Zeitschrift für Allgemeine Mikrobiologie	21	2	1981	125—130

(Institute of Applied Biology, Science University of Tokyo, Noda, 278, Japan)

Characterization of a radiation-resistant *Acinetobacter*

Y. Nishimura, E. Kairiyama[1]), M. Shimadzu and H. Iizuka

(Eingegangen am 27. 3. 1980)

For characterizing the radiation-resistant *Acinetobacter* strain FO-1 reported in the previous paper (1980), we investigated the structure of cell wall, the cellular fatty acid composition, and the detailed taxonomic characteristics. The results denoted that the cell division occurred by simple constriction with the formation of a slight septum and the intermediate dense layer between the outer membrane and the plasma membrane was seen, the main components of the cellular fatty acids were oleic acid, palmitic acid, and palmitoleic acid, and that the strain was tolerant to salt and could not produce acids from cellobiose, melibiose, lactose, and ribose.

In the previous paper (Kairiyama *et al.* 1980), we reported that the gram-negative bacterium strain FO-1 isolated from tampon's cotton sterilized by gamma radiation denoted the D_{10} value of 220 krad in air equilibrated 0.067 M phosphate buffer and belonged to the genus *Acinetobacter* from the taxonomic characteristics: Coccobacillus (pleomorphic), aerobic, nonmotile, non-sporeforming, catalase-positive, cytochrome oxidase-negative, non-fermentative, resistant to penicillin *etc.*

In this paper, we describe the structure of the cell wall, the cellular fatty acids and the further taxonomic characteristics of the strain FO-1.

Concerning the radiation-resistant *Acinetobacter*, there have been a few papers (Thornley and Glauert 1968, Glauert and Thornley 1971, Welch and Maxcy 1975). They reported in detail the fine structure of cell wall, but no detailed taxonomic characteristics.

Materials and methods

The intact cells obtained from cultivating with nutrient broth for 3.5 hours at 30 °C were fixed by the glutaraldehyde-osmium procedure described by Glauert and Thornley (1966). After embedding in epon 812, thin sections were cut on a ultramicrotome JEOL JUM 7. Sections were stained with uranyl acetate and lead citrate. Electron micrographs were taken with an electron microscope JEOL JEM 100 S, operating at 100 kV and at instrumental magnification of 20000.

The cellular fatty acid composition was determined by gas chromatography in Shimadzu gas chromatography apparatus GAMPF using a 3 m glass column of 15% diethylene-glycol succinate coated on 80–100 mesh chromosorb GAW under an isothermal run at 200 °C. The samples for gas chromatography were prepared by the method reported in the previous paper (Nishimura *et al.* 1979).

The carbon assimilation tests were carried out with the following medium: NH_4NO_3 0.3%, K_2HPO_4 0.2%, $MgSO_4 \cdot 7\,H_2O$ 0.1%, NaCl 0.05%, $FeSO_4 \cdot 7\,H_2O$ 0.001%, carbon source 1%, pH 7.0. The oxidation-fermentation tests were carried out according to the Hugh and Leifson method (1953). The nitrate reduction tests were performed for both succinate-nitrate medium and casitone nitrate medium. Methods in general use were employed in all other tests. The optimal temperature for growth was measured after incubating for 16 hours in nutrient broth with Toyo Kagaku Sangyo Temperature Gradient Model TX-3. Cultures were incubated at 30 °C except in the case of the temperature test for growth. Guanine plus cytosine content of DNA was calculated from the melting temperature (T_m) of purified DNA. Lysis of intact cells was done by sodium dodecyl sulfate (SDS) at 60 °C. The purification of DNA was performed by the pH 9 phenol buffer treatment of Saito and Miura (1963).

---

[1]) Present address: National Commission of Atomic Energy of Argentina, Intense Source of Radiation Department, Av. Libertador 8250, Buenos Aires, Argentina

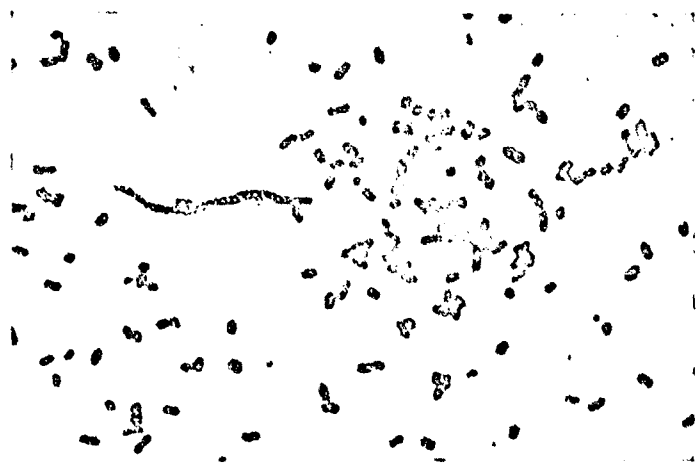

Fig. 1a

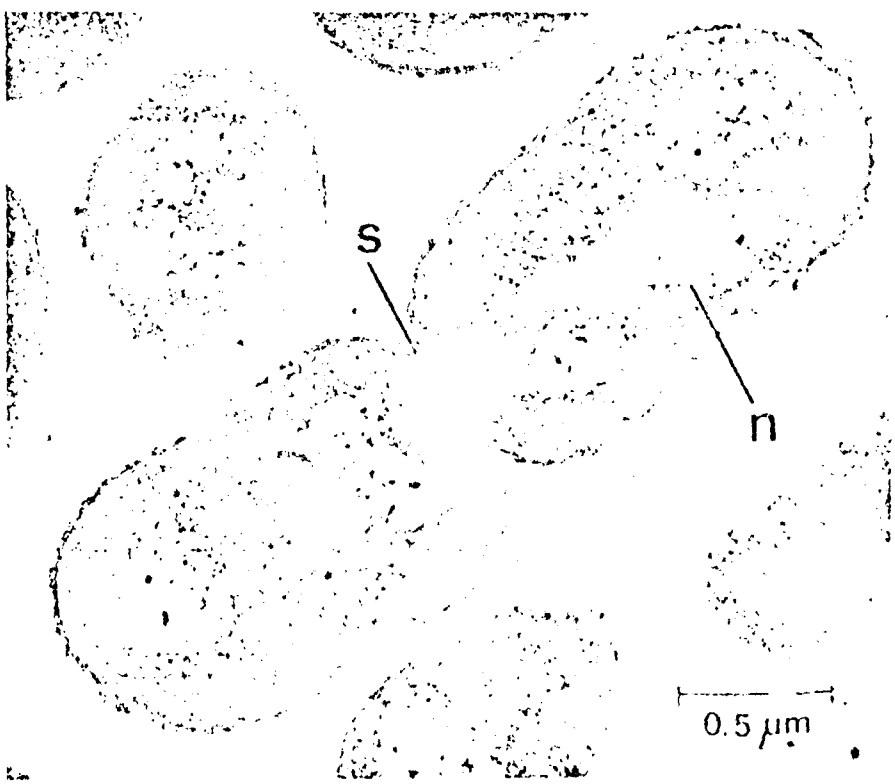

Fig. 1b

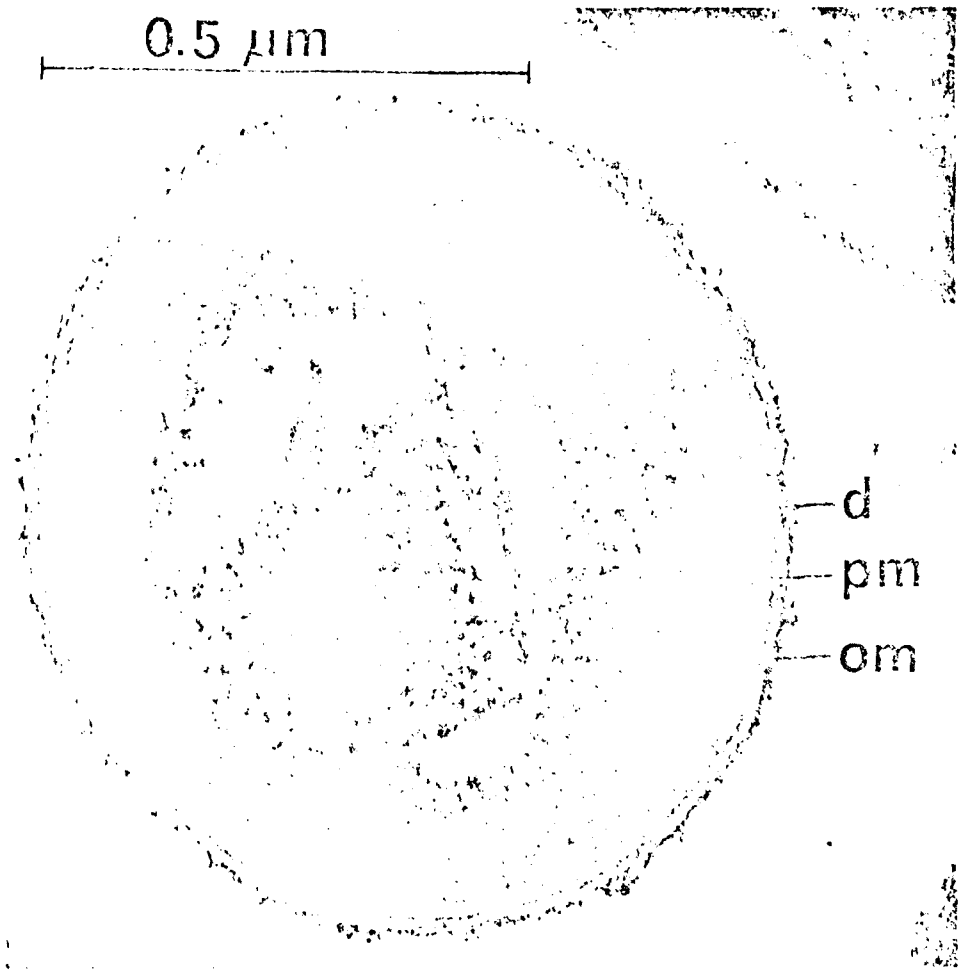

Fig. 1c

Fig. 1 (a). Micrograph of radiation-resistant strain FO-1 grown after 6 hr at 30 °C on nutrient agar medium and stained by crystal violet. Elongate form and plump form are seen.
(b). Electron micrograph of thin section of cells of strain FO-1. Cell division by constriction with formation of a slight septum (s) and fibrils of nuclear regions (n) are seen.
(c). Electron micrograph of the same strain. Intermediate dense layer (d) between outer membrane (om) and plasma membrane (pm).

Results and discussion

The micrograph of the strain FO-1 stained by crystal violet is shown in Fig. 1(a). The cells are cocci, coccoid rods or short rods, and occur mostly in pairs and occasionally in elongated cells. The electron micrographs of the strain are shown in Fig. 1 (b) and (c). Fig. 1(b) shows that the cell division occurs by simple constriction with the formation of a slight septum. The fibrils of the nuclear regions have been demonstrated.

Fig. 1(c) shows the existence of an intermediate dense layer between the outer membrane and the plasma membrane.

The main components of the cellular fatty acids in the strain FO-1 were oleic acid, palmitic acid, and palmitoleic acid (Table 1). This result coincided with the results for *Acinetobacter* species reported in our previous paper (NISHIMURA *et al.* 1979).

Table 1
Cellular fatty acid composition of radiation-resistant *Acinetobacter* FO-1

Fatty acids	Ratio of content (%)
Dodecanoic acid ($C_{12:0}$)	4.4
Tetradecanoic acid ($C_{14:0}$)	2.9
Palmitic acid ($C_{16:0}$)	20.1
Palmitoleic acid ($C_{16:1}$)	18.3
Heptadecanoic acid ($C_{17:0}$	T
Heptadecenoic acid($C_{17:1}$)	T
Stearic acid ($C_{18:0}$)	T
Oleic acid ($C_{18:1}$)	49.2
Linoleic acid ($C_{18:2}$)	T
Unidentified	1.8

Fatty acid occurring in amounts less than 1% are denoted by T

Taxonomic characteristics of the strain FO-1 are as follows: Coccoid rods 0.6 to 0.7 by 0.8 to 1.0 μm, pleomorphic: plump form 0.8 to 0.9 by 1.2 to 1.8 μm, elongate cells (15 μm or more). Non-spore forming, non-capsulated, non-motile, gram-negative, non-acid-fast.

Nutrient agar colonies: circular, smooth, slightly convex, entire, glistening, opaque, yellowish gray.

Nutrient broth: moderate growth, turbid, pellicle, slight sediment.

Nutrient agar slant: moderate growth, filiform, glistening, opaque, yellowish-gray.

Glutamate agar slant: moderate growth, filiform, yellowish-white to pale yellow.

Litmus milk: acid coagulation, no reduction of litmus. Indole and hydrogen sulfide not produced. Methyl-red test and Voges-Proskauer test: negative. Acid, but no gas from arabinose, xylose, rhamnose, galactose, glucose and mannose: no acid and no gas from ribose, cellobiose, melibiose, lactose, fructose, maltose, sucrose, trehalose and raffinose. Arabinose, xylose, galactose, fumarate, acetate, citrate, malonate, succinate, lactate, L-alanine, leucine, arginine, glutamate, aspartic acid, asparaginate, β-hydroxy butyrate, tridecane, hexadecane, kerosene and ethanol assimilated. Rhamnose, glucose, mannose, cellobiose, lactose, sucrose, trehalose, maltose, raffinose, adonitol, dulcitol, mannitol, sorbitol, gluconate, β-alanine, decane and methanol not assimilated.

Gelatinase, urease, phenyl alanine deaminase, lysine decarboxylase, ornithine decarboxylase and cytochrome oxidase: negative. Catalase and arginine dehydrolase: positive.

Nitrate reduced to nitrite in succinate-nitrate medium, but not reduced to nitrite in casitone-nitrate medium.

Penicillin resistance (100 IU): positive.

Haemolysis (sheep blood): negative.

Optimum temperature (Fig. 2): 27 to 30 °C.

Strict aerobic.

Guanine plus cytosine content of DNA: 44.5%

Comparing the data of strain FO-1 with those of the type strain of *A. calcoaceticus* ATCC 23055 the main taxonomic differences can be shown. Especially, the fact that the strain FO-1 could not produce acids from cellobiose, lactose, melibiose and ribose is important in the taxonomy of the *Acinetobacter* species. HUGH and REESE (1968) reported that 120 strains of *Bacterium anitratum* (*A. calcoaceticus*) produced acids from cellobiose, lactose, melibiose, and ribose. GILARDI (1971) reported that 255 strains of *Acinetobacter anitratum* (*A. calcoaceticus*) produced acids from lactose. NISHIMURA and IIZUKA (1976) reported that the three strains of hydrocarbon-utilizing, flavin

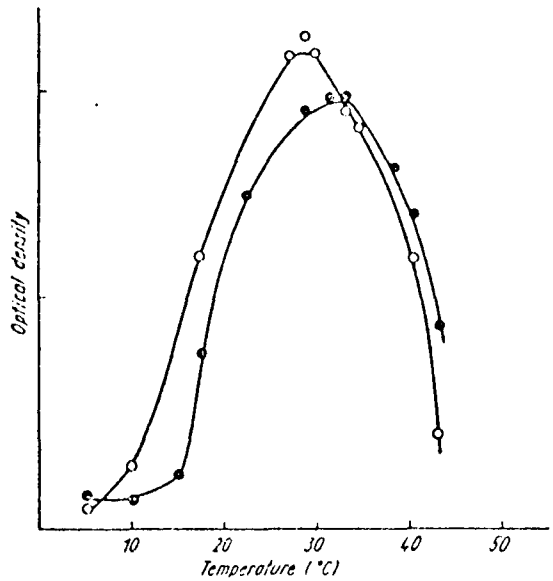

Fig. 2. Growth of radiation-resistant strain FO-1 and type strain ATCC 23055 of *Acinetobacter calcoaceticus* after 16 hr incubation at different temperatures. ○: strain FO-1, ●: strain ATCC 23055

nucleotide-forming *Acinetobacter anitratum* (*A. calcoaceticus*) produced acids from lactose and ribose. From the negative reaction with these sugars and the resistance to salt and radiation, we consider that the strain FO-1 should be understood to represent species separate from *A. calcoaceticus*.

Table 2

Different characteristics between radiation-resistant *Acinetobacter* FO-1 and *A. calcoaceticus* ATCC 23055

	ATCC 23055	FO-1
Oxidation of:		
D-Ribose	+	−
Cellobiose	+	−
Lactose	+	−
Melibiose	+	−
Assimilation of:		
Tridecane	−	+
Hexadecane	−	+
Kerosene	−	+
Urease	+	−
Gelatinase	+	−
Easy lysis by SDS	+	−
Growth in 5% NaCl broth	−	+
Radiation resistance	−	+
Optimal temperature (°C)	30 to 33	27 to 30
G + C content of DNA (moles %)	41.0	44.5

References

Gilardi, G. L., 1971 Characterization of non fermentative non fastidious gram negative bacteria encountered in medical bacteriology. J. appl. Bacteriol., 34, 623–644.

Glauert, A. M. and Thornley, M. J., 1966. Glutaraldehyde fixation of gram-negative bacteria. J. R. microsc. Soc., 85, 449–453.

Glauert, A. M. and Thornley, M. J., 1971. Fine structure and radiation resistance in *Acinetobacter*: a comparison of a range of strains. J. cell. Sci., 8, 19–41.
Hugh, R. and Leifson, E., 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J. Bacteriol., 66, 24–26.
Hugh, R. and Reese, R., 1968. A comparison of 120 strains of *Bacterium anitratum* Schaub and Hauber with the type strain of this species. Int. J. syst. Bacteriol., 18, 207–229.
Kairiyama, E., Lescano, H. G., Nishimura, Y. and Iizuka, H., 1980. Radio resistance of a gram negative bacterium. Z. Allg. Mikrobiol., 20, 149–152.
Nishimura, Y. and Iizuka, H., 1976. Microbiological studies of petroleum and natural gas. XIII. Identification of the flavin nucleotide-forming bacteria. J. gen. appl. Microbiol., 22, 79–84.
Nishimura, Y., Yamamoto, H. and Iizuka, H., 1979. Taxonomical studies of *Acinetobacter* species — Cellular fatty acid composition. Z. Allg. Mikrobiol., 19, 307–308.
Saito, H. and Miura, K., 1963. Preparation of transforming deoxyribonucleic acid by phenol treatment. Biochim. biophysica Acta, 72, 619–625.
Thornley, M. J. and Glauert, A. M., 1968. Fine structure and radiation resistance in *Acinetobacter*: studies on a resistant strain. J. cell. Sci., 3, 273–294.
Welch, A. B. and Maxcy, R. B., 1975. Characterization of radiation resistant vegetative bacteria in beef. Appl. Microbiol., 30, 242–250.

Mailing address: Dr. Y. Nishimura
Institute of Applied Biology
Science University of Tokyo
Noda, 278, Japan