NOTE

Base Composition of Deoxyribonucleic Acid from *Nannocystis exedens* (Myxobacterales)

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The base composition of the deoxyribonucleic acid of 11 strains of *Nannocystis exedens* varied between 70 and 72 mol% guanine plus cytosine (G+C). Therefore it falls into the G+C range known from other myxobacteria, i.e., 67 to 72 mol%.

Nannocystis was described some years ago as a new genus of myxobacteria; so far only one species, *N. exedens*, is recognized (9). The bacterium is very common in soil and has been isolated from many different environments all over the world. It is perhaps the most common

myxobacterium of all. This paper is intended to supply the still-lacking data on the deoxyribonucleic acid (DNA) base composition of the new organism.

The strains chosen for this investigation were all isolated in our laboratory with the

TABLE 1. Base composition of DNA from *Nannocystis exedens* and other myxobacteria

Strain	Origin of strain	Time of isolation	T_m^a (°C) (in degrees)	Mol%G+C from T_m	Buoyant density (g/cm ³)	Mol%G+C from buoyant density
<i>Nannocystis exedens</i> Na e1 ^b	Desert soil, Arizona	Oct. 1968	94.86	71.5	1.7298	70.9
<i>N. exedens</i> Na e3	Field soil, Nebraska	Feb. 1969	94.74	71.2	1.7293	70.3
<i>N. exedens</i> Na e7	Soil from secondary jungle, American Samoa	Dec. 1966	95.05	72.0	1.7297	70.8
<i>N. exedens</i> Na e10	Field soil, Kaiserstuhl Mountains, Germany	Sept. 1969	94.88	71.6	1.7297	70.7
<i>N. exedens</i> Na e14	Forest soil, Kaiserstuhl Mountains, Germany	Sept. 1969	94.47	70.6	1.7290	70.0
<i>N. exedens</i> Na e15 ^c	Grassland soil, Kaiserstuhl Mountains, Germany	Oct. 1969	94.56	70.8	1.7287	69.7
<i>N. exedens</i> Na e15 ^c			94.56	70.8	1.7288	69.8
<i>N. exedens</i> Na e15 ^d			94.59	70.9	1.7291	70.1
<i>N. exedens</i> Na e16	Grassland soil, Kaiserstuhl Mountains, Germany	Oct. 1969	94.54	70.8	1.7286	69.5
<i>N. exedens</i> Na e17	Grassland soil, Kaiserstuhl Mountains, Germany	Oct. 1969	94.56	70.8	1.7290	70.0
<i>N. exedens</i> Na 167 ^e	Garden soil, Bangkok, Thailand	June 1970	95.09	72.1	1.7297	70.7
<i>N. exedens</i> Na 283 ^e	Garden soil, Chiang Mai, Thailand	Sept. 1971	94.69	71.1	1.7296	70.7
<i>N. exedens</i> Na 290 ^e	Garden soil, Chiang Mai, Thailand	Sept. 1971	94.79	71.4	1.7297	70.8
<i>Myxococcus fulvus</i> Mx f16-1	Soil from Borobudur, Java	March 1971	94.37	70.4	1.7285	69.5
<i>Myxococcus xanthus</i> Mx NM	From R. P. Burchard Baltimore, non-motile mutant		94.07	69.7	1.7269	67.8
<i>Stigmatella aurantiaca</i> Sg a1	Rotten wood, Minneapolis, Minn.	Sept. 1966	92.78	66.7	1.7261	66.9

^a Data corrected for 0.1 M [Na⁺].

^b Type strain.

^c Two different determinations of the same DNA preparation.

^d From a separate DNA preparation.

^e Organisms which by their cytology and growth pattern were indistinguishable from *N. exedens* but could not be classified unequivocally because fruiting bodies were not obtained.

exception of strains Na e7 and Sg a1 which were isolated in the laboratory of Martin Dworkin in Minneapolis, Minn., and strain Mx NM which was kindly supplied by Robert P. Burchard, Baltimore, Md. Strain Na e1 is the type strain of *N. exedens*; it is deposited at the ATCC (American Type Culture Collection, Rockville, Md.) (number 25963), and at the DSM (German Collection of Microorganisms, Göttingen, Federal Republic of Germany; number DSM 71). Also, strain Na e17 is at the ATCC (number 25965). All stock cultures were kept on vy/2-agar: baker's yeast (0.5%; fresh weight of yeast cake), cyanocobalamine (0.5 µg/ml), CaCl₂ (anhydrous; 0.1%), agar (1.5%) (pH 7.2), autoclaved at 125°C for 30 min. For DNA preparation *N. exedens* was grown in MD1 liquid medium: Casitone (Difco; 0.3%), CaCl₂ (anhydrous; 0.05%), MgSO₄ (anhydrous; 0.1%), trace elements (0.5 ml of stock solution per 100 ml of medium) (trace element mixture as given in reference 2). Batches of 100 ml of MD1 liquid medium in 500-ml Erlenmeyer flasks were shaken with 160 cpm at 30°C with a stroke of 5 cm. The bacteria grew in the form of brightly colored minute flakes.

The other myxobacteria were grown in Casitone liquid medium: Casitone (1%), MgSO₄ (anhydrous; 0.1%), pH 6.8 unadjusted. Culture conditions were as above. These organisms formed homogeneous cell suspensions.

DNA was isolated by the method of Marmur (7) with some modifications. (i) Harvested cells were not washed before treatment with sodium dodecyl sulfate, because part of the DNA was already released during washing. (ii) The deproteinized precipitated DNA was dissolved in 1/10 strength tris(hydroxymethyl)aminomethane (Tris)-hydrochloride-Na⁺ buffer which was brought then to full strength (50 mM Tris-hydrochloride buffer, pH 7.65 + 10 mM NaCl). The dissolved DNA was treated with 50 µg of ribonuclease (RNase) A per ml (Boehringer, Mannheim, Germany) for 45 min at 37°C. This was followed by addition of ethylenediaminetetraacetic acid (10 mM) and treatment with 2 µg of RNase T₁ per ml (Boehringer) for 45 min at 37°C. Both RNases were heated before use for 15 min at 80°C to destroy any possible deoxyribonuclease activity. (iii) After RNase treatment, 0.5% sodium dodecyl sulfate and 25 µg of proteinase K per ml (Boehringer) were added. The mixture was kept at 37°C overnight and then deproteinized twice (7).

The guanine plus cytosine (G+C) content was determined in two ways. (i) From the "melting point" (8): Melting curves were measured with a Gilford model 250 spectrophotome-

ter equipped with a thermoprogrammer. Temperature increase was 0.5 degrees per min. DNA was dissolved at a concentration of 20 to 30 µg/ml in sodium phosphate buffer (pH 7.0, [Na⁺] 0.1 M). (ii) From the buoyant density (6): The buoyant density, ρ , was determined in a CsCl gradient on a Beckman model E analytical ultracentrifuge after 48 h at 44,000 rpm at 25°C. *Escherichia coli* DNA ($\rho = 1.71$ g/cm³) served as a reference. The guanine plus cytosine (G+C) content was calculated with the DeLey (1) formula: $G+C = 1038.47 (\rho - 1.6616)$. Usually DNA was prepared from each strain only once. With strain Na e15, however, two separate experiments were performed. As can be seen from Table 1 results were nearly identical.

As shown in Table 1, the investigated strains of *N. exedens* varied little in the G+C content of their DNA. Furthermore, the 69 to 72 mol% G+C found with different *Nannocystis* strains fell into the narrow range of 67 to 72 mol% G+C known for other myxobacteria (3-5).

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REPRINT REQUESTS

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