Physicochemical Characters of Ultra Violet Ray Resistant *Deinococcus* sp. Isolated from Air Dust

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Among a few number of UV-resistant bacteria isolated from various environmental sources (10), we made a comparative physio-chemoanalytical study on one of spherical bacteria isolated from air dust, presumably *Deinococcus* sp. (CM strain 29) with an UV resistant bacterium, *Deinococcus radiophilus* ATCC 27603 as the reference strain. Our isolate of UV resistant coccus, *Deinococcus* sp. CM 29 and *D. radiophilus* ATCC 27603 showed more than 75% matching coefficient in metabolic activity of various substrates. The most predominant cellular fatty acid of both strains was palmitoleic acid (C 16:1, cis 9), but the detail fatty acid profiles were slightly dissimilar to each other. Cell-bound orange pigment seemed to be an identical chemicals on spectrophotometric analysis. L-ornithine was detected as cell-wall amino acid in both strains. Galactose was detected as cell-wall sugar in *D. radiophilus* ATCC 27603, whereas glucose in *Deinococcus* sp. CM 29. G-C molar ratio of both strains was comparable, 63~65%.

KEY WORDS cell-wall amino acids, cellular fatty acids, G+C mol%, *Deinococcus* sp.

The genus *Deinococcus* has a number of interesting physiological and chemical properties. One of the most peculiar properties lies on highly resistant nature to ultra violet radiation and dessication, even more resistant than bacterial endopsores. Besides, the genus *Deinococcus* has an atypical ornithine in place of diamino acid of cell wall and cell-bound carotenoid pigment rendering bright orange or pink in colony color (14). The cell wall of Gram-positve *Deinococcus* is structurally complex, consisting of several layers including outer menbrane layer normally absent in the other Gram-positive bacteria (4, 16, 19). However, the Deinococcal outer menbrane layer is known to be chemically different from that of Gram-negative bacteria (11). The predominance of pamitoleic acid (C 16:1) of cellular fatty acids is also noted as a distinct property from the morphologically and physiologically resemble, but UV sensitive *Micrococcus roseus* (4, 6, 9).

Deinococcus spp. can be isolated from various environmental sources such as soil, creek water, ground meat, fish, fecal samples, sawdust and filtered air (11). There are four species of *Deinococcus* currently recognized according to Bergey's Mannual of Systematic Bacteriology (11). But it has not been excluded the possibility to find out some novel species of *Deinococcus* or new types of microorganism as resistant to UV radiation from natural habitats. In fact, there is a report on an UV radiation resistant, Gram negative, rod shaped bacterium, namely *Deinobacter grandis* isolated from elephant dung and fresh water fish (12). Thus, we made efforts to search out UV resistant bacteria from various environmental sources (10). Among a few isolates of UV resistant bacteria, we has chosen one spherical bacterium for its chemoanalysis in order to define the bacterial characters.

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MATERIALS AND METHODS

Bacterial strains

Deinococcus sp. CM 29 isolated from air dust (10) was used in the study. *Deinococcus radiophilus* ATCC 27603 purchased from ATCC, Rocksvill, Maryland, USA was employed as a reference strain. The choice of reference strain was made on its bacteriological similarity to *Deinococcuus* sp. CM 29 (10).

Culture conditions

Bacteria were grown on TYGM liquid medium (trypton 5g, yeast extract 3g, glucose 0.1% and DL-methionine 0.05%, pH 6.8) in a shaking incubator at 30°C unless specified (6, 10). Media

used in the study were either Difco products (Difco lab. Detroit, Mich., USA) or BBL products (BBL Microbiological System. Becton Dickinson and Co. Cockeysville, MD, USA). All of reagents used were above practical grade purchased from either Sigma Chemical Co., (St. Louis, MO, USA) or other place.

Detection of metabolic activity

Bacteria grown on TYGM medium were subjected to score metabolic acivity on BiologR GP plates of 96 wells (Biolog Inc., Hayward, CA, USA) as user's manual specified.

Analysis of cellular fatty acid

Cellular fatty acid was extracted as methylated form (MIDI Technical Note 101, May, 1990) and analysed by flame ionization detector-Gas Chromatography (MIDI Inc., Newark, DE, USA).

Absorption spectra of cell-bound pigment

Cell-bound pigment extracted with two phase method (1, 5) was spectrophotometrically analysed (Beckman DU-65, UV/Visible spectrophotometer). Beta-carotene (Type III, Sigma Chem. Co., St. Louis, MO, USA) dissolved in chloroform was also analysed as a reference.

Detection of cell-wall amino acids and sugars

Cell-wall amino acids and sugars were extracted as Staneck and Roberts (17) and Shleifer (13) described. Ca. 30 mg of dry cell was acid hydrolysed (6 N HCl, 100°C) for 18 hours in a sealed glass tube. Filtrate of hydrolysates through Whatmann no. 1 paper was dried at 100°C and dissolved in a small volume of distilled water. Five to 10 u*l* of prepared sample was subjected to ascending paper chromatography (3 mm whatmann cellulose paper, Whatman International Ltd., Maidstone, England) in a developing solution of n-buthanol-acetic acid-D-water (12:3:5, v/v). Dried Chromatogram was over-sprayed with 0.2% ninhydrine sol. in acetone for coloring amino acids. DL-diaminopimeric acid (DAP), L-ornitnine, L-lysine (1 u*l* of 1% sol) was employed as standards. Sugars were detected with silver nitrate-sodium hydroxide spray solution (7).

Estimation of G-C molar ratio

Bacterial DNA was extracted with a method by Bollet, *et al.* (2). Contaminated RNA was hydrolysed with ribonuclease T_1 as well as ribonuclease A (Sigma Chemical Co.). Deoxynucleotides obtained by hydrolysing DNA with nuclease P_1 and alkaline phosphatase (Sigma Chem. Co.) was subjected to reversed phase-HPLC (LC-4A Liquid Chromatograph, Shimadzu, Kyoto, Japan, Radial-pak C_{18} cartridge installed in Z Module, Water Associates, Lamda-Max Model 481 LC Spectrophotometer, Water Associates, Data analyser Chromatopac C-R2AX, Shimadzu) eluted with a mixture of 0.6 M $NH_4H_2PO_4$, pH 4.0, and acetonitrile (20:1, v/v) (18).

Table 1. *Metabolic activity of Deinococcus sp. CM 29 and D. radiophilus ATCC 27603*

Substrates utilized by both strains: β-cyclodextrin, dextrin, arbutin, cellobiose, D-fructose, D-galactose, D-gluconic acid, α-D-glucose, maltose, maltotriose, mannitol, D-mannose, palatinose, D-psicose, salicin, sedoheptulosan, D-sorbitol, tagatose, D-trehalose, D-xylose, methyl pyruvate, monomethyl succinate, pyruvic acid, glycerol, uridine, fructose-6-PO_4

Substrates not utilized by both strains: mannan, tween 80, inulin, N-acetyl-D-mannosamine, L-arabinose, D-arabitol, L-fucose, m-inocitol, α-D-lactose, D-melezitose, D-melibiose, α-methyl-D-galactose, D-ribose, β-methyl-D-glucose, α-methyl D-mannose, D-raffinose, D-rhamnose, stachypose, xylitol, α-, β-, γ-hydrobutyric acid, p-hydrophenyl acetic acid, α-ketoglutaric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, L-malic acid, D-malic acid, succinamic acid, succinic acid, alaninamide, glucose-6-PO_4, glucose-1-PO_4, thymidine, inosine, 2,3-butandiol, putrescine, glycyl-L-glutamic acid, L-pyroglutamic acid

Substrates utilized by only *Deinococcus* sp. CM 29: N-acetyl-D-glucosamine, amygdalin, α-methyl-D-glucose, gentibiose, tween 40, sucrose

Substrates utilized by only *D. radiophilus* ATCC 27603: glycogen, acetic acid, propionic acid, L-serine, L-glutamic acid, L-asparagine, 3-methyl glucose, α-ketovaleric acid, D-alanine, N-acetyl L-glutamic acid, L-alanyl-glycine, adenosine-5-PO_4

RESUTLS AND DISCUSSION

Detection of metabolic activity

Availability of commerical products of dye conjugated-substrates made us lessen a burden to detect metabolic activity of the bacteria on various substrates. As depicted in Table 1, both strains

Table 2. *Cellular fatty acids of UV resistant bacteria*

Strain	Fatty acids (%)
Deinococcus sp. CM 29	C 16:1, cis 9 (41.35)*
	C 16:0 (25.77)
	C 16:1 B*** (4.26)
	C 17:1 B (3.59)
	C 18:1, cis 9 (2.75)
D. radiophilus ATCC 27603	C 16:1, cis 9 (52.07)**
	C 17:1 B (15.82)
	C 16:1 B (12.29)
	C 15:1 A*** (3.94)
	C 18:1, cis 9 (2.69)

*: Among 97.61% named area for *Deinococcus* sp. CM 29

**: Among 77.76% named area for *D. radiophilus* ATCC 27603

***: Steric configuration is not known

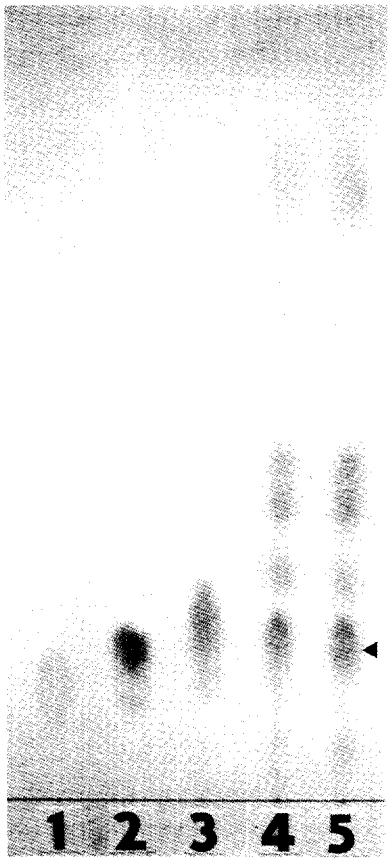

Fig. 1. *Paper-chromatograpgy of cell wall amino acids extracted by acid hydrolysis from UV resistant bacteria.*
Standard amino acids (1, 2, 3), 1: meso-diaminopimeric acid, 2: L-ornithine, 3: L-lysine, 4: cell wall amino acid from *D. radiophilus* ATCC 27603, 5: cell wall amino acid from *Deinococcus* CM 29. Both of UV resistant bacteria were appeared to possess L-ornithine in cell wall as pointed by an arrow.

showed an extent degree of common metabolic spectra against a number of substrates, but were not absolutely conincidental each other. They showed more than 75% matching coefficent (numbers of substrates either utilized by both strains or not utilized by both strains/total numbers of substrates tested×100)in metabolic diversity of various substrates. This fact suggested that both bacteria are regarded as the same genus.

Analysis of cellular fatty acid

Cellular fatty acid profiles are known to be rather characteristic to bacterial species, so it has been very valuable in bacterial identification (8). For instance, the predominant occurrence of

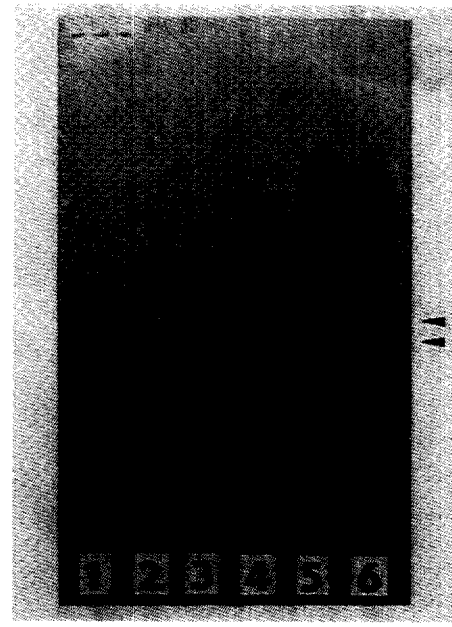

Fig. 2. *Paper chromatography of cell wall sugars extracted by acid hydrolysis from UV resistant bacteria. Standard sugars (1-4), 1: arabinose, 2: glucose, 3: galactose, 4: sucrose, 5: galactose from D. radiophilus ATCC 27603, 6: glucose from Deinococcus CM 29.*

palmioteic acid was noted a distinctive biochemical feature of the *Deinococcus* (9), compared with its absence in the morphologically resembled, red-pigmented *Micrococcus roseus*. As shown in Table 2, palmitoleic acid (C 16:1, cis 9) was the most predominant fatty acid in *Deinococcus* sp. CM 29 as well as in a reference UV resistant bacterium, *D. radiophilus* ATCC 27 603. But whole fatty acid profiles were slightly dissimilar to each other, suggesting that both bacteria are certainly belonged to the same genus but not to same species.

Absorption spectra of cell-bound pigment

Chloroform extract of cell-bound orange pigment of *Deinococcus* sp. CM 29 showed absorption peaks at 485, 455 and 413 nm wavelengths. These were similar to 484, 455 and 414 nm absorbed by those of *Deinococcus radiophilus* ATCC 27603 as a reference strain. We assumed that fat-soluble orange pigment of both species of the *Deinococcus* is caroteniods because beta-carotene (type III) of carrot purchased from Sigma chemical Co., showed similar absorption peaks.

Detection of cell-wall amino acids and sugars

As shown in Fig. 1, L-ornithine was found in cell wall hydrolysate of our isolate as in *D. radiophilus* ATCC 27603. The presence of

ornithine in cell wall is also known to be an unique features of the genus *Deinococcus* (14). Fig. 2 showed chromatogram of sugars in cell wall hydrolysate. Our isolate seemed to contain glucose whereas *D. radiophilus* ATCC 27603 seemed to contain galactose.

Estimation of G-C molar ratio

One distinctive molecular feature of the UV resistant *Deinococcus* sp. from *Micrococcus roseus* is different G-C molar ratio. The DNA of the genus *Deinococcus* has 62-70 G+C mol%, whereas that of the red-pigmented *Micrococcus roseus* has above 74 G+C mol%. Our isolate of *Deinococcus* showed 65 G+C mol% which is very close to 63 G+C mol% of the reference strain. Our data on G+C mol% of the refernce strain seemed to coincide with the value already reported by others (3).

Our results of physio-chemoanalytic study on the isolate of UV resistant bacterium from air dust in Chungbuk National Univeristy campus extended our confidence that the bacterium belongs to the genus *Deinococcus*, as comparable to *D. radiophilus* ATCC 27603, but not the same species. Further studies on chemotaxonomy of this isolate such as polar lipid profiles and 16s r-RNA sequence are being pursued. These would provide more concrete information for the identification of our UV resistant isolate.

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초 록: 대기에서 분리한 자외선 내성 세균, *Deinococcus* sp.의 생리-화학적 특성 연구

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본 연구자들이 대기에서 분리한 후 형태학적 특성과 자외선조사에 대한 현저한 내성에 근거하여 *Deinococcus* sp.로 추정하여 보고한 바 있는, 그람씨 양성, 구형 *Deinococcus* sp. CM strain 29을 보다 정확하게 동정하기 위하여, 여러가지 영양기질에 대한 이균의 대사능을 알아보고, 세포구성지방산, 세포벽 구성 아미노산과 당의 분석을 하고, 색소의 분광분석흡수파장을 측정하는 한편 핵산 구성 G+C 염기의 구성비율도 살펴보았다. 실험 결과 분리한 *Deinococcus* sp. CM 29는 본 연구에 대조균으로 사용한 자외선 내성 세균 *Deinococcus radiophilus* ATCC 27603과 같은 속에 속하는(the genus *Deinococcus*), 그러나 다른 종(species)으로 사료되었다.