Short Communication

RADIORESISTANCE OF AN *ACINETOBACTER* SPECIES

EULOGIA KAIRIYAMA,¹ YUKIMASA NISHIMURA,
AND HIROSHI IIZUKA

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

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In the course of studies on γ -radiation for the sterilization of tampon using as medical implement at the National Commission of Atomic Energy of Argentina, we isolated a radioresistant, Gram-negative bacterium. So far, there are many reports for the radioresistance of Gram-positive bacteria, but there are a few reports (1-3) for that of Gram-negative bacteria. They are for *Acinetobacter* of THORNLEY *et al.* (1), for *Pseudomonas radiora* of ITO and IIZUKA (2), and for the intermediate type of *Moraxella* and *Acinetobacter* of ITO *et al.* (3). The new isolate, strain FO-1, from cotton to make tampon is an *Acinetobacter* species, and the radioresistance of this strain is stronger than that of the above mentioned Gram-negative bacteria. In this paper we describe experimental studies on radioresistivity and identification of the strain FO-1.

The strain FO-1, isolated from the tampon's cotton sterilized by 3 Mrad irradiation of cobalt-60 γ -ray, and the type strain ATCC 23055 of *Acinetobacter calcoaceticus* as a comparison were used in this study.

The γ -ray source used was a 100 kCi cobalt-60 irradiator of Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute. The dose rate at the irradiation position determined by Fricke dosimetry was 400 krad/hr. The organisms were grown overnight in the nutrient broth, under constant aeration on a rotary shaker (220 rpm) at 30°. The cells were harvested and washed with 0.067 M phosphate buffer (pH 7.0) and resuspended in the same buffer.

Cell suspensions prepared were irradiated under air-bubbling, air-equilibrium, or under N₂-equilibrium conditions (wet state). In the case of the strain FO-1, the harvested cells were suspended in calf serum and 0.02 ml each of this suspension was packed in polyethylene-cellophane bags, dried in a desiccator, and irradiated in dry state at room temperature.

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

The carbon assimilation tests were carried out with the following medium: NH_4NO_3 , 0.3%; K_2HPO_4 , 0.2%; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1%; NaCl , 0.05%; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.001%; carbon source, 1%; pH 7.0. The oxidation-fermentation tests were carried out according to the HUGH-LEIFSON method (4). The nitrate reduction tests were carried out for both succinate-nitrate medium and casitonenitrate medium. The penicillin resistance test was performed by incubating the organism in the nutrient broth added with 100 IU of benzylpenicillin potassium. The optimal temperature for growth was measured after incubation for 16 hr in the nutrient broth using temperature gradient incubator Model TN-3 (Toyo Kagaku Sangyo). Guanine plus cytosine content of DNA was calculated from the melting temperature (T_m) of purified DNA. The purification of DNA was performed by

Fig. 1.

Fig. 2.

Fig. 1. Survival curves of *Acinetobacter* FO-1 under different conditions of gamma radiation.

The cells were suspended in 0.067 M phosphate buffer (pH 7.0): ●, air-bubbling; ⊙, air-equilibrium; ◐, N_2 -equilibrium. Dry state: ○, serum.

Fig. 2. Survival curves of *Acinetobacter calcoaceticus* ATCC 23055 under different conditions of gamma radiation.

The cells were suspended in 0.067 M phosphate buffer (pH 7.0).

●, air-bubbling; ⊙, air-equilibrium; ◐, N_2 -equilibrium.

Table 1. Radiosensitivity of strain FO-1 and *Acinetobacter calcoaceticus* ATCC 23055.

Condition of γ -ray irradiation	D ₁₀ value (krad)	
	Strain FO-1	Strain ATCC 23055
In 0.067 M phosphate buffer:		
air-bubbling	100	5.5
air-equilibrium	220	6.5
N ₂ -equilibrium	232	15.0
In dry state:		
serum	320

Fig. 3. Photographs of radioresistant *Acinetobacter* FO-1 grown at 30° on nutrient agar medium. Culture time: 6 and 20 hr. Elongated form is seen at left and plump form at right.

mixture of MARMUR method (5) and SAITO-MIURA method (6). The T_m was determined by Gilford Model 250 spectrophotometer with thermo-programmer.

For radiosensitivity studies, survival curves of the strain FO-1 under different irradiation conditions were determined and are shown in Fig. 1. Their shapes were exponential in air-bubbling and air-equilibrium, and sigmoidal in N₂-equilibrium and dry state. In the case of *Acinetobacter calcoaceticus* ATCC 23055, used as a reference, the shapes of survival curves were identical to those of the strain FO-1 respectively in air-bubbling, air-equilibrium, and N₂-equilibrium (Fig. 2). However, as shown in Table 1, the D₁₀ values under the above different irradiation conditions were very different, namely, in air-bubbling the D₁₀ value of the strain FO-1 was 18.2 times of that of the strain ATCC 23055, in air-equilibrium it was 33.8 times, and in N₂-equilibrium it was 15.5 times.

Taxonomical characteristics of the strain FO-1 are as follows: Coccoid rods

Table 2. Physiological characteristics and GC content of DNA in strain FO-1.

<p>Nutrient gelatin stab: No liquefaction. Litmus milk: Acid coagulation, no reduction of litmus. Indole not produced. Hydrogen sulfide not produced. Methyl Red test: Negative. Phenylalanine deaminase: Negative. Lysine decarboxylase: Negative. Urease: Negative. Catalase: Positive.</p> <p>Nitrate reduced to nitrite in succinate-nitrate medium but not in casitone-nitrate medium.</p> <p>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.</p> <p>Arabinose, xylose, galactose, fumarate, acetate, citrate, malonate, succinate, lactate, L-alanine, leucine, arginine, glutamate, aspartic acid, asparagine, β-hydroxybutyrate, tridecane, hexadecane, and ethanol assimilated. Rhamnose, glucose, mannose, cellobiose, lactose, sucrose, trehalose, maltose, raffinose, adonitol, dulcitol, mannitol, sorbitol, gluconate, β-alanine, decane, and methanol not assimilated.</p> <p>Growth in 5% NaCl broth and 15% sodium taurocholate. Strictly aerobic. Optimal temperature: 27° to 30°. GC-content of DNA: 44.5% (calculated from T_m).</p>
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0.6 to 0.7×0.8 to $1.0 \mu\text{m}$, pleomorphic; plump form 0.8 to 0.9×1.2 to $1.8 \mu\text{m}$, occur mostly in pairs, occasionally elongate cells ($15 \mu\text{m}$ or more) (Fig. 3). Non-spore forming, non-capsule, non-motile, Gram-negative, non-acid fast.

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

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

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

Nutrient broth: Moderate growth, turbid, pellicle, slightly sedimenting. Other physiological characteristics are shown in Table 2.

When we compare the radioresistance of the strain FO-1 with other radioresistant Gram-negative bacteria found in literature, under similar irradiation conditions, we can see (Table 3) that, the radioresistance of the strain FO-1 was about 4 times of that of the intermediate type of *Moraxella* and *Acinetobacter* (strains A-4 and A-10) (3) and about 1.6 times of that of *Pseudomonas radiora* O-1 in air-equilibrium, and it was about 1.7 times of that of *P. radiora* O-1 and about 2.2 times of that of *Acinetobacter* 199 A (7) in air-bubbling. *Acinetobacter* 199A was isolated from poultry by THORNLEY *et al.* (1) and assigned at first as *Achromobacter* strain. In 1967, from taxonomic study of *Acinetobacter* and related genera, THORNLEY grouped this strain into the phenone 4i as *Acinetobacter* strain, and described that the strain 199A was penicillin sensitive and oxidase positive (8). These characteristics differ from those of the genus *Acinetobacter*. On the other hand, D_{10} values of the radiosensitive *Acinetobacter*, *A. calcoaceticus* ATCC 23055 and ATCC 19606, and *A. anitratum* S5, were 6.5 krad,

Table 3. Comparative radiosensitivity of strain FO-1 and other Gram-negative bacteria in literature.

Microorganism	D ₁₀ value (krad)	
	in air-equilibrium	in air-bubbling
<i>Acinetobacter</i> FO-1	220	100
<i>Acinetobacter anitratum</i> S5 ^a	26 ^b	
<i>Acinetobacter calcoaceticus</i> ATCC 19606	12 ^c	
Intermediate type of <i>Moraxella</i> and <i>Acinetobacter</i> A-4	50 ^c	
A-10	54 ^c	
<i>Pseudomonas radiora</i> O-1	140 ^d	60 ^d
<i>Acinetobacter</i> 199A		46 ^e

^a Isolated (1972) and identified (1976) by NISHIMURA and IIZUKA (11).

^b Datum measured by YAMAMOTO (not reported).

^c Data reported by ITO *et al.* (3).

^d Data reported by ITO and IIZUKA (2).

^e This datum was obtained by GLAUERT and THORNLEY (7) from the cells suspended in broth and irradiated at 10°–13°.

12 krad, and 26 krad, respectively, in air-equilibrium (Tables 1, 3), which are from about 0.03 to 0.1 of that of the strain FO-1.

From the taxonomical characteristics, such as Gram-negative coccobacilli (pleomorphic), non-spore forming, strictly aerobic, catalase positive, cytochrome oxidase negative, resistant to penicillin, *etc.*, it is obvious that the strain FO-1 belongs to the genus *Acinetobacter*, but the strain FO-1 could not produce acids from cellobiose, lactose, melibiose, or ribose. HUGH and REESE (9) reported that 120 strains of *Bacterium anitratum* (*A. calcoaceticus*) produced acids from cellobiose, lactose, melibiose, or ribose. GILARDI (10) reported that 255 strains of *Acinetobacter anitratum* (*A. calcoaceticus*) produced acids from lactose. NISHIMURA and IIZUKA (11) reported that the three strains of hydrocarbon-utilizing, flavin nucleotide-forming *Acinetobacter anitratum* (*A. calcoaceticus*) produced acids from lactose or ribose. On the basis of these results for important characteristics, oxidation of sugars, we consider that the strain FO-1 should be treated as a new species of the genus *Acinetobacter*. We are now investigating the DNA-DNA homology among strains of *Acinetobacter* for the purpose of confirming that the strain FO-1 is a new species of the genus *Acinetobacter*.

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REFERENCES

- 1) M. J. THORNLEY, M. INGRAM, and E. M. BARNES, *J. Appl. Bacteriol.*, **23**, 487 (1960).
- 2) H. ITO and H. IIZUKA, *Agric. Biol. Chem. (Tokyo)*, **35**, 1566 (1971).
- 3) H. ITO, T. SATO and H. IIZUKA, *Agric. Biol. Chem. (Tokyo)*, **40**, 867 (1976).
- 4) R. HUGH and E. LEIFSON, *J. Bacteriol.*, **66**, 24 (1953).
- 5) J. MARMUR, *J. Mol. Biol.*, **3**, 208 (1961).
- 6) H. SAITO and K. MIURA, *Biochim. Biophys. Acta*, **72**, 619 (1963).
- 7) A. M. GLAUERT and M. J. THORNLEY, *J. Cell. Sci.*, **8**, 19 (1971).
- 8) M. J. THORNLEY, *J. Gen. Microbiol.*, **49**, 211 (1967).
- 9) R. HUGH and R. REESE, *Int. J. Syst. Bacteriol.*, **18**, 207 (1968).
- 10) G. L. GILARDI, *J. Appl. Bacteriol.*, **34**, 623 (1971).
- 11) Y. NISHIMURA and H. IIZUKA, *J. Gen. Appl. Microbiol.*, **22**, 79 (1976).