

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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Takeko Arai

Principal Investigator

May 30, 2014

Date





Antibacterial Activity Test

1. Client

Ohtagaki Laboratory Inc.

2. Sample

"Rappla@-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.

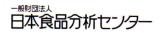




Table 2:	Viable cell	counts of the	test solutions

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 $^{\circ}$ C \pm 1 $^{\circ}$ 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 \pm 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 $35 \,^{\circ}\text{C} \pm 1 \,^{\circ}\text{C}$ 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

