. Shethna, Y. I., *Ind. J. Biochem.*, 1967, **4**, 34.
24. Van Gool, A. and Laudelout, H., *Biochim. Biophys. Acta*, 1966, **127**, 295.
25. Taylor, B. F. and Hoare, D. S., *J. Bacteriol.*, 1969, **100**, 487.
26. Hugh, R. and Leifson, E., *Ibid.*, 1953, **66**, 24.
27. McFadden, B. A., *Bacteriol. Rev.*, 1973, **37**, 289.

NEW EVIDENCE OF MAGNETIC POLE VARIATIONS

Scientists, including a team from the Australian National University in Canberra, are uncovering increasing evidence that the earth's magnetic poles have migrated dramatically in the past. The Australian National University's Research School of Earth Sciences research team has developed techniques which may allow them to reconstruct in detail the variations of the geomagnetic field in Australia over the past 30,000 years. The technique involves taking long, undisturbed oriented cores of sediment from the bottom of lakes and analysing their magnetic records. (Australian Information Service, Canberra, ACT 2600.)