IDENTIFICATION OF QUINOLINIC ACID- UTILIZING BACTERIA

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A microorganism utilizing quinolinic acid as sole carbon, nitrogen, and energy sources for growth, and producing a potent activity of quinolinate phosphoribosyltransferase in its cells was isolated. Based on its morphological, cultural, physiological, and biochemical characteristics, including the proper composition of cellular fatty acids, *Alcaligenes eutrophus* subsp. *quinolinicus* subsp. nov. KOUNO and IWAI is proposed for this organism.

Quinolinic acid (pyridine-2,3-dicarboxylic acid) has been known as a key intermediate in the *de novo* biosynthesis of nicotinamide adenine dinucleotide from tryptophan in mammals (1–3), *Neurospora crassa* (4), and aerobically grown yeast (5), or from aspartic acid and glycerol in plants (6, 7), and bacteria (8–12). It is converted to nicotinic acid mono-nucleotide in the presence of 5-phosphoribosyl-1-pyrophosphate and Mg^{2+} by an enzyme called quinolinate phosphoribosyltransferase (EC 2.4.2.19). This enzyme is widely distributed in animals, plants, and microorganisms such as *Neurospora*, mushroom, pseudomonad, and Baker's yeast (13). It was obtained as a highly purified preparation from bovine liver (14, 15), a Japanese mushroom (*Lentinus edodes*) (16), and castor bean endosperm (17), and as a crystalline preparation from *Pseudomonas* sp. (18) and hog liver (19). However, the properties of this enzyme are not yet fully investigated except the hog liver enzyme (20–23).

At an early stage of their studies on the enzyme for obtaining a potent enzyme producer, one of the authors (K.I.) and his co-workers attempted to isolate several strains from the soil by the enrichment culture technique according to PACKMAN and JAKOBY (24). One of the best producers, strain 6–3, seemed to belong to the genus *Alcaligenes*. Recently, the enzyme of this strain was isolated in a crystalline form (25). This bacterial enzyme was found to possess some charac-

teristic properties (26) differing from those reported previously. The present paper deals with identification of strain 6-3.

MATERIALS AND METHODS

Isolation of bacteria. For effective and selective isolation of quinolate-utilizing bacteria, the enrichment culture technique described by PACKMAN and JAKOBY (24), was applied, using medium of the following composition (quinolate liquid medium): Quinolinic acid, 1.5 g; NaH_2PO_4 , 0.5 g; K_2HPO_4 , 1.5 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g; tap water, 1,000 ml; pH 7.0. A small amount of soil (about 1 g) was added to a 500-ml Sakaguchi flask containing 20 ml of quinolate liquid medium, and incubated on a reciprocal shaker for 3 to 5 days at 30°. A loopful of the flask culture showing a substantial growth was streaked and incubated for 4 to 6 days on an agar plate containing 2% agar in quinolate liquid medium (quinolate agar medium). Representative colonies were picked up on a quinolate agar slant and purified by repeated serial platings on quinolate agar and on an agar medium of the following composition (nutrient agar medium): Peptone, 10 g; beef extract, 10 g; NaCl, 5 g; agar, 20 g; tap water, 1,000 ml; pH 7.0. The isolates were preserved on nutrient agar at 5° with transfer every 2 months.

Characterization of isolates. One- to 2-day-old culture on nutrient agar slant was used as the inoculum unless otherwise specified. All tests were carried out at 30° except in nutrient gelatin which was made at 20°.

The microorganism was cultured on nutrient agar or in quinolate liquid medium for 1-2 days and then stained flagella by the method of NISHIZAWA and SUGAWARA (27). A similar culture was also used for electron microscopic observation by chromium-shadowing and by negative staining. Other cultural, physiological, and biochemical characteristics were determined by the procedures described in manuals of COWAN and STEEL (28), SKERMAN (29), and PELCZAR *et al.* (30), including their original papers. Table 1 contains references of the relevant method of the tests (31-43). Assimilation of organic compounds, chemolithotrophic growth with hydrogen, arginine dihydrolase reaction, denitrification, and cleavage of an aromatic ring were tested mostly by the method of STANIER *et al.* (33). For the assimilation and growth tests, the original inoculation conditions were modified as follows: The cells were harvested from the nutrient agar slant and suspended in sterile distilled water to make a cell suspension of about 10^7 cells/ml and a loopful of this cell suspension was aseptically streaked on the test media. Nitrate reduction was examined both in nitrate broth (Difco) and in the STANIER medium (33). KOSER medium, CHRISTENSEN medium, and STANIER assimilation test medium was used for citrate utilization. Nitrogen fixation was judged by the KNOWLES method (31) based on the ability to reduce acetylene after 5 weeks of cultivation in a PAUKHURST tube (44). Malonate utilization test was

performed for 3 days. Hemolysis was tested by each blood agar medium of horse, sheep, and rabbit.

Table 1. Characteristics of *Alcaligenes eutrophus* subsp. *quinolinicus* 6-3.

Characteristics	References
Rod-shaped cells	+
Gram stain	—
Motility, Peritrichous flagella	+
Fixation of N ₂	—
Utilization of CO ₂ as a carbon source	—
Growth with H ₂	—
Gas production from carbohydrates ^a	—
Oxidative acid production from carbohydrates	—
Fermentative acid production from carbohydrates	—
Oxidase (Kovacs method)	+
Catalase	+
VP-MR test ^b	—
Indole production	—
H ₂ S production in TSI ^b and LA ^b agar (Difco)	—
Citrate utilization	+
Growth on SS agar (Eiken)	—
Growth on MacConkey agar (Eiken)	+
Denitrification	+
Nitrate reduction in nitrate-lactate broth	+
Nitrate reduction in nitrate broth (Difco)	—
Urease in Christensen medium	+
Soluble pigment in King A and B media	—
Growth on nutrient agar at 10° or 41°	—
Growth in nutrient broth at pH 4.5 or 9.2	—
Growth in KCN broth	—
2-Ketogluconate production from gluconate	—
Malonate utilization	+
3-Ketolactose production	—
Lysine and ornithine decarboxylase	—
Arginine dihydrolase	—
Arginine utilization	—
Phenylalanine and tryptophan deaminase	—
Phosphatase	—
DNase	—
Litmus milk alkaline	+
Gelatin liquefaction	—
Hydrolysis of starch, cellulose, and chitin	—
Hydrolysis of casein and Tween 80	—
Hemolysis	—
Egg-Yolk reaction	—
Digestion of Loeffler serum (Difco)	—
Hydrolysis of esculin	—

Table 1. (continued)

Characteristics	References
Penicillin G (2.5 UI/ml) sensitivity	— (42)
Terramycin (10 μ g/ml) sensitivity	— (42)
0/129 antibiotic sensitivity	— (42)
Arsenite oxidation	— (43)

^a Carbohydrates were glucose, fructose, D-arabinose, xylose, rhamnose, galactose, mannose, lactose, maltose, sucrose, trehalose, raffinose, inulin, salicin, glycerol, inositol, mannitol, sorbitol.

^b Abbreviations: VP-MR, Voges-Proskauer and Methyl Red tests; TSI, triple sugar iron; LA, lead acetate.

DNA was extracted by the method of MARMUR (45), and the base composition of DNA (GC content) was calculated from T_m (46).

Organisms. In the present work, the cellular fatty acid composition of strain 6-3 was compared with those of 3 cultures of *Pseudomonas acidovorans* AJ 3117 (ATCC 15668), *Alcaligenes eutrophus* AJ 3935 (ATCC 17697), and *Alcaligenes faecalis* ATCC 8750 as the reference strains. The former 2 type strains came from the Culture Collection of Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan, and the last reference strain from the American Type Culture Collection.

Analysis of cellular fatty acids by gas-liquid chromatography (GLC). The composition of cellular fatty acids was examined chiefly by the method (A) of MOSS *et al.* (47) and subsidiarily by the procedure of UETA *et al.* (48) and YAMAKAWA *et al.* (49). The microorganism was cultivated in 100 ml of Trypticase Soy Broth (BBL) on a reciprocal shaker for 40 hr. Yanagimoto GCG 550 FTP gas chromatography equipped with a stainless steel column (3 $\times$ 225 mm) filled with 15% polyethylene glycol succinate-coated 80/100 mesh Chromosorb W.AW was employed for GLC analysis. The temperatures of the injector, detector, and column were maintained at 220, 230, and 180°, respectively. At a flow rate of 15 ml/min of carrier nitrogen gas, relative retention time of the samples to that of standards (BC-Mix L and BC-Mix 1; Applied Science Laboratories, State College, U.S.A.) was measured. Fatty acid components were identified on the basis of results of MOSS *et al.* (47) and BRIAN and GARDNER (50), including GLC-mass spectrometry.

Identification of strain 6-3. Bergey's Manual of Determinative Bacteriology, 8th ed. (1974) (51), literature of HENDRIE *et al.* (52) and of DAVIS *et al.* (53), and the chemotaxonomical studies of bacterial fatty acids by MOSS *et al.* (54) and YANO *et al.* (55, 56) were referred to bacterial identification.

RESULTS

Based on results of the preliminary screening, 5 bacterial strains were chosen

as promising candidates for producing a substantial amount of quinolinate phosphoribosyltransferase. After further enzymological and microbiological studies on these five strains of bacteria, a strain tentatively numbered 6-3, which was isolated from a soil sample from Kyoto, was proved to be the best producer and therefore subjected to the identification.

Morphological characteristics

The strain 6-3 is a Gram-negative, asporogenous rod, and is motile with 1 to 4 peritrichous flagella, as shown Fig. 1. The cells on nutrient agar, which exist singly or in pairs ($0.3\text{--}0.5 \times 0.8\text{--}1.2\ \mu\text{m}$), are smaller than those on quinolinate agar ($0.6\text{--}0.8 \times 0.7\text{--}1.8\ \mu\text{m}$) at 20 hr of incubation. No pleomorphic cells are observed. Abundant accumulation of poly- β -hydroxybutyrate is confirmed by staining with Sudan Black B.

Fig. 1. Photograph of *A. eutrophus* subsp. *quinolinicus* 6-3.

Electron micrograph of negative stained preparation showing a cell with peritrichous flagella. The cell was grown in nutrient broth for 18 hr, fixed in 4% formaldehyde, washed with distilled water, and stained with 1% sodium phosphotungstate. Bar represents $1\ \mu\text{m}$.

Cultural characteristics

Strain 6-3 produces a homogeneous turbidity in nutrient broth 3-5 days after inoculation. Ring, but no pellicle, forms on the surface of nutrient broth, without specific odor. Casitone-yeast extract broth and OF-fructose medium do not support growth of this microorganism under anaerobic condition, but do well under aerobic condition. No growth factor is necessary to serially maintain the strain in quinolinate broth. After 2 days of incubation, colonies on nutrient agar are punctiform, raised, undulate, smooth, glistening, colorless to white, and translucent, while on quinolinate agar they are circular, convex, entire,

smooth, glistening, white, and translucent. Growth developing 2 days after incubation on nutrient agar slant is abundant, filiform, butyrous, and pale yellow or pale brownish yellow. No soluble pigment is produced in either KING A or B medium. Litmus milk (Difco) turns alkaline a few days after incubation and remains alkaline for 25 days, and neither coagulation nor peptonization is observed.

Physiological and biochemical characteristics

Activities of catalase, oxidase, and urease, utilization of citrate, and denitrification are positive, but other characteristics tested are mostly negative. The physiological and biochemical characteristics are summarized in Table 1.

The strain reduces nitrate to nitrite in the STANIER medium supplemented with sodium lactate in which moderate growth without formation of nitrogen gas is observed under anaerobic condition, but does not reduce nitrate in nitrate broth (Difco). This organism can neither grow at the expense of H_2 nor in the LORD carbon-free ammonium phosphate liquid medium (32). Ability for nitrogen fixation seems to be negative. Arginine test according to the technique of THORNLEY (38) gives negative. The strain cannot grow at 10° or 40° on nutrient agar or at pH 4.5 and 9.2 in nutrient broth. In the fermentation test with 20 kinds of carbohydrate in the OF medium, no production of gas or acid is observed, and pH of the medium changes to alkalinity in 14 kinds of carbohydrate. Protocatechuate is cleaved through the *ortho*-cleavage pathway. The GC content of DNA of this strain is 64.6 mol %.

Result of the assimilation test

The following 55 substrates are well utilized: D-fructose, gluconate, propionate, butyrate, isobutyrate, valerate, caproate, caprate, glutarate, adipate, suberate, azelate, sebacate, D-(−)-tartrate, L-(+)-tartrate, *meso*-tartrate, DL- β -hydroxybutyrate, DL-lactate, glycollate, pyruvate, laevulinate, itaconate, mesaconate, glycerol, ethanol, propanol, butanol, isobutanol, D-mandelate, benzoate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, phenylacetate, phenol, quinate, L-leucine, L-isoleucine, L-valine, L-glutamate, L-histidine, L-proline, L-tyrosine, L-phenylalanine, L-tryptophan, kynurenate, anthranilate, benzylamine, butylamine, α -amylamine, betaine, hippurate, pantothenate, nicotinate, and quinolinate.

Weakly utilized are 33 substrates (2-ketogluconate, mucate, acetate, oxalate, malonate, succinate, maleate, fumarate, pimelate, D-malate, L-malate, DL-glycerate, citrate, α -ketoglutarate, aconitate, citraconate, ethyleneglycol, L-mandelate, benzoylformate, testosterone, glycine, L-alanine, D-alanine, β -alanine, L-serine, L-threonine, DL-norleucine, L-aspartate, γ -aminobutyrate, acetamide, glutathione, L-asparagine, and L-glutamine).

Among the negative substrates tested, the following 57 compounds are included: D-ribose, D-xylose, D-arabinose, L-arabinose, D-fucose, L-rhamnose,

D-glucose, D-mannose, D-galactose, sucrose, trehalose, maltose, cellobiose, lactose, starch, inulin, salicin, isovalerate, heptanoate, caprylate, pelargonate, erythritol, mannitol, sorbitol, *meso*-inositol, adonitol, propyleneglycol, 2,3-butyleneglycol, methanol, isopropanol, geraniol, phthalate, isophthalate, terephthalate, naphthalene, L-lysine, DL-arginine, DL-ornithine, DL-citrulline, DL- α -aminobutyrate, DL- α -aminovalerate, δ -aminovalerate, D-tryptophan, *m*-aminobenzoate, *p*-aminobenzoate, methylamine, ethanolamine, putrescine, histamine, tryptamine, sarcosine, creatine, trigonelline, *n*-dodecane, *n*-hexadecane, DL-methionine, and aniline.

Composition of cellular fatty acids

Cellular fatty acids of the strain 6-3, *P. acidovorans*, *A. faecalis*, and *A. eutrophus* were analyzed by both the methods of UETA *et al.* (48) and Moss *et al.* (47) (Tables 2 and 3). Patterns of GLC analysis by the Moss method are also shown in Fig. 2. The major fatty acid components of strain 6-3 are straight-chain acids of C_{14:0}, C_{16:0}, C_{16:1}, and C_{18:1}, and C₁₇ cyclopropanoic fatty acid, and 2-

Table 2. Percentage composition of cellular fatty acids in strain 6-3 and 3 reference strains by the method of UETA *et al.*^a

Fatty acid	rRT ^b	Strain 6-3 (%)	<i>P. acidovorans</i> ATCC 15668 (%)	<i>A. faecalis</i> ATCC 8750 (%)	<i>A. eutrophus</i> ATCC 17697 (%)
C _{12:0}	0.30	—	2.7	1.5	—
C _{14:0}	0.56	1.8	1.0	1.0	3.6
C _{15:0}	0.75	0.7	0.8	0.7	1.2
C _{16:0}	1.00	22.6	37.4	30.8	24.5
C _{17:0}	1.34	0.7	0.9	1.2	0.9
C _{18:0}	1.80	0.5	0.6	2.4	0.6
C _{16:1}	1.22	28.7	21.2	7.8	17.6
C _{18:1}	2.16	29.5	13.7	11.0	22.6
C ₁₇ cyclic	1.63	3.7	9.9	17.3	10.5
C ₁₉ cyclic	2.86	0.2	—	—	1.1
2-OH-C _{12:0}	1.39	—	—	4.1	—
2-OH-C _{14:0}	2.49	3.3	—	—	3.6
3-OH-C _{10:0}	1.11	—	2.0	—	—
3-OH-C _{14:0}	3.49	4.0	—	6.7	3.8
Unknown A	0.93	0.2	—	—	trace
Unknown B	1.09	0.2	1.3	2.3	1.3
Unknown E	1.45	trace	0.6	—	0.3
Unknown G	1.97	trace	—	—	0.3
Unknown F	2.54	2.8	5.9	9.3	6.1
Unknown C	3.49	0.7	1.2	3.0	1.9
Unknown H	4.81	—	0.8	0.9	—
Unknown D	5.28	0.3	—	—	—

^a Transesterification: dry cells were methylated with 3% HCl-methanol for 3 hr at 100°.

^b rRT: relative retention time (C_{16:0}=1.00).

Table 3. Percentage composition of cellular fatty acids in strain 6-3 and 3 reference strains by the saponification method^a of Moss *et al.*

Fatty acid	rRT ^b	Strain 6-3 (%)	<i>P. acidovorans</i> ATCC 15668 (%)	<i>A. faecalis</i> ATCC 8750 (%)	<i>A. eutrophus</i> ATCC 17697 (%)
C _{12:0}	0.31	—	2.3	1.2	—
C _{14:0}	0.56	2.0	1.0	1.1	3.3
C _{15:0}	0.75	0.9	0.8	0.6	1.3
C _{16:0}	1.00	21.0	36.6	28.7	23.3
C _{17:0}	1.33	1.8	0.6	—	1.6
C _{18:0}	1.79	1.3	1.0	2.5	1.1
C _{18:1}	1.22	27.8	22.3	7.8	17.3
C _{18:1}	2.15	27.3	13.5	10.9	20.9
C ₁₇ cyclic	1.62	6.9	20.3	32.3	18.9
C ₁₉ cyclic	2.86	1.3	—	1.4	2.0
2-OH-C _{12:0}	1.40	—	—	3.3	—
2-OH-C _{14:0}	2.47	2.3	—	—	2.4
3-OH-C _{10:0}	1.12	—	1.6	—	—
3-OH-C _{14:0}	3.48	7.4	—	10.1	7.9

^a Saponification: dry cells were saponified with 5% NaOH in 50% methanol for 1 hr at 100°, and methylated with boron trichloride-methanol reagent for 5 min at 85°.

^b rRT: relative retention time (C_{16:0}=1.00).

Fig. 2. Composition of cellular fatty acids according to the method (A) of Moss *et al.*

hydroxytetradecanoic and 3-hydroxytetradecanoic acids are detected. The analytical data of *P. acidovorans* ATCC 15668 agree with the results of Moss *et al.* (54). 2-Hydroxy-C_{12:0} and 3-hydroxy-C_{14:0} acids in the cellular fatty acid components of *A. faecalis* ATCC 8750, and 2- and 3-hydroxy-C_{14:0} acids in those of *A. eutrophus* ATCC 17697 are recognized. These findings coincide with the results of YANO *et al.* (55).

DISCUSSION

The strain 6-3 is clearly distinguished from the members of *Pseudomonas*, *Enterobacteriaceae*, *Acetobacter*, *Agrobacterium*, and the *Acinetobacter-Morexella* group. Recent studies on the composition of cellular fatty acids of bacteria have stressed its importance in the identification of genera and species, and more particularly Moss *et al.* (54) and YANO *et al.* (55, 56) have stated that the presence of one or more hydroxy-fatty acids in cellular fatty acids is a useful tool to determine various species of *Alcaligenes* and *Pseudomonas*.

When cellular fatty acids were prepared by the method of UETA and that of Moss, a significant difference was observed in assay results. That is, compared with the cellular fatty acid sample prepared according to the procedure of Moss *et al.* (47), the method of UETA *et al.* (48) yielded an unknown component (F) and a broader peak of 3-hydroxy-C_{14:0} fatty acid. To find the reason for this difference, the said two samples were further treated with trifluoroacetic anhydride in chloroform and again analysed by GLC. In the sample prepared by the Moss method, the peak of 3-hydroxy-C_{14:0} fatty acid disappeared and a new peak of 3-trifluoroacetoxy-C_{14:0} appeared. In the sample prepared by the method of UETA *et al.*, in contrast, a substantial but smaller peak of an unknown component (C) was still detected at the site of 3-hydroxy-C_{14:0} fatty acid, together with a new peak of 3-trifluoroacetoxy-C_{14:0} fatty acid and the unchanged peak of component (F). Further analysis of the two samples by GLC-mass spectrometry and nuclear magnetic resonance spectrum suggested that components (C) and (F) might be isomeric methoxy-fatty acids having a molecular weight of 314. In brief, components (C) and (F) will be artifacts probably derived from C₁₇ cyclopropane fatty acid by methylation in the method of UETA *et al.* It is interesting to note that SHIOIRI-NAKANO and TADOKORO described the presence of artifacts derived from cellular C₁₉ cyclopropane fatty acid (57). It seems probable that minor components (*i.e.*, A, B, and D in Table 2) are also artifacts. Based on those findings, the saponification method of Moss *et al.* (47) was mainly employed for the subsequent comparison of microorganisms in the composition of cellular fatty acids (Table 3 and Fig. 2).

The analytical data of *P. acidovorans*, which confirm the results of Moss *et al.* (54), significantly differ from those of strain 6-3 in the presence of straight chain fatty acid of C_{12:0} and 3-hydroxy-C_{10:0} fatty acid, and in the absence of C₁₉

cyclopropane fatty acid and 2- and 3-hydroxy- $C_{14:0}$ fatty acids. It should be noticed that, as is the case in this paper, when the decision of identification based on flagellation is difficult or when some exceptions in the differentiation test of *Pseudomonas-Alcaligenes* group on the basis of arginine metabolism are suspected, the analysis of cellular fatty acid can be effective for determination of genera and species.

Based on the morphological, cultural, physiological, and biochemical properties of the strain 6-3, including the GC content, it is considered that this organism belong to the genus *Alcaligenes*. Among others, *A. faecalis* seemed very similar, though the GC content (58.9%) reported by HOLDING and SHEWAN (51) was smaller than 64.6% in the strain 6-3. As to the utilization of many carbon compounds and the urease reaction of *A. faecalis*, there is no detailed information, except for those by HENDRIE, HOLDING, and SHEWAN (52). COUN (58) and BOARD (59) had stated that this microorganism could not utilize urea, while RHODES (60) identified an aniline-utilizing strain of *A. faecalis* which hydrolyzed urea and starch, and had a GC content of 67.3%. The strain 6-3 utilizes urea but not aniline or starch. At any rate, the strain 6-3 resembles *A. faecalis* to some extent in general characteristics. As is the case in *P. acidovorans*, GLC analysis of cellular fatty acids has again proved effective for taxonomic study. In comparison with the composition of cellular fatty acids of *A. faecalis*, the clear difference in the strain 6-3 is found in peaks of straight-chain fatty acids of 2-hydroxy- $C_{12:0}$, $C_{12:0}$, and 2-hydroxy- $C_{14:0}$ (Tables 2 and 3 and Fig. 2). As reported by YANO *et al.* (55, 56), the characteristic appearance of 2- and 3-hydroxy fatty acids components in *A. faecalis* is confirmed as shown in Fig. 2. Therefore, strain 6-3 is concluded not to be *A. faecalis*.

The strain 6-3 shares most morphological, cultural, physiological, and biochemical properties with the type culture of *A. eutrophus* which was discriminated by DAVIS *et al.* (53). For example, positive nitrate reduction in nitrate-sodium lactate broth but not in nitrate broth; positive cleavage of protocatechuate by the *ortho* pathway; intracellular accumulation of poly- β -hydroxybutyrate; utilization pattern of carbon sources including the sole usage of fructose among many sugars, *etc.*, can be cited. However, differences are observed in the fact that the type culture of *A. eutrophus* cannot utilize glycerol, quinate, pantothenate, L-serine, and ethanol, while trigonelline is assimilated. *A. eutrophus* had been known to be able to grow at the expense of hydrogen, even though its utilization has recently been recognized to be facultative (53). The isolate 6-3 cannot grow with hydrogen as a sole energy source. The GC content of the DNA of the strain 6-3 (64.6 mol%) also indicates a closer relationship to *A. eutrophus* (66.3–66.8 mol% by HOLDING and SHEWAN (51)) than to *A. faecalis* (58.9 mol% (51)). As is apparent from Table 3 and Fig. 2, an identical GLC pattern of cellular fatty acids is recorded for the type culture of *A. eutrophus* ATCC 17697 and the strain 6-3. 2-Hydroxy- $C_{14:0}$ and 3-hydroxy- $C_{14:0}$ fatty acids, which are reported to be signifi-

cant in this species by YANO *et al.* (55), are clearly located.

Although some differences are noticed in the utilization of some carbon compounds, it seems reasonable to assign the strain 6-3 to *A. eutrophus*, particularly based on the composition of cellular fatty acids, and the differences noted above will be enough to establish a new subspecies for the strain 6-3. Thus, the isolated strain tentatively numbered 6-3 is proposed to be named *Alcaligenes eutrophus* subsp. *quinolinicus* subsp. nov. Kouno and Iwai, which has been deposited with the accession number of IAM 12305 at the Institute of Applied Microbiology, University of Tokyo, Japan. The original type culture of *Alcaligenes eutrophus* described by DAVIS *et al.* (53) should be called *Alcaligenes eutrophus* subsp. *eutrophus* (WITTENBERGER and REPASKE, 1958) Davis *et al.* according to the International Code of Nomenclature of Bacteria (61).

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