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Nutritional flexibility of oligotrophic and copiotrophic Antarctic bacteria with respect to organic substrates

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1. SUMMARY

The nutritional flexibility of oligotrophic and copiotrophic bacteria from an Antarctic freshwater lake sediment was investigated. Bacteria isolated on plates of oligotrophic and copiotrophic media were replica plated onto media containing different substrates, and their ability to utilise the different substrates was determined. The oligotrophs were shown to be able to utilise a significantly broader range of organic substrates than the copiotrophs, consistent with the idea that nutritional flexibility is adaptive for oligotrophic bacteria.

2. INTRODUCTION

Two principal definitions of oligotrophic bacteria have been proposed. Kuznetsov et al. [1] tentatively classified bacteria isolated from oligotrophic habitats as those that developed at the first cultivation on media with minimal organic matter of about 1–15 mg $C.l^{-1}$ and that grow on such media at subsequent recultivations although they can grow on richer media. Poindexter [2] found it more appropriate to define a habitat in terms of the average flux of energy through the environment, and conceived oligotrophic bacteria as those which can multiply in habitats of low energy flux of a fraction of a mg $C.l^{-1} \cdot d^{-1}$. In copiotrophic environments, the nutrient flux is at least fifty times greater and does not drop to zero for prolonged periods.

These concepts may be summarised as a 'fast and famine' oligotrophic existence [2] and a copiotrophic 'feast and famine' existence [3]. In reality bacteria in the two groups are probably not separated into distinct groups [4,5] but represent extremities of a continuous scale covering the complete range of physiological ability.

The growth of oligotrophic bacteria is primarily limited by the availability of assimilable organic substances. Poindexter [2] proposed that bacteria adapted to oligotrophic conditions might be expected to possess uptake systems for a more diverse variety of substrates than bacteria adapted to copiotrophic conditions, so maximising simultaneous utilisation of a multiplicity of substrates. The characteristics of oligotrophic bacteria, and

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mechanisms facilitating survival in oligotrophic environments, have been extensively reviewed [6–9]. This present work investigated whether bacteria isolated on an oligotrophic medium were able to use a greater variety of organic substrates than those isolated on a copiotrophic medium.

3. MATERIALS AND METHODS

3.1. Isolation of oligotrophic and copiotrophic bacteria

Source of isolates. Freshwater sediment was collected in February 1985 in a sterile 250 ml polypropylene bottle from Heywood Lake, Signy Island (60°40′S, 45°35′W), a member of the South Orkney Islands in the maritime Antarctic. The sample was stored at −20°C prior to use.

Culture of isolates. The freshwater sediment was thawed overnight at 4°C before a ten-fold dilution series was prepared aseptically. The diluent was the Griffiths and Morita [10] replication medium with the carbon source and agar omitted and the volume of the distilled water in the basal medium adjusted to account for any reduction in volume due to the ommissions. Subsamples (0.1 ml) of each dilution were aseptically spread with sterile glass spreaders onto triplicate pre-cooled plates of casein peptone starch (CPS) [11] and oligotrophic enrichment (OEMS) [12] isolation media. The copiotrophic isolation plates were incubated for 10 weeks (the first 4 weeks at 10°C then at 4°C) and the oligotrophic isolation plates for 13 weeks at 10°C until there were no further increases in the number of colonies on the plates.

3.2. Isolation media

The copiotrophic CPS medium employed was a casein peptone starch medium modified from the CPSA medium of Wynn-Williams [11]. The basal medium contained, in 905 ml distilled water, casein hydrolysate, 0.5 g; bacteriological peptone, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.05 g; glycerol, 1.0 ml; $FeCl_3$ solution (0.01% w/v), 0.1 ml; and NaCl, 0.1 g. The pH was adjusted to 6.8 and 12 g agar (number 3; Oxoid Limited, Basingstoke, U.K.) added. The medium was autoclaved for 15 min at 121°C. On cooling, filter sterilised cycloheximide solution (0.5% w/v), 10 ml; vitamin solution [13], 5 ml; and trace element solution (consisting of [$g \cdot l^{-1}$] EDTA, 1.5; $MnSO_4$, 0.5; $FeSO_4$, 0.1; $CaCl_2$, 0.1; $CoCl_2$, 0.1; $ZnSO_4$, 0.1; $CuSO_4$, 0.01; $AlK[SO_4]_2$, 0.01; and Na_2MoO_4, 0.01), 9 ml were added. To complete, autoclaved (15 min at 121°C) K_2HPO_4 solution (1.0% w/v), 20 ml; and soluble starch solution (1.0% w/v), 50 ml were added aseptically.

The oligotrophic medium (OEMS) was based on Bold's basal medium [12]. The basal medium contained 10 ml each of the 1 $g \cdot 400\ ml^{-1}$ solutions of $NaNO_3$, $CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$, K_2HPO_4 and KH_2PO_4 in 936 ml distilled water. To the 986 ml NaCl, 0.1 g; NH_4Cl, 0.173 g; glucose, 5 mg; and arabitol, 5 mg were added with 1 ml each of Bold's EDTA, iron, boron, and micronutrient solutions [12]. The pH was adjusted to 6.8, agar (12 g) added and the medium autoclaved as in the CPS medium. On cooling, filter sterilised cycloheximide solution (0.5% w/v), 10 ml; vitamin B_6 solution (1×10^{-6} $g \cdot l^{-1}$), 25 μl; and vitamin B_{12} solution ($1 \times 10^{-5} g \cdot l^{-1}$), 25 μl were added aseptically.

3.3. Replication of the isolates onto the test media

The oligotrophic and copiotrophic freshwater replication series were replicated from master plates that were chosen from a dilution (10^{-1}) which gave between 20 and 60 well separated colonies on each plate. The colonies on the initial isolation spread plates were replicated onto pre-cooled agar plates of a number of media which contained different organic substrates (Table 1). Replica plating was carried out using sterile velvet material held tightly onto a rubber bung (85 mm diameter) with a large jubilee clip. Prior to replication the pad was autoclaved, dried and cooled to 10°C.

All manipulations were carried out in a laminar flow cabinet (M.D.H. Ltd., Andover, U.K.) and on an ice tray similar to that of Wiebe and Hendricks [14] to minimise contamination and maintain a low temperature. Accurate replication was achieved by placing the velvet pad onto the agar surface of the spread plate and in turn onto each of the pre-cooled replica plates 1 to 11 (Table 1). The first and final replication plates (1 and 11) were the same medium as the master spread plate

Table 1

The media onto which the oligotrophic and copiotrophic series of freshwater bacteria were replicated

Replication Medium Code	OEMS Spread Plates	CPS Spread Plates
1	OEMS	CPS
2	CPS	OEMS
3	FC2	FC2
4	G&M + arabitol	G&M + arabitol
5	G&M + ribitol	G&M + ribitol
6	G&M + glycerol	G&M + glycerol
7	G&M + acetate	G&M + acetate
8	G&M + citrate	G&M + citrate
9	G&M + glucose	G&M + glucose
10	G&M + mannitol	G&M + mannitol
11	OEMS	CPS

to ensure that the bacteria being replicated had remained viable on the velvet pad throughout the replication series.

3.4. Replication media

The FC2 replication medium was modified from that by Tanner [15]. The basal medium contained, in 985 ml distilled water, Na_2HPO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; nitrilotriacetic acid, 0.05 g; NH_4Cl, 1.0 g; glycerol, 1.0 g; and NaCl, 0.1 g. The pH was adjusted to 6.8 and agar (12 g) added as in the CPS medium. The medium was autoclaved for 20 min at 115°C. On cooling, filter sterilised cycloheximide solution (0.5% w/v), 10 ml; and 1 ml of each trace element solution: A, ([g · l^{-1}] $CaCl_2 \cdot 2H_2O$, 4.0; $MnCl_2 \cdot 4H_2O$, 4.0; $FeCl_3 \cdot 6H_2O$, 2.8); B, ([g · l^{-1}] $CoCl_2 \cdot 6H_2O$, 0.2; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.2); C, ([g · l^{-1}] $ZnSO_4 \cdot 7H_2O$, 0.1; $SrCl_2 \cdot 6H_2O$, 0.2; $NiCl_2 \cdot 6H_2O$, 0.2; H_3BO_3, 0.2; KI, 0.1); and growth factor solution ([g · l^{-1}] myo-inositol, 20; pyridoxine HCl, 0.5; calcium pantothenate HCl, 2.0; nicotinic acid, 4.0; thiamine HCl, 0.4; and biotin, 0.001) was added aseptically.

Modified Griffiths and Morita (G&M) medium [10] contained, in 960 ml distilled water, $(NH_4)_2SO_4$, 2 g; KCl, 0.1 g; KH_2PO_4, 0.1 g; K_2HPO_4, 0.5 g; and NaCl, 0.1 g. The pH was adjusted to 6.8 and agar (12 g) added to solidify the medium. The medium was autoclaved for 20 min at 121°C. On cooling, the cycloheximide, vitamin and trace element solutions were added aseptically together with a $MgSO_4 \cdot 7H_2O$ solution (2.4% w/v), 5 ml. Finally, 10 ml of a solution of organic substrate (10% w/v) was added to bring the final carbon source concentration of the medium to 0.1% (1 g · l^{-1}). The single carbon sources employed were arabitol, ribitol, glycerol, acetate, citrate, glucose and mannitol.

3.5. Scoring of the growth

The two series of replica plates were incubated for 2.5 weeks at 10°C prior to growth on each organic substrate being scored. Growth was scored only for those colonies for which positive growth was exhibited on plates 1 and 11 of each replication series (Table 1) to ensure the viability of the inoculum throughout the procedure. The growth of these colonies on plates 2 to 10 was recorded simply as growth or no growth, and noted as 1 or 0 respectively. Subjectiveness was reduced by comparing each colony's growth on any replica plate to that on plates 1 and 11.

3.6. Statistical analysis

For each initially-isolated colony, growth on replica plates 2 to 10 was recorded and averaged over the nine replication media to give the 'nutritional flexibility' index,

$$\text{'Nutritional flexibility' index} = \frac{\Sigma G}{n}$$

where ΣG is the number of substrates which supported growth; and n is the number of substrates tested.

The median 'nutritional flexibility' index values for the series of oligotrophic and copiotrophic bacteria were analysed for significant differences using the Mann-Whitney U-test [16]. The null hypothesis of the analysis was that the two series of isolates were drawn from populations having the same ability to utilise the range of substrates tested. A two tailed analysis was carried out and the critical values of the normal deviate of U determined from statistical tables [17].

4. RESULTS AND DISCUSSION

The ability of each bacterial isolate to grow on each of the organic substrates tested, and their

Table 2

The ability of copiotrophic and oligotrophic freshwater bacteria to grow on the replication media. Growth is denoted by a value of 1 and no growth by 0

Isolate	Replication Medium Code									Number of substrates that supported growth	'Nutritional flexibility' index
	2	3	4	5	6	7	8	9	10		
Copiotrophic isolates											
1	0	0	0	0	0	0	0	0	0	0	0.000
2	0	0	0	0	0	0	0	0	0	0	0.000
3	0	1	0	0	1	0	0	0	0	2	0.222
4	0	0	0	0	0	0	0	0	0	0	0.000
5	0	0	0	1	0	0	0	0	1	2	0.222
6	0	0	0	0	0	0	0	0	0	0	0.000
7	0	0	0	0	0	0	0	0	0	0	0.000
8	0	0	0	0	0	0	0	0	0	0	0.000
9	0	1	0	0	1	1	1	1	0	5	0.556
10	0	1	1	0	1	1	1	1	1	7	0.778
11	0	0	0	0	0	0	0	0	0	0	0.000
12	0	0	0	0	0	0	0	0	0	0	0.000
13	1	1	1	1	1	0	1	1	1	8	0.889
14	0	0	0	0	1	1	1	1	0	4	0.444
15	0	0	1	0	1	1	1	1	0	5	0.556
16	0	0	0	1	1	1	1	1	0	5	0.556
17	0	0	0	0	0	0	0	0	0	0	0.000
18	0	0	0	0	1	0	0	1	0	2	0.222
19	0	1	0	0	0	0	1	0	0	2	0.222
20	0	0	0	0	0	0	1	0	0	1	0.111
21	0	0	0	0	0	0	0	0	0	0	0.000
22	0	0	0	0	0	0	0	0	0	0	0.000
23	0	0	0	0	0	0	0	0	0	0	0.000
24	0	0	0	0	0	0	0	0	0	0	0.000
25	0	1	1	1	1	1	1	1	0	7	0.778
26	1	1	1	1	1	1	1	1	1	9	1.000
27	0	1	1	1	1	1	1	1	1	8	0.889
28	1	1	1	1	1	1	1	1	0	8	0.889
29	0	0	0	1	1	0	1	1	0	4	0.444
30	0	1	1	1	1	0	1	1	0	6	0.667
31	0	0	0	0	0	0	0	0	0	0	0.000
Oligotrophic isolates											
1	1	0	0	0	0	0	0	0	0	1	0.111
2	1	1	1	1	1	0	1	1	1	8	0.889
3	1	1	1	1	1	1	1	1	1	9	1.000
4	1	1	1	1	1	0	1	1	1	8	0.889
5	0	1	1	1	1	1	1	1	1	8	0.889
6	0	1	1	1	1	1	1	1	1	8	0.889
7	0	1	1	1	1	1	1	1	1	8	0.889
8	0	1	1	1	1	0	1	1	1	7	0.778
9	1	1	1	1	1	1	1	1	1	9	1.000
10	0	1	1	1	1	1	0	1	1	7	0.778
11	1	1	1	1	1	1	0	1	1	8	0.889
12	1	1	1	1	1	1	0	1	1	8	0.889
13	1	1	1	1	1	0	0	1	1	7	0.778
14	1	1	1	1	1	0	0	1	1	7	0.778

Table 3

The median 'nutritional flexibility' indices of the copiotrophic and oligotrophic series of freshwater bacteria

Isolate series	Sample size	Median 'nutritional flexibility' index	Interquartile range
Copiotrophs	31	0.222	0.000 to 0.556
Oligotrophs	14	0.889	0.778 to 0.889

'nutritional flexibility' indices, are shown in Table 2. The median 'nutritional flexibility' index values for the oligotrophic and copiotrophic bacteria were calculated and are shown in Table 3. The microorganisms isolated on an 'oligotrophic' medium had a significantly higher 'nutritional flexibility' index than those isolated on a 'copiotrophic' medium. The amount of assimilable organic carbon present in the agar used in the media was unknown, and it is possible that the OEMS medium was not 'oligotrophic' in the strict terms described by Kuznetsov et al. [1]. However, the 'oligotrophic' medium did have less organic carbon than the 'copiotrophic' medium.

Five out of the fourteen oligotrophic isolates were shown to be unable to grow on the copiotrophic CPS medium (Table 2). This may suggest that they were obligate oligotrophs, although Martin and MacLeod [5] have cautioned that the ability to use high or low concentrations of substrates may depend upon the substrate, or mixture of substrates, available. They showed that an apparently obligate oligotroph was inhibited by high concentrations of succinate and aspartate. Inhibition at high peptone concentrations was due to the presence of these inhibitors, and not to the presence of high concentration of organic carbon per se. Hattori [18] has also demonstrated that the ability of oligotrophic soil microorganisms to utilise a substrate may be influenced by the presence either of different amino acids or by changes of concentrations of inorganic salts.

Our data show that bacteria isolated on a low nutrient oligotrophic medium from a freshwater Antarctic lake (Heywood Lake) could utilise a significantly broader range of organic carbon sources than those isolated on a richer copiotrophic medium ($P < 0.0001$). Harder and Dijkhuizen [19] have pointed out that in microorganisms from oligotrophic environments there tends to be an increase in the affinity of uptake systems for substrates; while there is a decrease in the substrate specificity of these uptake systems. It has also been demonstrated [20] that the presence of multiple substrates may influence the uptake kinetics of individual substrates. These may all be adaptations to low energy habitats where the ability to utilise, possibly simultaneously, a wide range of organic substrates may be of survival value. Our findings tend to confirm Poindexter's hypothesis that oligotrophic bacteria are more nutritionally flexible than copiotrophic bacteria.

Horowitz et al. [21] have previously reported an investigation into the versatility of oligotrophs to different carbon sources. Plates of different nutritional composition, with final substrate concentrations between 0.1 and 0.15% (w/v), were inoculated with bacteria previously isolated on low and high nutrient media from Cook Inlet, Alaska. The presence of growth was observed to assess the nutritional versatility of the isolates. The bacteria isolated on low nutrient media were found to be nutritionally far more versatile than those isolated on high nutrient media, having significantly higher 'nutritional utilization indices' for alcohols, carboxylic acids, amino acids and hydrocarbon substrates. No significant difference was shown for the ability to utilise a number of carbohydrates. The data obtained in the present study therefore corroborates the findings of Horowitz et al. Both studies are consistent with the hypothesis of Poindexter [2] that bacteria adapted to oligotrophic environments will be more nutritionally flexible than bacteria adapted to copiotrophic environments.

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