Short Communication

Competency Development of *Escherichia coli* in Foodstuffs

SUMIO MAEDA^{1*}, NOZOMI KAKIHARA^{1**} and YUMI KOISHI^{1**}

¹ Food Safety Laboratory, Faculty of Human Life and Environment, Nara Women's University, Kitauoyanishi-machi, Nara 630–8506, Japan

(Received November 25, 2002—Accepted April 28, 2003)

We report here that certain kinds of foods can act as media that induce genetic competency of *Escherichia coli*. Of 42 food samples tested, more than 10 showed an ability to induce competency. Among them, tofu supernatant showed the highest activity, corresponding to about one half of the activity obtained with 100 mM CaCl₂. These results suggest that some populations of *E. coli* in food environments exist as “competent cells”. There were no clear correlations between transformation frequencies and the chemical indices of foods (Ca²⁺ or Mg²⁺ concentrations and pH), suggesting that complex factors in foods affect competency development.

Key words: *Escherichia coli*, genetic competency, transformation, food

Human foods are good culture media for many bacteria. However, little attention has been given to the effects of foods on bacterial physiology other than growth and survival. In this study, we investigated the possibility that foods act as media for bacterial genetic transformation. Foods often contain millimolar concentrations of divalent metal ions (Ca²⁺, Mg²⁺, etc.). In addition, foods are often stored in a refrigerator or freezer, and warmed rapidly (“heat shocked”). It should be noted that these conditions are conducive to the development of genetic competency in *E. coli*^{3,5,7,8}. This idea was partly demonstrated by Bauer *et al.*², who showed that plasmid transformation of *E. coli* could occur in several kinds of foodstuffs². Transformation of *E. coli* in drinking water obtained from natural springs was also demonstrated by Baur *et al.*³. To further test this idea, we have examined the abilities of 42 kinds of foods to induce transformation of *E. coli* under temperature conditions that are used in daily food manipulations.

Amp, tet, kan, PBS tablets, and LB (Luria Bertani, Lennox) powder were purchased from Sigma (USA). Distilled water (molecular biology grade) was purchased from Invitrogen (USA). Other general reagents were obtained from Wako (Japan). All the food samples used in this study were obtained from daily commercial sources in Japan. For convenience, most of them were either beverages or semi-liquids in bottles or cartons, and several were instant liquid foods or drinks that were prepared from dry powders or granules. In this report, we do not identify the brand names of the foods because of the possibility that our results could be mis-interpreted by the public, however, we are ready to offer this information on request to individual researchers.

For the transformation assay, we used tet- and kan-resistant *E. coli*, SURETM (Stratagene, USA) as the host strain, and amp-resistant plasmid pBluescript KS- (Stratagene) as the donor DNA. Plasmid DNAs for transformation were prepared by the conventional alkali-lysis method⁸. DNA concentrations of plasmid solutions were measured by fluorometer (DyNA Quant 200, Pharmacia, USA) with Hoechst 33342. Development of competency was measured by the appearance of triple-resistant transformants (tet^r, kan^r, amp^r). Because triple-resistant bacteria are rare, the

* Corresponding author; E-mail: smaeda@cc.nara-wu.ac.jp, Tel/Fax: +81–742–20–3967

** These authors were contributed equally to this work.

Abbreviations: PBS, phosphate-buffered saline; amp, ampicillin; kan, kanamycin; tet, tetracycline.

procedures for sterilizing the food samples used in this study could be kept to a minimum. We confirmed that there were no contaminants in the resulting transformants by the isolation of plasmids and restriction-enzyme analysis (data not shown). Each food sample was prepared as follows. Dry powders or granules of miso soup (19 g/200 ml), soupstock (2 g/300 ml), curry soup (3 g/175 ml), instant coffee (2.5 g/150 ml), and cocoa (4 g/140 ml) were dissolved in distilled water, and the resulting solutions were autoclaved (120°C, 15 min). Green tea and black tea were extracted by immersing a tea bag in 200 ml of hot water (90°C) for 2 min, and the resulting extracts were autoclaved (120°C, 15 min). All other food samples in bottles or cartons were used without further sterilization. Tomato and vegetable juices were centrifuged (8000 g, 10 min) once and the supernatants were used. Tofu supernatant was prepared by the freezing (−50°C, 12 hr), thawing (25°C, 1 hr), and subsequent centrifugation (8000 g, 10 min) of a tofu block, which was sealed in a plastic package. All the prepared samples were stored at 4°C, and used within 2 weeks. The concentrations of Ca²⁺ and Mg²⁺ in the food samples were determined with AquaMerck kits (Merck, Germany). The pH of food samples was measured with a pH meter. The data on Ca²⁺, Mg²⁺ and pH are summarized in Table 1. In the following parts and figures, we indicate samples by their names and/or the number described in Table 1.

The standard transformation protocol we constructed is as follows. In order to simulate the conditions in ordinary food manipulations as much as possible, we adopted 25°C both for preparing cultures of competent cells and for heat shock, instead of the usual temperatures of 37°C and 42°C, respectively. A freeze-stock of SURE (100 µl) was inoculated into 120 ml of LB broth containing 30 µg/ml kan and cultured with shaking until OD₆₀₀=0.5 (for 14–16 h) at 25°C. Cells were collected by centrifugation (5000 g, 7 min) and washed once with PBS (1.47 mM potassium phosphate monobasic, 8.1 mM sodium phosphate dibasic, 2.7 mM potassium chloride and 137 mM sodium chloride, pH 7.4 at 25°C). The cells were resuspended in 5 ml of PBS, and aliquots containing 3.5×10⁹ cells were put into 1.5 ml Eppendorf tubes. After a brief centrifugation (5000 g, 1 min) at 4°C and removal of the supernatant, ice-cold food samples (1 ml) were added to each tube, and the pellets were resuspended by vortexing. To develop competency, the cell suspensions were incubated at 4°C for 60 min, mixed with 2 µg of plasmid DNA (pBluescript), incubated at 4°C for 20 min, and then incubated at 25°C for 10 min for heat shock. After a brief centrifugation and removal of the supernatant, 400 µl of LB was added to the cell pellet, and

Table 1. Ca²⁺ and Mg²⁺ concentrations and pH of food samples.

no.	food samples	Ca (mM)	Mg (mM)	pH
1	natural water (E) P ^a	2.6	0.1	7.7
2	natural water (E) E ^a	1.6	1.4	7.9
3	natural water (E) V ^a	0.3	0.4	8.1
4	natural water (A) C ^a	0.1	0.6	8.1
5	natural water (J) M ^a	0.2	0.3	8.1
6	natural water (J) R ^a	0.5	0.4	8.3
7	natural water (J) I ^a	0.1	0.0	6.9
8	natural water (J) A ^a	0.1	0.4	8.1
9	deep ocean water U ^a	1.8	12.4	6.8
10	deep ocean water M ^a	0.1	0.3	7.1
11	deep ocean water H ^a	0.6	3.0	7.5
12	black tea	1.0	0.6	5.2
13	green tea (extracted)	0.0	0.6	5.3
14	green tea (bottled)	0.5	0.0	6.4
15	isotonic drink P	0.5	0.1	3.9
16	isotonic drink A	5.0	2.7	3.8
17	isotonic drink E	0.6	1.5	3.9
18	milk	32.0	16.4	6.8
19	Calcium-enriched milk	52.4	3.0	6.9
20	skim milk	29.9	3.6	6.7
21	Calcium-enriched skim milk	28.4	8.3	6.6
22	coffee milk	14.1	5.9	6.7
23	fruits milk	11.1	3.1	4.9
24	half-and-half	6.1	1.1	7.3
25	carbonated drink C	1.2	0.5	3.1
26	carbonated drink S	5.0	0.3	3.6
27	carbonated water	0.9	0.6	4.7
28	orange juice	4.8	0.8	4.4
29	apple juice	4.3	0.7	4.1
30	vegetable-mixed juice	3.1	6.2	4.7
31	tomato juice	1.5	3.9	4.7
32	Calcium-added drink T	25.3	0.1	3.9
33	Calcium-added drink C	18.5	0.8	4.3
34	instant coffee	0.5	3.4	4.9
35	cocoa	5.0	0.6	6.7
36	soy sauce	41.9	4.6	4.8
37	miso soup	1.8	1.7	5.2
38	tofu supernatant	1.8	16.2	5.9
39	soy drink	2.5	10.0	6.7
40	worcester sauce	22.5	18.3	3.9
41	soupstock	0.6	0.2	6.8
42	curry soup	0.8	0.8	5.6

^a No. 1–3 are European waters (all from France), no. 4 is a USA water, no. 5–8 are Japanese waters (no. 5: from Yamanashi, no. 6: from Kobe, no. 7: from Niigata, and no. 8: from Nagano), and no. 9–11 are deep ocean waters (no. 9, 10: from Kochi, Japan and no. 11: from Hawaii, USA).

the samples were incubated at 37°C for 45 min for gene expression. Then aliquots of the samples were plated onto LB-agar plates containing 100 µg/ml amp, 30 µg/ml kan, and 25 µg/ml tet. The plates were incubated at 37°C for 18–22 h and the colonies that appeared were counted. Averages and standard deviations for at least three similar experiments are shown in Figs. 1 and 2.

Baur *et al.*⁽³⁾ demonstrated that several commercially available natural drinking waters had modest abilities to develop competency of *E. coli*. We tested waters, which were derived from Japan (no. 5–8 in Table 1 and Fig. 1) or the USA (no. 4), and deep-ocean waters (no. 9–11), in addition to European waters (no. 1–3) that were similar to those used by Baur *et al.*⁽³⁾. It was revealed that not only European waters but also US and deep-ocean waters showed modest transformation activities (Fig. 1b). Their activities were around one fiftieth of the activity obtained with 100 mM CaCl₂ (no. 43, Fig. 1a). On the other hand, Japanese waters appeared to show less activity, although the differences

between Japanese and other waters were not significant.

Next, we tested 24 samples of beverages (no. 12–35), and found that 3 isotonic drinks (no. 15–17), 2 carbonated drinks (no. 26, 27), 3 juices (no. 29–31), and 1 calcium-added drink (no. 32) showed abilities to induce competency (Fig. 1b). Their activities ranged from about one fiftieth to one seventh of the activity obtained with 100 mM CaCl₂ (no. 43, Fig. 1a). The results for the 3 juices are consistent with the results of Bauer *et al.*⁽²⁾. However, milk samples (no. 18–24) showed little activity, which is inconsistent with the results of Bauer *et al.*⁽²⁾.

We also tested several Japanese soy foods (no. 36–39), as well as several popular seasoning solutions (no. 40–42). Although most of them showed little or no activity, tofu supernatant (no. 38, Fig. 1a) showed a very high level of activity, more than half that obtained with 100 mM CaCl₂.

In laboratory conditions, it was reported that Ca²⁺ ion plays an important role in the competency development of *E. coli*^(5,7,8). Mg²⁺ ion was also reported to induce competency, although its activity was weaker than that of Ca²⁺ ion^(3,5). In Fig. 2a and 2b, we sorted the transformation data in Fig. 1 using the data on Ca²⁺/Mg²⁺ concentrations in Table 1, but there were no obvious correlations between Ca²⁺/Mg²⁺ concentrations and transformation frequencies.

Under laboratory conditions, pH was also reported to influence transformation of *E. coli*^(1,6). Sorting of the data in Fig. 1 using the data on pH in Table 1 (Fig. 2c) revealed that there were no simple linear correlations between pH values and transformation frequencies in the pH range 3–8. However, active samples that bore more than 10 transformants (no. 26, 16, 32, 15, 17, 29, 31, and 27) tended to gather in the pH range 3–4, implying the presence of some effect of low pH. Nevertheless, there were many exceptions: *e.g.*, the most active sample (no. 38) had a higher pH (5.9), and there were many low-activity samples (no. 25, 40, 33, 28, 36, 23, 34) at pH 3–4. Therefore, based only on this data, we could not draw a conclusion as to the effect of pH.

E. coli is a normal component of the intestinal flora of humans and animals. Because it is also a common food contaminant, it is of interest to determine whether *E. coli* can be transformed in foods. Our results have demonstrated that at least certain kinds of popular foods can be transformation media for *E. coli*. Notably, tofu supernatant showed prominent activity, which was comparable to half the activity of 100 mM CaCl₂ under our experimental conditions (Fig. 1). Our results as well as the results of Bauer *et al.*⁽²⁾ suggest that some populations of *E. coli* in food environments exist as “competent cells”. It should be noted that a similar effect of foods to conduct transformation has been

Fig. 1. Transformation of *E. coli* in 42 foodstuffs. For convenience, the same data were presented in panel a (on a large scale) and panel b (on a small scale). Numbers along the X axis are sample numbers that correspond to those in Table 1. No. 43, 44, and 45 are 100 mM CaCl₂ (positive control), PBS (negative control), and H₂O (negative control), respectively.

Fig. 2. Correlations between Ca^{2+} concentration (a)/ Mg^{2+} concentration (b)/ pH (c) and transformation frequencies. Numbers on each X axis are sample numbers that correspond to those in Table 1.

reported with *Bacillus Subtilis*^{4,9)}. Therefore, transformation in foods could occur with other bacteria to a greater or lesser degree. Whether this process is actually involved in the genetic changes to bacteria in human living environments is a subject worth investigating.

The concentrations of Ca^{2+} and Mg^{2+} in the foodstuffs were not correlated with the transformation frequencies (Fig. 2a, 2b). Bauer *et al.*²⁾ reported a similar result. This may be because foods usually contain complex constituents

and additives, many of which could affect physiological functions of *E. coli*. Competency development would also be influenced by numerous factors in foods, directly or indirectly, and positively or negatively. The data in Fig. 2c implied that the pH of foods may have some relevance to competency development. At present, however, we do not have enough data to draw a definite conclusion. More detailed studies are needed to elucidate what chemical factors in foods promote or suppress competency development of *E. coli*, and by what mechanisms they act on *E. coli* cells. These analyses will also help to clarify by what mechanisms *E. coli* uptakes extracellular DNAs.

Acknowledgements

This work was supported by a grant from the Sumitomo Foundation.

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