Japan Food Research Laboratories

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REPORT

Client: Ohtagaki Laboratory Inc.
1067-39 Karuizawa, Karuizawa-machi, Kitasaku-gun, Nagano 389-0102, Japan

Sample(s): "Rappla②-kun" Corrosion Protection Agent

Title: Antibacterial Activity Test

Received date of sample(s): April 17, 2014

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May 30, 2014
Date

JAPAN FOOD RESEARCH LABORATORIES

Antibacterial Activity Test

1. Client

Ohtagaki Laboratory Inc.

2. Sample

"Rappla②-kun" Corrosion Protection Agent

3. Purpose

The antibacterial activity of the sample is evaluated.

4. Outline of the method

Suspensions of *Campylobacter jejuni* and *Escherichia coli* (Serovar O157:H7, Shiga toxin I & II-producing *Escherichia coli*) were inoculated into each of the sample solutions (hereafter called "the test solutions) and stored at room temperature. After 5 minutes and after 3 hours (only after 5 minutes for *Campylobacter jejuni*), the viable cell counts of the test solutions were determined.

The test method for determining viable cell counts was validated by a preliminary test.

5. Results

Tables 1 and 2 show the test results.

The results of the preliminary test showed that the test solutions should be diluted by 10-fold with SCDLP broth to remove the effects of the sample.

Table 1: Viable cell counts of the test solutions

Test strain	Test specimen	Concentration	Viable cell count (/mL)	
			Initial*	After 5 minutes
Campylobacter jejuni	Sample	10 g/L	4.4×10^6	<100
	Control	–	4.4×10^6	3.4×10^6

<100: Not detected

Storage temperature: Room temperature

Control: Purified water

* The viable cell count of the control immediately after inoculation was defined as the viable cell count at the initial point.

Table 2: Viable cell counts of the test solutions

Test strain	Test specimen	Concentration	Viable cell count (/mL)		
			Initial*	After 5 minutes	After 3 hours
Escherichia coli (O157:H7)	Sample	10 g/L	6.3×10^5	<10	<10
	Control	–	6.3×10^5	6.6×10^5	6.8×10^5

<10: Not detected
Storage temperature: Room temperature
Control: Purified water
* The viable cell count of the control immediately after inoculation was defined as the viable cell count at the initial point.

6. Methods

1) Test strains

a) *Campylobacter jejuni* subsp. *jejuni* ATCC 33560

b) *Escherichia coli* ATCC 43895 (Serovar O157:H7, Shiga toxin I & II-producing *Escherichia coli*)

2) Medium for determination of viable cell counts and incubation conditions

Test strain a): Blood Agar Base No. 2 including 5 % defibrinated horse blood (OXOID); Spread plate method; Incubation, at 35 °C ± 1 °C for 6 days under microaerobic conditions

Test strain b): SCDLP Agar (Nihon Pharmaceutical Co., Ltd.); Pour plate method; Incubation, at 35 °C ± 1 °C for 2 days under aerobic conditions

3) Preparation of cell suspensions

Test strain a): The test strain was incubated on Blood Agar Base No. 2 including 5 % defibrinated horse blood at 35 °C ± 1 °C for 2 to 3 days under microaerobic conditions. Then, the cells were suspended in physiological saline to a concentration of about 10^8 to 10^9/mL.

Test strain b): The test strain was incubated on Nutrient Agar (Eiken Chemical Co., Ltd.) at 35 °C ± 1 °C for 18 to 24 hours under aerobic conditions. Then, the cells were suspended in purified water to a concentration of 10^7 to 10^8/mL.

4) Procedure

A 0.1-mL portion of the cell suspension was inoculated into the sample solution (mix up 10 g of the sample and 1 L of purified water), and this solution was used as the test solution. The test solution containing the test strain a) was stored at room temperature for 5 minutes, and the test solution containing the test strain b) was stored for 5 minutes and for 3 hours. At each of the measurement points, the test solutions were immediately diluted by 10-fold with SCDLP broth (Nihon Pharmaceutical Co., Ltd.). Then, the viable cell counts of the test solutions were measured.

As a control, purified water was prepared in the same manner. The viable cell count of the control was determined at the initial point, after 5 minutes and after 3 hours.

End of Report