Note

Use of a Tumbling-Defective Mutant of *Escherichia coli* for Evaluation of Pollutant Toxicity by Computer-Assisted Image Analysis of Bacterial Motion

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For the evaluation of pollutant toxicity in the aquatic environment, a novel principle of a dynamic image analysis of bacterial motion by using a tumbling-ability-defective mutant of *Escherichia coli* K-12, OW22, isolated as a laboratory indicator organism, is proposed. The average linear motion speed of intact cells of this mutant was approximately 32 $\mu\text{m/s}$ and decreased markedly by the presence of model chemical pollutants tested. It was suggested that the mutant should be useful as an indicator organism for the evaluation of pollutant toxicity by the dynamic image analysis method.

Key words : *Escherichia coli*/Tumbling-defective mutant/Pollutant toxicity/Image analysis/Bacterial motion.

Motile bacterial cells move by rotating their flagella and demonstrate taxis (Macnab, 1987; Khan, 1990). In general, depending upon the direction of flagellum rotation, a cell exhibits two modes of movement, i.e. linear swimming and tumbling, in the case of many kinds of bacteria including *E. coli*. Cells require proton motive force as an energy source for both modes of movement. If a cell is exposed to the stress of pollution in its aquatic environment, it should become less motile or non-motile due to the reduction or depletion of its energy level. Based upon this principle, the measurement of bacterial motility should be useful for rapid monitoring of water pollution, although other biomonitoring methods have been proposed (Loeb and Spacie, 1994).

By using a dynamic image analysis system consisting of a motion analyzer and a microscope, we have already reported the motion characteristics of stressed cells of a wild-type *Bacillus subtilis* 168 and its possible application to the evaluation of bacterial

injury' (Tsuchido et al., 1994). However, this preliminary system had two practical problems in the evaluation of injury: first, tumbling gave rise to a great variation of cellular motion speed; second, its motility was rapidly reduced during the observation period due to the aerobic property of indicator organism tested, *B. subtilis* (Tsuchido, 1993; Tsuchido et al., 1994).

In this study, therefore, we switched to the use of *E. coli*, which is a facultatively anaerobic bacterium and also a general hygienic indicator bacterium for assessing microbial contamination levels in the food industry and the pollution of aquatic environments. In addition, we attempted to isolate its tumbling-defective mutant to discriminate the loss of motility caused by exposure to a chemical pollutant from the tumbling as a normal mode of motion. The purpose of this study is to analyze the motion of the cells of this mutant exposed to chemical pollutants and further propose the principle of a novel bioassay method for the evaluation of aquatic pollution.

Several tumbling-defective mutants of *E. coli* were isolated as described previously (Parkinson, 1976).

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The cell suspension of wild type OW6 strain (*pro*) (Miyake et al., 1993) was spread on the swarm agar plates of L broth consisting of 10 g tryptone (Difco), 5 g yeast extract (Difco), 5 g NaCl and 4 g agar powder per 1 l of distilled water supplemented with 0.2% (w/v) glucose. The plates were exposed to UV light and then incubated at 37°C. The relatively large resultant colonies were picked up. Several tumbling-defective strains were selected from those colonies by observation under a microscope (Olympus, BH2) and one of those was designated OW22 and employed in this study.

An overnight culture of OW22 strain was inoculated into a 100-ml flask containing 20 ml of L broth. The flask was then shaken at 37°C at a speed of 80 rev/min. When the OD at a wavelength of 650 nm (OD_{650}) of the culture reached 0.3, potassium cyanide or cetyltrimethylammonium bromide (CTAB), as a model pollutant, was added. Both compounds were purchased from Wako Pure Chemical Co.

A sample (1 ml) was withdrawn and poured into a test tube (16mm×160mm) and Tween 80 was added to this tube at a final concentration of 0.01% to avoid cell adsorption to the glass coverslips described below. A portion (4 μ l) was withdrawn from the cell suspension, immediately placed on a glass slide prewarmed at 37°C, and overlaid with a cover-slip (18mm×18mm). The edges were then sealed with manicure paste.

Microscopy and image analysis of cell motion were performed as described previously (Tsuchido et al., 1994). The above preparation was mounted on the stage of a phase contrast microscope (BH-2, Olympus, 12V 100W HAL-L). A heat reflection filter was used to avoid an increase in temperature during the observation period. An NEC CCD camera (TI-23A) was attached to the microscope by means of a spacing adapter (Olympus, MTV-3) and connected

FIG. 1. Histograms of the distribution of the average linear motion speed of cells of a wild-type OW6 strain (A) and a tumbling-defective OW22 mutant (B). The total numbers of observed cells were 81 and 54, respectively.

directly to a Keio Motion Analysis System (MD-20R, Keio Electronic Ind. Co. Ltd., Ibaraki, Osaka). Images were digitized in real-time at 20 frames/s (0.05-s intervals during a 1-s period) and were detected and analyzed on a personal computer (NEC, PC9801) by a motion analysis program, MAS version 1.22 (Keio Electronic Ind.). Image areas of dark bacterial cells on a light background were processed by the analyzer and recorded on a grid of 241 vertical by 256 horizontal pixels (6.25 pixels/ μ m). A total of 8 to 15 scenes, each containing one to five cells, were image-processed.

The motion speed of all of the cells killed by heat treatment at 90°C for 10 min was below 6.0 μ m/s, and thus regarded to the maximum Brownian motion level. A normal distribution of the motion speed was obtained for a population of motile cells growing in the

TABLE 1. Effects of CTAB and potassium cyanide on the motility of *E. coli* OW22 strain

Concn (μ M)	Observed cell no.	Avg motion speed $\pm$ S.D. (μ m/s)	Percentage of motile cells (%)
CTAB			
0	46	31.8 $\pm$ 6.2 (100) ^a	95
2	44	25.6 $\pm$ 6.7 (81)	91
4	40	21.2 $\pm$ 6.4 (67)	88
6	36	19.6 $\pm$ 5.4 (62)	86
8	31	13.4 $\pm$ 5.1 (42)	68
Potassium cyanide			
0	50	30.0 $\pm$ 6.6 (100)	96
10	36	21.5 $\pm$ 6.3 (49)	67
100	31	13.8 $\pm$ 5.6 (37)	58

^a percentage.

medium at 37°C (Fig. 1). On the contrary to the wild-type OW6 strain, there were only few cells showing a motion speed of less than 6.0 $\mu\text{m/s}$ in OW22 strain. In the representative data (Fig. 1), the average motion speed of motile cells was $32.3 \pm 7.8 \mu\text{m/s}$ (S.D.), whereas that of the wild type OW6 was $27.2 \pm 9.4 \mu\text{m/s}$ (S.D.). These average values varied from experiment to experiment in ranges from 31 to 33 $\mu\text{m/s}$ and from 27 to 29 $\mu\text{m/s}$, respectively.

We analyzed the motility of cells of OW22 mutant exposed to a surface active agent, CTAB, and potassium cyanide as model chemical pollutants. Increasing concentrations of CTAB and potassium cyanide caused a marked reduction in the average motion speed of the cells that was accompanied by a decreased percentage of motile cells (Table 1).

An apparently high motion speed of OW22 mutant and an extraordinary high percentage of motile cells were substantially due to the non-tumbling property of this strain and thus the reduction in the motion speed as well as in the percentage of motile cells can be considered to correspond directly to the degree of pollution stress experienced by the cell population. OW22 strain was therefore suggested to be an excellent indicator bacterium for the aquatic pollution bioassay. The reduction in motion speed may reflect the lowering of the cellular energy level, although the possibility of the release of flagella from the cell during the encounter with a pollutant cannot be ruled out.

We have already suggested that this image analysis system of cell motion can be applied to the rapid and simple evaluation of bacterial injury for microbiological control in food, pharmaceutical, and fermentation industries (Tsuchido et al., 1994). An additional case of application to the efficacy evaluation of antimicrobial compounds is presented elsewhere (Tsuchido et al., 1996). We further propose here that the principle of this system can be applied to the biological monitoring of chemical pollution as a novel, efficient and rapid method. The study of the practical use of this method for biomonitoring is under investigation.

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