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HORIZONTAL TRANSFER OF PLASMID DNA BETWEEN DIFFERENT BACTERIA SPECIES UNDER MICROBIAL INTERACTIONS

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ABSTRACT: Horizontal transfer of plasmid DNA was investigated under phytoplankton metabolites / zooplankton predation exposure condition, to obtain some basic information about the prosperity and decay of GMO (genetically modified microorganisms) in filed release was investigated in this study. *Escherichia coli* HB101, *E.coli* C600, *E.coli* S17-1, *Pseudomonas aeruginosa* PAO1 (gram-negative) and *Bacillus cereus* MC (gram-positive) as recipient strain of plasmid DNA, *E.coli* HB101/pBR325, *E.coli* C600/RP4 and *E.coli* S17-1/pSUP104 as donor of plasmid DNA, were supplied. As phytoplankton, *Microcystis aeruginosa* (cyanophyceae), *Melosira varians* (bacillariophyceae) and *Scenedesmus quadricauda* (chlorophyceae) collected from Lake Tega as donor of metabolites, were supplied. As zooplankton, *Tetrahymena pyriformis* (cilliata) and *Philodina erythrophthalma* (rotifer) collected from Lake Tega as predator were supplied. The results can be concluded as follows; 1) Phytoplankton metabolites leads acceleration of horizontal transfer between not only same strains but also different strain in spite of whether transmissible or not, 2) Zooplankton predation leads decrease of bacterial individual number and horizontal transfer of plasmid DNA, and 3) Horizontal plasmid DNA transfer is influenced greatly, because the natural ecosystem includes phytoplankton as producer and zooplankton as consumer in the same time.

Keywords: plasmid DNA, horizontal transfer, phytoplankton, zooplankton, eutrophicated lake, bioremediation

1. INTRODUCTION

The practical utilization of GMO (genetically modified microorganism) has been in real, and some GMO is released in market in fact, such as microbial pesticides and so on. The environmental effect of GMO has been much discussed in these 30 years, such as the prosperity and decay, i.e., fate of GMO in a case of the field release [1][2]. However, how not only the genetically modified microorganisms but also the modified gene behave in the environment, how the impact to the environment is given, is not still made clear [3]-[8].

Horizontal transfer of plasmid DNA between different bacterial species was investigated in this study, under phytoplankton metabolites and zooplankton predation exposure condition, to obtain some basic information about the prosperity and decay of GMO in filed release such as bioremediation technology.

2. MATERIALS AND METHODS

2.1 Bacterial Strains

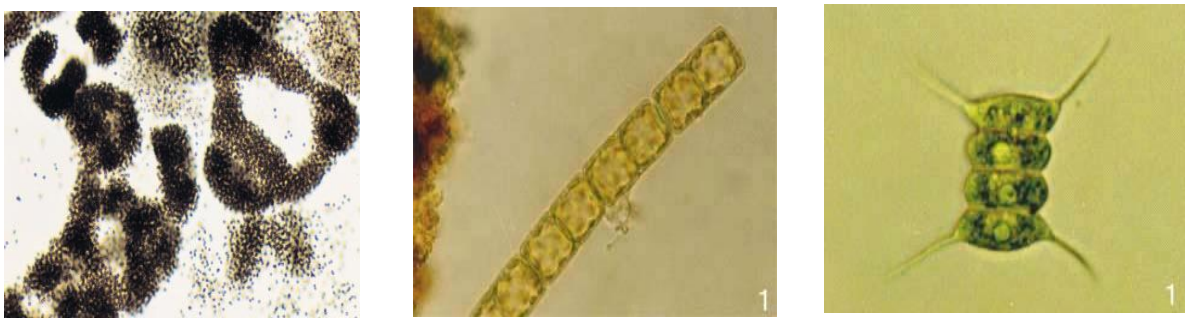
Escherichia coli HB101, *E.coli* C600, *E.coli* S17-1, *Pseudomonas aeruginosa* PAO1 (gram-negative) and *Bacillus cereus* MC (gram-positive) as recipient strain of plasmid DNA, *E.coli* HB101/pBR325, *E.coli* C600/RP4 and *E.coli* S17-1/pSUP104 as donor of plasmid DNA, were supplied. The appearance of these banteria is shown in Photo 1. Plasmid DNA pBR325 (Cm^r, Tc^r, Ap^r) is non-transmissible, RP4 (Ap^r, Tc^r, Km^r) is transmissible, and pSUP104 (Cm^r, Tc^r) is mobilized transmissible, respectively [1].

2.2 Phytoplankton Strains

Microcystis aeruginosa (cyanophyceae), *Melosira varians* (bacillariophyceae) and *Scenedesmus quadricauda* (chlorophyceae) as donor of metabolites, were supplied. The appearance of these phytoplankton were shown in Photo 2. These phytoplankton were collected from Lake Tega which is well known as one of the most eutrophicated lakes in Japan, and the occurrence of water bloom (Aoko) is observed in summer. *M.aeruginosa* is one of the dominant species in summer, *M.varians* is in autumn and winter, and

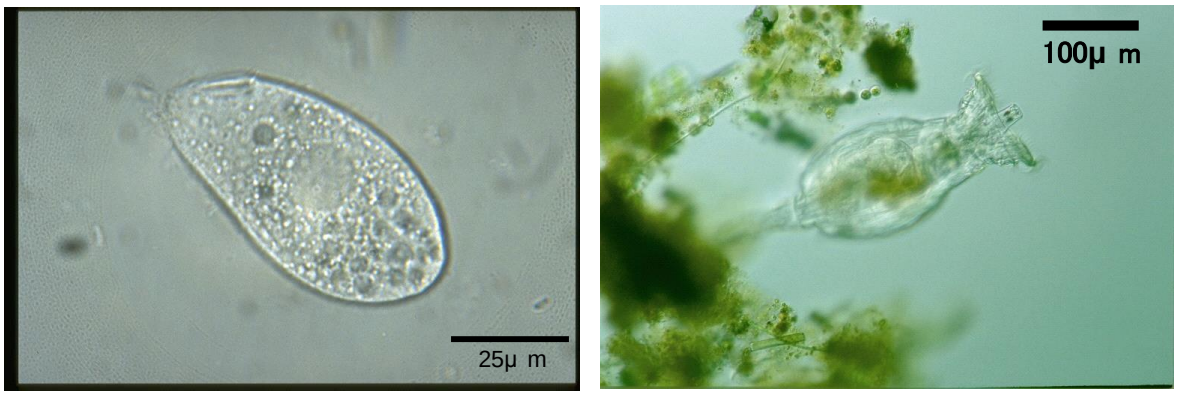
a) *Escherichia coli* (gram negative)
b) *Pseudomonas aeruginosa* (gram negative)
c) *Bacillus cereus* (gram positive)

Photo 1 Bacterial strains supplied in this study (colony on agar plate)



a) *Microcystis aeruginosa* (cyanophyceae)
b) *Melosira varians* (bacillariophyceae)
c) *Scenedesmus quadricauda* (chlorophyceae)

Photo 2 Phytoplankton strains supplied in this study



a) *Tetrahymena pyriformis* (ciliata)
b) *Philodina erythrophthalma* (rotifer)

Photo 3 Zooplankton strains supplied in this study

S.quadricauda is in spring [9][10].

2.3 Zooplankton Strains

Tetrahymena pyriformis (cilliata) and *Philodina erythrophthalma* (rotifer) as predator were supplied. The appearance of these zooplankton were shown in Photo 3. These zooplankton were collected from Lake Tega which is well known as one of the most eutrophicated lakes in Japan. *T.pyriformis* and *P.erythrophthalma* are ordinary observed in not only Lake Tega but also Japanese lakes and marshes through a year [11][12].

2.4 Experimental Procedure

Each bacterial strain was pre-cultured in liquid culture medium under dark, 30℃ and shaking condition for 12hr. with and/or without antibiotics, and the log-phased bacteria was collected by centrifugation. Each phytoplankton strains was pre-cultured under light (20,000lux), 30℃ and non-shaking condition for 2 weeks in modified M11 basic medium. Each zooplankton strains was pre-cultured under dark condition and 20℃ for 5 days and/or 2 weeks.

Pre-cultured each bacterial strain was supplied in combination of experimental series to liquid culture medium containing each phytoplankton metabolite adjusted to phased concentration (dilution rate; ×1, ×2, ×10) and/or polypeptone to investigate the effect of phytoplankton metabolite on plasmid DNA transfer, and containing each zooplankton species (initial individual number; 100 N・ml^{-1} in *T.pyriformis*, 10 N・ml^{-1} in *P.erythrophthalma*) to investigate the effect of zooplankton predation on plasmid DNA transfer.

Plasmid transfer culturing was conducted under dark, 30℃ and shaking condition, considering with the maximum growth rate of bacteria. Colony forming units (CFU) was counted by selection medium plate containing each antibiotic, and transfer rate was calculated to estimate the phytoplankton/zooplankton effect on plasmid DNA transfer.

3. RESULTS AND DISCUSSION

3.1 Effect of Phytoplankton Species on Plasmid DNA Transfer

As results, about the effect of phytoplankton species, in the case of same bacterial strain, that is, the combination of i) *E.coli* HB101 + *E.coli* HB101/pBR325, ii) *E.coli* C600 + *E.coli* C600/RP4, iii) *E.coli* S17-1 + *E.coli* S17-1/pSUP104, the effect of acceleration of horizontal plasmid DNA transfer was higher in *M.aeruginosa* metabolite and lower in *S.quadricauda* metabolite, which shows the influence is different in different phytoplankton species. In the case of different bacterial strain, that is, the combination of iv) *E.coli* HB101/pBR325 + *P.aeruginosa* PAO1, v) *E.coli* HB101/pBR325 + *B.cereus* MC, vi) *E.coli* C600/RP4 + *P.aeruginosa* PAO1, vii) *E.coli* C600/RP4 + *B.cereus* MC, viii) *E.coli* S17-1/pSUP104 + *P.aeruginosa* PAO1, ix) *E.coli* S17-1/pSUP104 + *B.cereus* MC, the effect of acceleration of horizontal plasmid DNA transfer was higher in *M.aeruginosa* and lower in *M.varians* and *S.quadricauda*, which shows the influence is also different in different phytoplankton species to different bacterial strains. The plasmid DNA transfer rate (%) in each combination of donor and recipient bacteria were shown in Table 1.

The horizontal transferring mechanism between different bacterial species such as gram-negative and gram-positive is not made clear. However, the possibility of outer releasing of plasmid DNA from donor bacterial body after its death and/or body solution, and intaking into recipient bacterial body during its growth, were considered. Form these outcomes, it was made clear that phytoplankton metabolites leads acceleration of horizontal transfer between not only same strains but also different strain in spite of whether transmissible or not.

3.2 Effect of Metabolite Concentration on Plasmid DNA Transfer

The effect of metabolite concentration was also investigated. In the case of same bacterial strain, the horizontal plasmid DNA transfer was accelerated under the highest concentration exposure of *M.aeruginosa* metabolite. The other hand, polypeptone, instead of phytoplankton metabolite, did not show any influence on the transfer rate. This indicates that horizontal plasmid DNA transfer is most frequent in summer season when water bloom, that is, Aoko is occurred. The plasmid DNA transfer rate (%) in each metabolite concentration were shown in Table 2.

The horizontal plasmid DNA transfer was also accelerated under the highest concentration exposure of *M.aeruginosa* metabolite between different bacterial strains. Polypeptone did not show any influence in this case. This indicates that not only the quantity but also the quality of phytoplankton metabolite influences much to horizontal transfer of plasmid DNA. In addition, the possibility that GMO which holds artificially modified DNA, survive with changing the host strain in eutrophicated lake where water bloom such as *M.aeruginosa* occurs in summer season, was suggested.

Table 1 Effect of phytoplankton metabolite on plasmid DNA transferring rate

	M.aeruginosa	*M.varians*	*S.quadricauda*	polypeptone
E.coli HB101/pBR325 + *E.coli* HB101	13.4	11.8	5.7	3.0
E.coli C600/RP4 + *E.coli* C600	98.3	92.7	89.1	90.0
E.coli S17-1/pSUP104 + *E.coli* S17-1	85.7	69.1	60.1	67.4
E.coli HB101/pBR325 + *P.aeruginosa* PAO1	14.2	9.3	6.7	1.2
E.coli HB101/pBR325 + *B.cereus* MC	6.4	3.4	1.6	1.1
E.coli C600/RP4 + *P.aeruginosa* PAO1	72.2	54.8	42.6	45.2
E.coli C600/RP4 + *B.cereus* MC	8.8	6.1	2.6	2.1
E.coli S17-1/pSUP104 + *P.aeruginosa* PAO1	80.1	67.1	67.0	68.4
E.coli S17-1/pSUP104 + *B.cereus* MC	9.1	7.2	2.9	2.2

(unit : %)

Table 2 Effect of metabolite concentrations on Plasmid DNA transferring rate

	×1	×2	×10	polypeptone
E.coli HB101/pBR325 + *E.coli* HB101	13.4	4.1	2.1	3.0
E.coli C600/RP4 + *E.coli* C600	98.3	93.2	90.5	90.0
E.coli S17-1/pSUP104 + *E.coli* S17-1	85.7	68.8	65.1	67.4
E.coli HB101/pBR325 + *P.aeruginosa* PAO1	14.2	6.8	1.4	1.2
E.coli HB101/pBR325 + *B.cereus* MC	6.4	2.1	1.1	1.1
E.coli C600/RP4 + *P.aeruginosa* PAO1	72.2	65.1	45.9	45.2
E.coli C600/RP4 + *B.cereus* MC	8.8	7.1	4.6	2.1
E.coli S17-1/pSUP104 + *P.aeruginosa* PAO1	80.1	69.2	68.7	68.4
E.coli S17-1/pSUP104 + *B.cereus* MC	9.1	3.2	3.1	1.7

(unit : %)

Table 3 Effect of zooplankton predation on Plasmid DNA transferring rate

	Tetrahymena pyriformis			*Philodina erythrophthalma*		
	μ max	Nmax	transfer rate	μ max	Nmax	transfer rate
E.coli HB101/pBR325 + *E.coli* HB101	4.0	51,000	1.5	0.36	3,100	1.7
E.coli C600/RP4 + *E.coli* C600	4.1	50,000	67.1	0.35	3,100	76.2
E.coli S17-1/pSUP104 + *E.coli* S17-1	4.0	50,000	55.7	0.31	3,000	61.8
E.coli HB101/pBR325 + *P.aeruginosa* PAO1	4.0	48,000	1.7	0.32	3,100	1.8
E.coli HB101/pBR325 + *B.cereus* MC	3.8	46,000	1.9	0.28	2,800	1.8
E.coli C600/RP4 + *P.aeruginosa* PAO1	4.0	48,000	54.2	0.33	3,000	55.9
E.coli C600/RP4 + *B.cereus* MC	3.8	46,000	10.5	0.28	2,800	9.8
E.coli S17-1/pSUP104 + *P.aeruginosa* PAO1	3.9	46,000	49.2	0.30	2,900	46.3
E.coli S17-1/pSUP104 + *B.cereus* MC	3.7	47,000	11.6	0.30	2,600	10.2
	(unit : day^{-1})	(unit : N/ml)	(unit : %)	(unit : day^{-1})	(unit : N/ml)	(unit : %)

3.3 Effect of Zooplankton Species on Plasmid DNA Transfer

As results, about the effect of zooplankton species, in the case of same bacterial strain, that is, the combination of i) *E.coli* HB101 + *E.coli* HB101/pBR325, ii) *E.coli* C600 + *E.coli* C600/RP4, iii) *E.coli* S17-1 + *E.coli* S17-1/pSUP104, *T.pyriformis* and *P.erythrophthalma* grew rapidly in all cases. All bacteria decreased in their individual number as food source for *T.pyriformis* and *P.erythrophthalma*. In the case of different bacterial strain, that is, the combination of iv) *E.coli* HB101/pBR325 + *P.aeruginosa* PAO1, v) *E.coli* HB101/pBR325 + *B.cereus* MC, vi) *E.coli* C600/RP4 + *P.aeruginosa* PAO1, vii) *E.coli* C600/RP4 + *B.cereus* MC, viii) *E.coli* S17-1/pSUP104 + *P.aeruginosa* PAO1, ix) *E.coli* S17-1/pSUP104 + *B.cereus* MC, the predator *T.pyriformis* and *P.erythrophthalma* grew rapidly in all cases. All bacteria decreased in their individual number as food source for *T.pyriformis* and *P.erythrophthalma*. The specific growth rate (μ) and the maximum individual number (N_{max}) of each zooplankton as predator and the plasmid DNA transfer rate (%) in each combination of donor and recipient bacteria were shown in Table 3.

As described above, all bacterial strains were good food source for *T.pyriformis* and *P.erythrophthalma*, in any culture combination.

3.4 Effect of Predation on Plasmid DNA Transfer

As results, about the effect of zooplankton species, in the case of same bacterial strain, that is, the combination of i) *E.coli* HB101 + *E.coli* HB101/pBR325, ii) *E.coli* C600 + *E.coli* C600/RP4, iii) *E.coli* S17-1 + *E.coli* S17-1/pSUP104, *T.pyriformis* and *P.erythrophthalma* grew rapidly in all cases. All bacteria decreased in their individual number as food source for *T.pyriformis* and *P.erythrophthalma*. In the case of different bacterial strain, that is, the combination of iv) *E.coli* HB101/pBR325 + *P.aeruginosa* PAO1, v) *E.coli* HB101/pBR325 + *B.cereus* MC, vi) *E.coli* C600/RP4 + *P.aeruginosa* PAO1, vii) *E.coli* C600/RP4 + *B.cereus* MC, viii) *E.coli* S17-1/pSUP104 + *P.aeruginosa* PAO1, ix) *E.coli* S17-1/pSUP104 + *B.cereus* MC, *T.pyriformis* and *P.erythrophthalma* grew rapidly in all cases. All bacteria decreased in their individual number as food source for *T.pyriformis* and *P.erythrophthalma*.

As described above, all bacterial strains were good food source for *T.pyriformis* and *P.erythrophthalma*, in any culture combination. Same results were obtained in the case of one bacterial strain containing different kind of plasmid DNA, and the case of some different kind of host bacterial strain containing one same plasmid DNA as food source for ciliate *T.pyriformis* and *Colpidium campylum*, rotifer *P.eruthrophthalma* and oligochaeta *Aeolosoma hemprichi* as predator [1]. This result suggests that the apitude for food source of micro animals is dependent on the kind of host bacterial strain not on the kind of DNA information coding on plasmid. From these outcomes, it was made clear that zooplankton predation leads decrease of bacterial individual number and horizontal transfer of plasmid DNA. On the other hand, ciliates rapidly enhance the frequency of conjugation between *E.coli* strains through bacterial accumulation in vesicles is reportrd [6][8]. More information should be obtained to discuss the prosperity and decay of GMO in natural ecosystem.

4. CONCLUSIONS

This study was conducted to investigate horizontal transfer of plasmid DNA under phytoplankton metabolites and zooplankton predation exposure condition, to obtain some basic information about the prosperity and decay of GMO in filed release. The results can be concluded as follows;

1) Phytoplankton metabolites leads acceleration of horizontal transfer between not only same strains but also different strain in spite of whether transmissible or not.

2) Zooplankton predation leads decrease of bacterial individual number and horizontal transfer of plasmid DNA.

3) Plasmid DNA transferring between different bacterial strains is influenced greatly by biological interaction, because the natural ecosystem includes phytoplankton as producer and zooplankton as consumer in the same time.

4) Monitoring of the prosperity and decay of not only the genetically engineered microorganisms but also the modified gene itself is necessary for wise use of bioremediation technology.

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