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Effect of Environmental Factors on Growth Characteristics of The Ciliate Protozoan *Colpidium campylum*

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Abstract Ciliate protozoan (Hymenostomatida) *Colpidium campylum* is one of the typical protozoa found frequently in activated sludge and bio-film from waste-water treatment process. Effects of environmental factors on growth characteristics of *C. campylum* were investigated in this study. Using a bacterial strain *Acinetobacter calcoaceticus* as the food source, the effects of culture temperature, pH, phosphate buffer solution (PBS) concentration, shake stress and food concentration on the monoxenic growth rate of *C. campylum* were measured. Optimum temperature for *C. campylum* were 30℃. Activation energy estimated from Arrhenius plot was determined as 76.8 $kJ \cdot mol^{-1}$. PBS concentrations, pH conditions, bacterial concentrations were not serious for growth of *C. campylum* within the range which we investigated. The endurance of *C. campylum* against shake stress was higher than Peritrichida, Rotatoria and Oligochaeta. *C. campylum* was able to grow by feeding 7 bacterial strains within our 11 strains of investigations. Maximam specific growth rate (μmax) was 2.6 day^{-1}, and Ks value estimated from Monod kinetics was 20 $mg \cdot l^{-1}$.

Key words: Protozoa, *Colpidium campylum, Dexiostoma campyla,* Growth characteristics, Environmental factor.

The biological waste-water treatment processes generally utilize a complex ecosystem composed of bacteria, fungi, protozoa and small metazoa (Hynes, 1960; Bick, 1972; Curds *et al.*, 1975). In these processes, bacteria degrade polluting organic materials to carbon dioxide and other small molecules (Sudo and Aiba, 1984). Protozoa and small metazoa graze on the bacteria and, in turn, become prey for higher order species in food web such as fly larvae (Sudo and Aiba, 1984). Protozoa have been found in various types of biological waste-water treatment processes that are operated successfully (Ward, 1978; Brenda, 1984). There are some studies about the role of protozoa appearing on waste-water treatment processes (Taylor and Berger, 1976; Güde, 1979; Curds, 1982; Liang *et al.*, 1982; Sinclair and Alexander, 1984). However, these experimental studies have been restricted within few species. Therefore it is still unclear how they contribute to the waste-water treatment process.

We focused on ciliate protozoan (Hymenostomatida) *Colpidium campylum* (*Syn. Dexiostoma campyla*). This species is well known as frequently appearing in various kinds of biological waste-water treatment facilities. Furthermore this species has been taken up through physiological approach (Dive *et al.*, 1976, 1980; Foissner, 1977; Lynn and Sogin, 1988; Bonnemain and Dive, 1990; Greenwood *et al.*, 1991; Sekkat *et al.*, 1992). Nevertheless effects of environmental factors are still unsolved assignment. In the present paper, we shall try to clarify the effects of some environmental factors, such as water temperature, pH, qualities and quantities of bacteria as food and so forth, on growth characteristics of ciliate protozoan *Colpidium campylum.*

Materials and Methods

1. Protozoa strain

Colpidium campylum (Stokes, 1886) isolated from biofilm samples in a waste-water treatment plant, was used throughout this study. We picked it up by using capillary pipette from biofilm samples, and rinsed in Phosphate buffer solution (PBS) repeatedly until another potozoa is absent. Then monoxenic *C. campylum* strain had been replanted with known bacterial strains in PBS where any bacteria cannot grow (Inamori *et al.*, 1990).

C. campylum belongs to the phylum Ciliophora, class Oligohymenophorea, subclass Hymenostomatia, order Hymenostomatida, suborder Tetrahymenia, family Turaniellidae of Protozoa (Kahl, 1931). *Dexiostoma campyla* is a synonym of *C. campylum* (Foissner *et al.*, 1994). *C. campylum* is characterized by : a body size is about 50–120 μm in length and 20–45 μm in width, and body outline shape is oval with ventral side concave, dorsal surface convex, and oral aperture located about quarter of way down body in the concavity of the ventral surface, described as Figure 1. *C. campylum* preys bacteria and small protozoa, such as flagellate. *C. campylum* propagates asexually by means of transverse division.

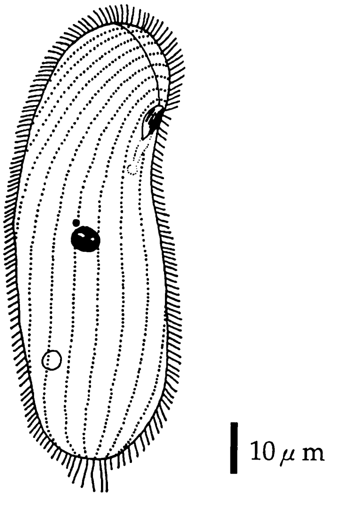

Fig. 1. Habit of Protozoa "*Colpidium campylum*".

2. Bacterial strains

The bacterial strains used as prey were obtained from the Institute of Molecular and Cellular Bioscience (IAM), University of Tokyo, Japan, and also from the Institute of Fermentation (IFO), Osaka, Japan. These strains of bacteria were well known as frequently isolated from biofilm and activated sludge sample taken from a waste-water treatment plant. The characteristics of these bacterial strain are shown in Table 1.

3. Experimental conditions

From preliminary growth experiments with *C. campylum*, we chose 1/750 M PBS, 20℃, Potential of hydrogen (pH) 6.5, dark and static conditions. We fed to *C. campylum* with complex of above known bacterial mixed resting cells for food, as basic condition for estimate various kind of environmental conditions as follows: (1) water temperature 5, 10, 15, 20, 25, 30, 35 and 40℃; (2) pH ranged form 4.2 to 9.7; (3) buffer concentration ranged from 0 to 40 mM; (4) bacterial species as food 11 bacterial strains shown in Table 1; (5) bacterial food concentration (initial bacterial concentration) ranged form 5 to 5,000 $mg \cdot l^{-1}$; (6) shake stress ranged from 0 (static) to 0.9 G by rotary and reciprocate shaker. Growth characteristics were tested in 5.6 cm diameter petri dish with initial 50 individuals of *C. campylum* by triplicated of batch cultures.

4. Growth measurements

The growth of *C. campylum* was monitored frequently during the 6 days incubation period, and the specific growth rates in the logarithmic phase of growth were calculated using the following equation:

$$\mu = 2.303 \log (N - N_0)/(t - t_0)$$

where μ = the specific growth rate (day^{-1}), N = the population number at time t (individuals ml^{-1}), and N_0 = the population number at time t_0 (individuals ml^{-1}).

Results and Disscussions

1. Water temperature

Water temperature is well known as im-

Table 1. Bacterial strains used as food for *C. campylum.*

Strain	Strain Lot.*	Gram stain	Cell	Oxygen Demand	Characeristics
Pseudomonas putida	IAM1002	Negative	Rod	Aerobe	Glugonate oxidation
Bacillus cereus	IAM1029	Positive	Rod	Aerobe	Endospore, *Bacillus cereus toxin*
Bacillus subtilis	IAM1069	Positive	Rod	Aerobe	Endospore
Escherichia coli	IAM1239	Negative	Rod	Facultative anaerobe	No endospore
Acinetobacter calcoaceticus	IAM1517	Negative	Rod	Aerobe	No movement, Catalase positive, Oxidase negative
Micrococcus luteus	IAM1313	Positive	Coccus	Aerobe	Catalase positive, Salt tolerant, Dry tolerant
Klebsiera pneumoniae	IAM1102	Negative	Rod	Facultative anaerobe	No movement, No endospore
Achromobacer cycloclastus	IAM1013	Negative	Rod	Aerobe	Nitrogen fixing abilitiy
Flavobacterium luteus	IAM1667	Negative	Rod	Aerobe	Catalase positive, Oxidase positive
Flavobacterium suaveolens	IFO3752	Negative	Rod	Aerobe	Catalase positive, Oxidase positive
Streptococcus acidominius	—	Positive	Coccus	Facultative anaerobe	Catalase negative, Oxidase negative

* IAM: Institite of Molecular and Cellular Bioscience (Univ. of Tokyo). IFO: Institute for Fermentation, Osaka. —: isoleted from biofilm.

portant environmental factor for growth characteristics of protozoa (Sudo and Aiba, 1984). Figure 2 shows the effect of temperature on growth of *C. campylum* ranged from 5 to 40℃ by using Arrhenius plot (Hall, 1953). *C. campylum* was acclimatized for each water temperature at every series of cultures. Figure 2 suggests that optimum water temperature condition was 30℃, and specific growth rate of *C. campylum* at 30℃ indicated 3.8 days^{-1}. Activation energy (Sudo and Aiba, 1984) calculated from linear relation-

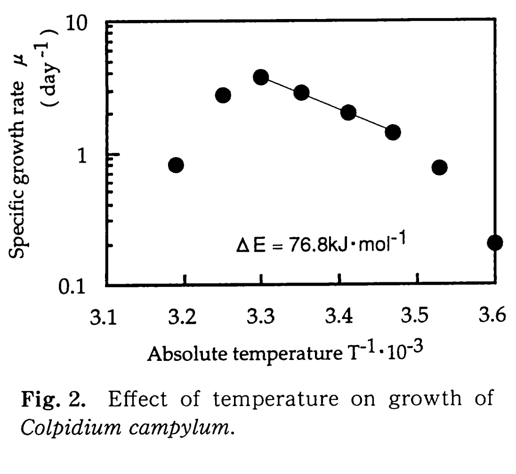

Fig. 2. Effect of temperature on growth of *Colpidium campylum.*

ship within the limits described in Figure 2 is 76.8 kJ·mol^{-1}. There are some approaches about activation energy. Because we do not have enough space to describe every thing about activation energy, we will introduce a few typical examples about frequently appearing species in waste-water treatment processes. Activation energy of Protozoan ciliata *Vorticella microstoma* (Peritrichida) and *V. convallaria* were reported as 76.8 kJ·mol^{-1} and 67.1 kJ·mol^{-1} respectively (Hayashi *et al.*, 1999). Similarly, activation energy of Oligochaeta *Aeolosoma hemprichi, Pristina longiseta*, and *Nais variabilis* were reported as 52.3 kJ·mol^{-1} 77.4 kJ·mol^{-1}, and 75.3 kJ·mol^{-1}, respectively. Similarly, activation energy of Rotifera *Phiolodina erythrophthalma, Rotatira rotatoria* and *Lecane luna* were reported as 74.9 kJ·mol^{-1}, 78.8 kJ·mol^{-1} 82.4 kJ·mol^{-1} respectively (Hayashi *et al.*, 1998). These data and length of linear relationship described in Fig. 2 suggest us sensitivity of *C. campylum* against water temperature is identical with Protozoan ciliata *Vorticella microstoma* (Peritrichida). It means *C. campylum* will be able to grow under wide ranged water temperature conditions, and *C.*

campylum is not sensitive rather than Rotifera *Rotatira rotatoria, Lecane luna* and Oligochaeta *Pristina longiseta.* This means that *C. campylum* will be able to appear in waste-water treatment processes regardless of water temperature condition.

2. pH

Conditions of pH are also well known as important environmental factor for growth characteristics of protozoa (Sudo and Aiba, 1984). Figure 3 shows the effect of pH on growth of *C. campylum* initial pH ranged from 4.2 to 9.7. *C. campylum* could grow every initial pH conditions within our investigation. Growth of protozoan ciliata *Vorticella microstoma* was restricted at pH under 5.2 and also over 8.2 conditions (Hayashi *et al.*, 1998). In the case of Oligochaeta, growth of *Aeolosoma hemprichi, Pristina longiseta* and *Nais variabilis* were restricted under pH 4.7 conditions (Kuniyasu *et al.*, 1997). On the other hand, growth of Rotatoria *Lecane luna* was restricted over pH 9.2 conditions (Hayashi *et al.*, 1998). These results suggest that pH condition is not important factor for growth of *C. campylum.* This means that *C. campylum* will be able to appear in waste-water treatment processes regardless of pH condition.

3. Buffer concentration

Figure 4 shows the effect of buffer concentration on growth of *C. campylum* ranged from 0 to 40 mM under pH 6.5 conditions. *C. campylum* could grow every conditions of buffer concentration within our investigation.

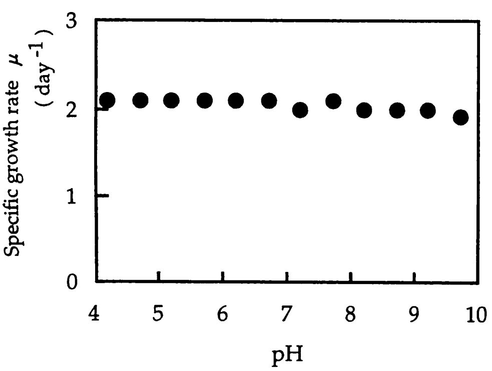

Fig. 3. Effect of initial pH condition on growth of *Colpidium campylum.*

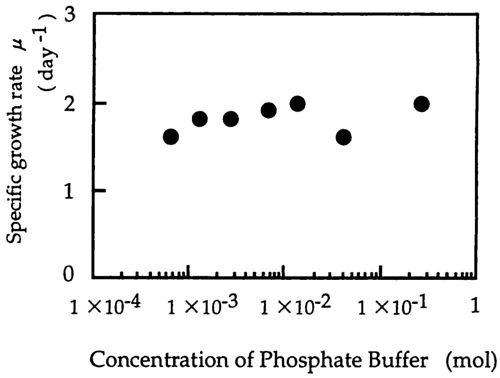

Fig. 4. Effect of phosphate buffer on growth of *Colpidium campylum.*

Growth of protozoan ciliata *Vorticella microstoma* was restricted at under 1.3×10^{-3} M and also over 4×10^{-3} M conditions (Hayashi *et al.*, 1999). In the case of Oligochaeta, growth of *Aeolosoma hemprichi, Pristina longiseta* and *Nais variabilis* were restricted over 1.3×10^{-2} M (*A. hemprichi*) or over 4×10^{-3} M (*P. longiseta* and *N. variabilis*) conditions (Kuniyasu *et al.*, 1997). On the other hand, Rotatoria *Philodina erythrophthalma, Rotaria rotatoria* and *Lecane luna* could grow every conditions as buffer concentration ranged from 0 to 40 mM under pH 6.5 conditions (Hayashi *et al.*, 1998). These results suggest us condition of buffer concentration is not important factor for growth of *C. campylum. C. campylum* is known as they could appear under high loading condition at waste-water treatment facilities (Curds *et al.*, 1975; Curds 1982). These facts support our result about high tolerance against wide ranged buffer concentrations.

4. Bacterial species as food

C. campylum was conducted using various strains of bacteria as prey to test the prey specificity. *C. campylum* could grow by feeding on the following 7 bacterial species as shown in Figure 5: *Escherichia coli, Pseudomonas putida, Acinetobacter calcoaceticus, Klebsiera pneumoniae, Flabobacterium suaveolens, Flavobacterium luteus,* and *Achromobacer cycloclastus.* The specific growth rate of *C. campylum* with these bacterial strain was in the ranged from 0.13 to 2.2 day^{-1}. Other four bacterial strains such as *Streptococcus*

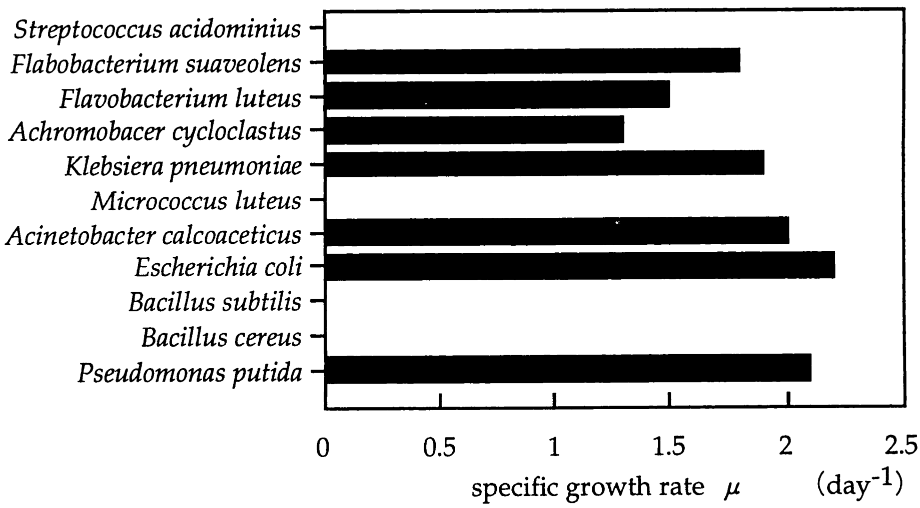

Fig. 5. Relationship between variety of bacterial strain as food source and growth rate of *Colpidium campylum.*

acidominius, Micrococcus luteus, Bacillus cereus, and *Bacillus subtilis*, could not become food for *C. campylum.* These results indicate *C. campylum* has its preference about food. In case of other researches about Oligochaeta (Kuniyasu *et al.*, 1997) or Rotatoria (Hayashi *et al.*, 1998), there are same liking about food. We try to consider some factor about bacteria such as bacterial size, bacterial shape, flocculate characteristics, and gram positive or negative, as a clue to elucidate of food preferences (Table 1). However we could not clarified about preferences in bacterial food. We have to research food preferences about many other protozoa and small metazoa as basic informations. Especially we will have to focus about metabolic materials from bacterial strains in next steps. After that, we will be able to clarify why some bacteria is not suitable for *C. campylum's* taste.

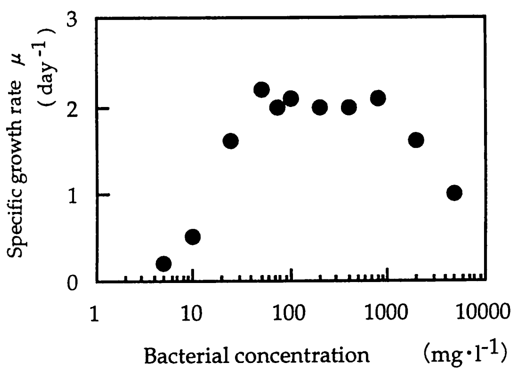

Fig. 6. Effect of bacterial food concentration on growth of *Colpidium campylum.*

5. Bacterial food concentration

Figure 6 shows the effect of bacterial food concentration on growth of *C. campylum.* Initial bacterial concentrations were ranged form 5 to 5,000 $mg \cdot l^{-1}$. There are hyperbolas relation between bacterial concentrations and specific growth rate of *C. campylum.* However an increase in the bacterial concentration beyond a critical level resulted in a decrease in specific growth rate unlike the situation predicted by conventional Monod kinetics. We tried to apply Monod kinetics about these hyperbolas relationship. Assuming the Monod type saturation model, maximum specific growth rate (μmax) and saturation constant (Ks) could be estimated from Lineweaver-Burk plots (Hayashi *et al.*, 1998) as follows:

$\mu max = 2.6\ day^{-1}$, $Ks = 20\ mg \cdot l^{-1}$

In the case of Protozoan *Vorticella microstoma* and *V. convallaria*, their μmax and Ks value were reported as 2.3 day^{-1}, 2.9 day^{-1} (μmax) and 33 $mg \cdot l^{-1}$, 38 $mg \cdot l^{-1}$ (Ks) respectively (Hayashi *et al.*, 1999). The Ks value of *C. campylum* is clearly low rather than *Vorticella microstoma* and also *V. convallaria.* This means that affinity of *C. campylum* for bacteria is lower than *Vorticella microstoma* and *V.*

convallaria. It will be able to explain from grazing impact. Namely *C. campylum* graze while moving by itself, but *Vorticella microstoma* and *V. convallaria* are able to draw food near themselves by using their cilia around the corona. In the case of Rotatoria *Philodina erythrophthalma, Rotaria rotatoria* and *Lecane luna*, their μmax and Ks value were reported as 0.54 day^{-1}, 0.50 day^{-1}, 0.19 day^{-1} (μmax) and 24 $mg \cdot l^{-1}$, 27 $mg \cdot l^{-1}$, 23 $mg \cdot l^{-1}$ (Ks) respectively (Hayashi *et al.*, 1998). These results support the discussion above. Because *Lecane luna* graze while swimming similar to *C. campylum*. On the other hand, other Rotatoria has two corona using for moving and feeding. This is a reason why Rotatoria achieves high Ks. Therefore bacterial food concentration is important factor for growth of *C. campylum*.

6. Shake stress

Both rotary and reciprocate shake intensities are converted for maximum acceleration. Figure 7 shows relationship between shake stress as maximum acceleration and specific growth rate of *C. campylum*. Maximum acceleration ranged form 0 to 0.94 G did not influence on specific growth rate of *C. campylum*. In the case of *Vorticella microstoma* and *V. convallaria*, specific growth rate are clearly decrease over 0.3 G shake stress conditions (Hayashi *et al.*, 1999). Furthermore growth activities were perfectly blocking over 0.6 G (*V. convallaria*) and 0.94 G (*V. microstoma*) shake stress conditions respectively (Hayashi *et al.*, 1999). Similarly, growth activities of Oligochaeta *Pristina longiseta* and *Nais variabilis* were perfectly blocking under 0.94 G shake stress conditions (Kuniyasu *et al.*, 1997). These results suggest that *C. campylum* is not sensitive about shake stresses as an environmental factor. This means that *C. campylum* will be able to appear in waste-water treatment processes regardless of shake stress condition.

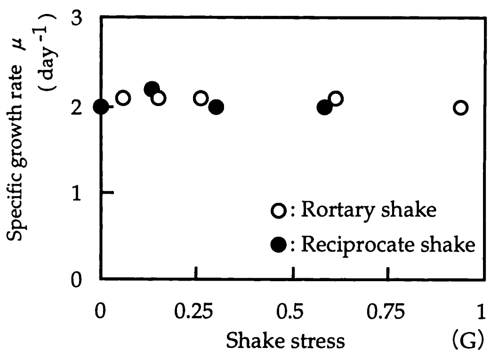

Fig. 7. Effect of shake stress on growth of *Colpidium campylum*.

Conclusion

Effect of environmental factors on growth characteristics of ciliate protozoan *Colpidium campylum* was investigated. Results can be summarized as follows; (1) *C. campylum* is able to grow on wide ranged water temperature within the waste-water ranged from 5 to 40°C. (2) Buffer concentration, pH condition and shake stress are not important factors for growth of *C. campylum*. (3) *C. campylum* could grow by feeding on 7 bacterial species, such as *Escherichia coli, Pseudomonas putida, Acinetobacter calcoaceticus, Klebsiera pneumoniae, Flabobacterium suaveolens, Flavobacterium luteus*, and *Achromobacer cycloclastu*, from 11 tested bacterial strains. (4) Variety and quantity of bacteria as food are important factors for growth characteristics of *C. campylum*. (5) Maximam specific growth rate (μmax) is 2.6 day^{-1}, Ks value estimated from Monod kinetics is 20 $mg \cdot l^{-1}$. (6) *C. campylum* can survive in wide ranged environmental conditions as described above. This is one of reasons why *C. campylum* is frequently appearing in waste-water treatment processes.

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原生動物 *Colpidium campylum* の増殖に及ぼす環境因子の影響

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原生動物織毛虫類に属する *Colpidium campylum* は，排水の生物処理施設において活性汚泥および生物膜中にしばしば大量に出現する微小動物として知られる．しかしながら，その増殖に及ぼす環境因子の影響に関しては明らかにされていない点が多い．そこで本研究では，その生理・生態学的諸特性にかかわる基礎

的知見を得ることを目的として実験的検討を行なった．得られた知見は次のようにまとめられる．(1) 水温の至適条件は，30℃であり，夏期の高水温時に増殖活性が高まるが，冬期の5℃程度の低水温時にも増殖は十分可能である．アレニウスプロットから算出した活性化エネルギーは，76.8 kJ·mol^{-1}である．(2) 塩濃度は，リン酸緩衝液濃度で0〜40 mMの範囲において増殖に大きな影響を及ぼさない．(3) pHは，4.2から9.7の範囲において増殖に大きな影響を及ぼさない．(4) 食物源としての細菌濃度の影響を，Monod式の適用できる範囲で計算すると，最大比増殖速度は2.6 day^{-1}，Ks値は20 mg·l^{-1}．(5) 攪拌強度への耐性は，原生動物縁毛類，袋形動物輪虫類，環形動物貧毛類に比較して高く，供試最大Gである9.4 Gまで増殖に全く影響を及ぼさない．(6) 食物源としての細菌の種類は，供試細菌11種の内，*Pseudomonas putida, Escherichia coli, Acinetobacter calcoaceticus, Klebsiera pneumoniae, Achromobacer cycloclastus, Flavobacterium luteus, Flavobacterium suaveolens*の7種の単独細菌を用いた培養で増殖可能であり，*Bacillus cereus, Bacillus subtilis, Streptococcus acidominius, Micrococcus luteus*の4種の単独細菌では増殖できなかった．