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NOTE

Nutritional Flexibility of Oligotrophic and Copiotrophic Bacteria Isolated from Deionzed-ultrapure Water Made by High-purity Water Manufacturing System in A Semiconductor Manufacturing Company

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Bacteria isolated from ultrapure water made by a high-purity water manufacturing system in a semiconductor manufacturing plant were classified into two groups which either grew in diluted nutrient broth medium (oligotrophic bacteria) or could not grow (copiotrophic bacteria). The nutritional flexibility of oligotrophic and copiotrophic bacteria was investigated. The oligotrophic bacteria were shown to be able to utilize a significantly broader range of organic substrates than the copiotrophic bateria. This finding substantiates the hypothesis that nutritional flexibility is adaptive for oligotrophic bacteria.

Oligotrophic bacteria are still relatively unknown to many microbiologists, especially industrial microbiologists (16, 18). Oligotrophs are ubiquitous in the environment and have been isolated from soil, rivers, lakes, oceans, and tap water lacking organic substances (8, 10, 14, 15). Some oligotrophic isolates can even grow in distilled water. Two different types of oligotrophs have been distinguished. Those oligotrophs that can grow on only a low concentration of carbon are called obligate oligotrophs (1, 5, 8). Those that are able to grow in the presence of both low and high concentrations of organic substances are called facultative oligotrophs (9). Poindexter hypothesized that bacteria adapted to oligotrophic conditions might be able to use more diverse substrates than bacteria adapted to copiotrophic conditions (13). The nutritional availability to bacteria of various nutrients has been expressed as a nutritional flexibility index as follows: Nutritional flexibility index=$\Sigma G/n$ where ΣG is the number of substrates which supported growth and n is the number of substrates tested (15).

Semiconductor device manufacturing lines require stringent water quality control because contaminated organic and inorganic materials have been shown to be a major cause of manufacturing problems and process failure. In particular bacteria, as living organisms, contain metals and are particulate which are detrimental to integrated circuit manufacturing (4). Deionized-ultrapure water is made by high-purity water manufacturing systems consisting of sand filters, active carbon filters, reverse osmosis filters, ion-exchange resins, 254-and 185-nm UV, and finally ultrafilters. The concentrations of total organic carbon and metal ions in the final product are strictly controlled to less than 0.1 PPB and less than 0.05 PPB, respectively. Although deionized water in the semiconductor industry is an extreme oligotrophic environment, microorganisms can survive, grow and multiply even in these conditions (3, 7, 11, 12).

Little has been learned of the physiological characteristics of oligotrophs and copiotrophs recovered from ultrapure water made by high-purity water manufacturing systems used in the semiconductor manufacturing company. In the present study we investigated the frequency of isolation of oligotrophs and copiotrophs from ultrapure water in the high-purity water distribution system of a semiconductor manufactrung company (Samsung Electronics Co., LTD.) and the nutritional flexibility of the isolated bacteria.

Frequency of Isolation of Oligotrophic Bacteria and Copiotrophic Bacteria in Deionized-ultrapure Water

Bacteria present in deionized water were collected aseptically by filtration (0.45 μm porosity, milipore GS filter) through a sampling valve on the main pipe line. One liter of deionized water per each filter was filtered at the same velocity (1 liter per 2 min) as the flowing

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stream. 6 filters were aseptically transferred onto one hundred-fold diluted nutrient broth agar medium (NB; Difco) which was designated NA/100 and incubated for 7 days at 30°C. 127 colonies were isolated from the filters. Colonies grown on NA/100 were tested for their capacity to grow on NB agar (NA) and diluted NB agars (NA/100, NA/1,000, and NA/10,000) and divided into four groups (Table 1). Class I bacteria, which can grow on all media tested, numbered 102 out of 127 isolates. Class II bacteria, which can grow on NA, NA/100, and NA/1,000, but not on NA/10,000, numbered 10. Class III bacteria, which can grow on NA and NA/100, but not on NA/1,000 and NA/10,000, numbered 14. Class IV bacterum, which can grow on NA/100, NA/1,000, and NA/10,000, but not on NA, numbered 1.

Criteria for oligotrophy have been discussed in several papers. Concentration of energy-yielding nutrients required by oligotrophic bacteria were considered to be in the order of 1 to 15 mg carbon per liter (10) or 5 to 10 mg carbon per liter (13). Recently, the concept of oligotrophic bacteria was modified to include a group of bacteria capable of growing on a medium containing less than 1 mg carbon per liter (17). The NA medium used in our experiments contained 3347 mg carbon per liter, thus a 10^{-4} dilution is assumed to contain approximately 0.33 mg carbon per liter. Based on the above assumptions, Class I bacteria and Class IV bacteria were designated as oligotrophic bacteria. Class II bacteria and Class III bacteria were designated as copiotrophic bacteria. Using the above criteria, 84% of heterotrophic bacteria were oligotrophs which can grow on NA/10,000 medium. 16% of bacteria were copiotrophs which can not grow on NA/10,000 media.

Nutritional Flexibility of Oligotrophic and Copiotrophic Bacteria

Among the oligotrophic and copiotrophic bacteria isolated from deionized water 30 strains of oligotrophic bacteria and 25 strains of copiotrophic bacteria were randomly selected and separately toothpicked onto NA/100 plate at a concentration of 15 strains per each plate. They were incubated at 30°C for 5 days. The colonies on the plates were replicated onto pre-cooled agar plates of a number of media containing different organic carbon sources (Table 2). Replica plating was carried out using sterile velvet pad (85 mm diameter). Prior to replication the pad was autoclaved, dried and cooled to room temperature. Accurate replication was achieved by placing the velvet pad onto the agar surface of the colonies grown on NA/100 plate and in turn onto each of the pre-cooled replica plates 1 to 15. The first and final replication plates (1 and 15) were the same medium as the master plates to ensure that the bacteria being replicated had remained viable on the velvet pad throughout the replication series. The replica plates were incubated for 14 days at 30°C prior to growth on each organic substrates being scored. The replica plate experiment was triplicated. Growth was scored only for those colonies for which positive growth was exhibited on plates 1 and 15 of each replication series to confirm the viability of the inoculum throughout the procedure. The growth of these colonies on plates 2 to 14 was recorded simply as growth or no growth, and noted as 1 or 0, respectively. Nutritional flexibility index of each strain was calculated (Table 3).

The mean nutritional flexibility index values for the series of oligotrophic and copiotrophic bacteria were analyzed for significant differences using the t-test procedure in Microsoft Excell for Windows 95 (Version 7.0). The null hypothesis of the analysis was that the two series of isolates had the same ability to utilize the range of substrates tested. The significance of probability of the t-test was 0.00000000515 ($P < 0.0001$), indicating that oligotrophic bacteria had a significantly higher nutritional flexibility index than copiotrophic bacteria (Table 4). This data shows that oligotrophic bacteria isolated from artificial oligotrophic conditions such as from deionized water made by a high-purity manufacturing system can utilize a significantly broader range of or-

Table 1. Classification of isolates based on growth patterns in different concentration of nutrient agar media.

Type	Growth on different media				Number of isolates
	NA	NA/100	NA/1,000	NA/10,000	
I	+	+	+	+	159
II	+	+	+	−	12
III	+	+	−	−	18
IV	−	+	+	+	1
				Total	190

Growth is denoted by a symbol of + and no growth by −.

Table 2. The media used to determine the nutritional flexibility of the oligotrophic and copiotrophic bacteria.

Replication medium code	Medium content	Replication medium code	Medium content
1	NA/100	2	G&M+glucose
3	G&M+sucrose	4	G&M+xylose
5	G&M+rhamnose	6	G&M+raffinose
7	G&M+melezitose	8	G&M+maltose
9	G&M+xylitol	10	G&M+meso-inositol
11	G&M+glycerol	12	G&M+mannitol
13	G&M+adonitol	14	G&M+Na-acetate
15	NA/100		

Modified Griffiths and Morita (G&M) medium consisted of (%, w/v): $(NH_4)_2SO_4$, 0.2; KCl, 0.01; KH_2PO_4, 0.01; K_2HPO_4, 0.05, and NaCl, 0.01 (2).

Table 3. The ability of oligotrophic and copiotrophic bacteria to grow on the replication media.

Isolate	Replication medium code													Number of substrates that supported growth	Nutritional flexbility index
	2	3	4	5	6	7	8	9	10	11	12	13	14		
Oligotrophic bacteria															
1	1	1	1	0	1	0	1	0	1	1	1	0	0	8	0.615
2	1	1	0	0	0	0	1	0	0	1	1	1	1	7	0.538
3	1	1	0	0	0	0	1	0	1	1	1	1	1	8	0.615
4	1	1	0	0	0	0	1	0	0	1	1	1	1	7	0.538
5	1	1	0	0	0	0	1	1	1	1	1	1	1	9	0.692
6	1	1	0	1	1	0	1	1	1	1	1	1	1	11	0.846
7	1	1	0	0	0	0	0	1	0	1	1	1	1	7	0.538
8	1	1	0	0	0	0	1	0	0	1	1	1	1	7	0.538
9	1	1	1	0	0	0	1	0	0	1	1	1	1	8	0.615
10	1	1	1	0	1	1	1	1	1	1	1	1	1	12	0.923
11	1	1	0	1	1	0	1	1	0	1	1	1	1	10	0.769
12	1	1	0	1	1	0	1	1	1	1	1	1	1	11	0.846
13	1	1	1	0	0	0	1	0	1	1	1	1	1	9	0.692
14	1	1	0	0	0	0	1	0	1	1	1	1	1	8	0.615
15	1	1	1	1	1	0	1	0	1	1	0	1	1	10	0.769
16	1	1	1	0	0	0	1	0	1	1	0	1	1	8	0.615
17	1	1	1	0	0	0	1	0	1	1	1	1	1	9	0.692
18	1	1	0	0	1	0	1	0	0	1	1	1	1	8	0.615
19	1	1	0	0	0	0	1	0	0	1	1	1	1	7	0.538
20	1	1	1	1	1	1	1	1	1	1	1	1	1	13	1.000
21	1	1	1	0	0	1	1	0	1	1	1	1	1	10	0.769
22	0	1	0	0	0	1	0	0	0	1	1	1	1	6	0.462
23	1	1	0	0	0	1	0	1	0	1	1	1	1	8	0.615
24	1	1	0	0	0	0	0	0	0	1	1	1	1	6	0.462
25	1	1	1	0	0	0	0	0	1	1	1	1	1	8	0.615
26	1	1	1	0	0	1	0	0	1	1	1	1	1	9	0.692
27	1	1	1	0	1	1	0	0	1	1	1	1	1	10	0.769
28	1	1	1	1	1	1	1	1	1	1	1	1	1	13	1.000
29	1	1	1	1	0	0	1	1	0	1	1	1	1	10	0.769
30	1	1	1	1	1	1	1	1	1	1	1	1	1	13	1.000
Copiotrophic bacteria															
1	1	0	0	0	0	0	0	0	0	1	1	1	1	6	0.462
2	1	0	0	0	0	1	1	0	0	0	1	1	1	7	0.538
3	1	0	0	0	0	1	1	0	0	1	1	1	1	8	0.615
4	1	0	0	0	0	1	1	0	0	0	1	1	1	7	0.538
5	1	0	0	0	0	0	1	0	0	1	1	1	1	7	0.538
6	1	0	0	0	0	0	1	0	0	1	1	1	1	6	0.462
7	1	0	0	0	0	0	0	0	0	1	1	1	1	6	0.462
8	1	0	0	0	0	0	1	0	0	1	1	1	1	7	0.538
9	1	0	0	0	1	0	1	0	0	1	1	1	1	8	0.615
10	1	1	1	0	0	0	0	0	0	1	1	1	1	7	0.538
11	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0.077
12	1	0	0	0	0	0	0	0	0	1	0	0	1	3	0.231
13	1	0	0	0	0	0	0	0	0	1	1	1	1	6	0.462
14	1	0	0	0	0	0	0	0	0	1	1	1	1	6	0.462
15	1	0	0	0	0	0	0	0	0	1	1	1	1	6	0.462
16	1	0	0	0	0	0	0	1	0	1	1	1	1	6	0.462
17	1	0	0	0	0	0	0	0	0	1	1	1	1	6	0.462
18	1	0	0	0	0	0	0	0	0	1	1	1	1	6	0.462
19	0	0	0	0	0	0	0	0	1	0	0	0	1	2	0.154
20	0	0	0	0	0	0	0	0	1	0	0	0	1	2	0.154
21	0	0	0	0	0	0	0	0	1	0	0	0	1	2	0.154
22	0	0	0	0	0	0	0	0	1	0	0	0	1	2	0.154
23	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0.077
24	1	0	0	0	0	0	0	0	0	1	0	0	1	3	0.231
25	1	0	0	0	0	0	0	0	0	1	0	0	1	3	0.231

Growth is denoted by a value of 1 and no growth by 0.

Table 4. The mean nutritional flexibility indices of the oligotrophic and copiotrophic bacteria isolated from ultrapure water.

Isolate series	Sample size	Mean nutritional flexibility index
Oligotrophs	30	0.692
Copiotrophs	25	0.385

ganic carbon sources than copiotrophic bacteria.

There have been some reports regarding the nutritional flexibility of microorganisms isolated from a natural oligotrophic environment such as freshwater of antartic lakes and subartic marine environments. Bacteria isolated from a low nutrient oligotrophic medium such as a freshwater of antartic lake (Heywood lake) could utilize a significantly broader range of organic carbon sources than those isolated from a richer copiotrophic medium (15). Bacteria isolated from a low nutrient media such as Cook Inlet, Alaska was found to be nutritionally far more versatile than those isolated from high nutrient media, showing significantly higher nutritional utilization indices for alcohols, carboxylic acids, amino acids, and hydrocarbon substrates (6).

The data obtained in the present study corroborates the hypothesis of Poindexter that bacteria adapted to oligotrophic environments will be more nutritionally flexible than bacteria adapted to copiotrophic environments (13).

In summary, we reported here on the oligotrophic characteristics of heterotrophic bacteria isolated from deionized-ultrapure water, a man-made extreme condition, found in the semiconductor manufacturing industry. The higher nutritional flexibility of oligotrophic bacteria isolated from an artificial nutrient poor environment was the same as that of oligotrophic bacteria isolated from natural oligotrophic environments.

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REFERENCES

1. Fry, J. C. 1990. Oligotrophs, p. 93-116, *In* C. Edwards (ed.), *Microbiology of extreme environments*. Open University Press, Milton Keynes, United Kingdom.
2. Griffiths, R. P. and R. Y. Morita. 1973. Salinity effects on glucose uptake and catabolism in the obligately psychrophilic bacterium, *Vibrio marinus. Mar. Biol.* **23**: 177-182.
3. Harfst, W. F. 1992. Back to basics: fundamentals in microbiology for high-purity water treatment. *Ultrapure water* **July/August**: 33-35.
4. Harned, W. 1986. Bacteria as a particle source in wafer processing equipment. *J. Environ. Sci.* **May/June**: 32-34.
5. Hirsch, P. 1979. Life under conditions of low nutrient concentrations, p. 357-372, *In* M. Shillo (ed.), *Strategies of microbial life in extreme environments*. Dahlem Konferenzen Life Sciencies Research Report 13. Weinheim: Verlag Chemie.
6. Horowitz, A., M. I. Krichevsky, and R. M. Atlas. 1983. Characteristics and diversity of subartic marine oligotrophic, stenoheterotrophic, and euryheterotrophic bacterial populations. *Can. J. Microbiol.* **29**: 527-535.
7. Husted, G. R. and A. A. Rutkowski. 1994. Microbials: response of oligotrophic biofilm bacteria in high-purity water system to stepwise nutrient supplementation. *Ultrapure Water* **September**: 43-50.
8. Ishida, Y. and H. Kadota. 1981. Growth patterns and substrate requirements of naturally occurring obligate oligotrophs. *Microbiol. Ecol.* **7**: 123-130.
9. Ishida, Y., Y. Imai, T. Miyagai, and H. Katoda. 1982. Growth and uptake kinetics of a facultative oligotrophic bacterium at low nutrient concentration. *Microbiol. Ecol.* **8**: 23-32.
10. Kuznetsov, S. I., G. A. Dubinnina, and N. A. Lapteva. 1979. Biology of oligotrophic bacteria. *Ann. Rev. Microbiol.* **33**: 377-387.
11. McFeters, G. A., B. H. Pyle, K. K. Siu, and Y. Egozy. 1993. Microorganisms: Bacterial ecology of operating laboratory water purification systems. *Ultrapure water* **September**: 32-37.
12. Michael, K., P. E. Patterson, G. R. Husted, and A. Rutkowski. 1991. Bacteria: isolation, identification, and microscopic properties of biofilms in high-purity water distribution systems. *Ultrapure Water* **May/June**: 18-24.
13. Poindexter, J. S. 1981. Oligotrophy: fast and famine existence. *Adv. Micro. Ecol.* **5**: 63-89.
14. Singh, R. P. and K. S. Yadav. 1990. Oligotrophic bacteria from barley rhizosphere. *Indian J. Microbiol.* **30**: 491-493.
15. Upton, A. C. and D. B. Nedwell. 1989. Nutritional flexibility of oligotrophic and copiotrophic antartic bacteria with respect to organic substrates. *FEMS Microbiol. Ecol.* **62**: 1-6.
16. Wainwright, M., F. Barakah, I. al-Turk, and T. Ali. 1991. Oligotrophic organisms in industry, medicine and the envirinment. *Sci. Prog.* **75**: 313-322.
17. Whang, K. and T. Hattori. 1988. Oligotrophic bacteria from rendzina forest soil. *Antonie van Leeuwenhoek J. Microbiol.* **54**: 19-37.
18. Yoshida, T., M. Ihmori, and J. Yamguchi. 1995. Oligotrophic bacteria isolated from clinical materials. *J. Clin. Microbiol.* **33**: 493-494.

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