

Report

Test of Efficacy of the Microorganisms Commercially Mixed (Biowish)
to Inhibit Food-Borne Pathogenic Bacteria

Dr. Warapha Mahakanhanakun

Science Technology Department, Industrial Agriculture Faculty,
Kasetsart University



Certified True and Correct Translation

Arree Saphantanaet
Arree Saphantanaet
INTERLANGUAGE TRANSLATION CENTER
Tel. 243-2018, 243-2109 Fax. 243-5686

Efficacy of Biowish against Food borne Pathogenic Bacteria

Warapha Mahakanchanakun and Jirapha Wongwathanachoti
Food Science and Technology Department, Industrial Agriculture Faculty, Kasetsart
University,
Tel. 02-562-031, fax 02-562-5021, email: fagwpm@ku.ac.th

Abstract

Commercially antagonistic microorganisms in the powder form of Biowish can result in inhibiting microorganisms that cause negative-gram bacteria are *Salmonella Typhimurium*, *Vibrio parahaemolyticus* and *Escherichia coli*, when testing the bacteria by Biowish powder that is soaked water overnight at the concentration of 1% and diluted to the concentration of 0.5% (1:1) and 0.25% (1:3), inhibited at the periods of 10, 30, 60 and 90 minutes. Moreover, the Biowish powder can result in inhibiting the positive-gram bacteria are *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium perfringens*. Upon testing the bacteria in Biowish powder soaked in culturing TBS food overnight at 1% concentration for 120 minutes and 24 hours; at the concentrations of 0.5% and 0.25% for 24 hours, it can be said the powder Biowish at the concentration of 1% has a tendency to be applied for control and inhibit pathogenic bacteria especially the negative-gram and positive-gram bacteria as mentioned above for more safety in the various types of food.



Certified True and Correct Translation

Anon Suphiantanon
INTERLANGUAGE TRANSLATION CENTER
Tel. 243-2018, 243-2109 Fax. 243-5686

Introduction

Some bacteria are very important in terms of food safety, if contaminated in food and consumption may cause digestive diseases, i.e. diarrhea, nausea and fever, etc. These bacteria are *Escherichia coli*, *Salmonella spp.*, *Vibrio parahaemolyticus* and *Listeria monocytogenes*, and some bacteria cells not only causing diseases but build up toxins in food, when eaten may cause digestive diseases, they are: *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium perfringens*.

At present the most popular method of controlling and eliminating these diseases is the use of heat, i.e. pasteurization and sterilization, etc. However, sometimes some foods, i.e. fresh vegetables cannot use heat to kill the germs, because of the popularity of eating raw. Use of chemicals as sterilization, i.e. chlorine, sodium hypochlorite at the concentration of 200 ppm can reduce the number of *Salmonella Typhimurium* by 1.6-3.3 log CFU/g, but causing the meat in contact with slides of tomato soft and cut-up carrot to be pale (Monthakan, 2002). Use of chemicals sometimes has good effectiveness of killing germs, but may change the food to the unwanted characteristics. Also the bacteria that can build spores and toxins, e.g. *Clostridium perfringens* and *Bacillus cereus* contaminated in food can withstand disinfectant better than the minus gram bacteria, cannot be easily eliminated by using heat or chemical. Use of natural extracts that produced by microorganisms or bringing other microorganisms to grow competing with germ microorganisms may be another method to control and prevent germ microorganisms from food courier, thus the objective of this experiment is to test and find the effectiveness of commercially antagonistic microorganisms in Biowish powder to inhibit pathogenic bacteria to be applied in food later.

Equipment and methods of testing

1. Microorganisms that used in the experiment

- *Salmonella Typhimurium* S. 13311
- *Escherichia coli* ATCC 8739
- *Vibrio parahaemolyticus* VP-293
- *Staphylococcus aureus* ATCC 25923
- *Bacillus cereus* DMST 5040
- *Listeria monocytogenes* L-Scott A
- *Clostridium perfringens* DMST 16178

2. Preparation of bacteria solution

Put 1 loop from the Tryptic Soy Agar (TSA) in the Tryptic Soy Broth (TSB) 10 ml (for *V. parahaemolyticus* in the TSB-3% NaCl and *L. monocytogenes*



3.3.2 Dilute the germ solution in No. 2 with 0.1% peptone water to reach the required dilution. Spread the plate to have the starting germs at 3 log CFU on the TSA-culture plate for a while. Then make a hole with the cork border No. 2 by germ-free method and drop Biowish extract from No. 3.3.1 with 30 micro liters in the hole and cure at $35 \pm 2^\circ \text{C}$ for 24 hours, then notice the clear zone occurs around the hole.

Results and review of the experiment

Table 1 Efficacy of Biowish powder in inhibiting pathogenic bacteria that causes digestive disease by the spiral plate

Type of bacteria tested	Time (Minute)	Number of microorganisms (log CFU/ml)			
		Control (TSB)	Concentration of Biowish		
			1%	0.5%	0.25%
<i>S. Typhimurium</i> *	10	2.62	0	0	0
	30	3.64	0	0	0
	60	4.48	0	0	0
	90	5.00	0	0	0
	24 hours	7.75	ND	7.15	7.50
<i>V. parahaemolyticus</i> *	10	3.30	0	0	0
	30	6.63	0	0	0
	60	ND	0	0	0
	90	ND	0	0	0
	24 hours	6.95	ND	5.49	6.12
<i>E. coli</i> *	10	3.72	0	0	0
	20	3.95	0	0	0
	40	3.80	0	0	0
	60	3.80	0	0	0
	24 hours	7.33	ND	6.30	7.43
<i>S. aureus</i> **	10	3.32	3.00	3.21	4.00
	30	3.18	3.41	3.41	3.41
	60	3.18	3.13	3.13	3.51
	90	3.30	0	3.51	3.42
	120	3.19	0	0	0



[Handwritten Signature]

<i>B. cereus</i> **	10	3.25	TNTC	TNTC	TNTC
	30	3.08	TNTC	TNTC	TNTC
	60	3.10	TNTC	TNTC	TNTC
	90	3.27	TNTC	TNTC	TNTC
	120	3.16	TNTC	TNTC	TNTC

Table 1 Efficacy of Biowish powder in inhibiting bacteria by the spiral plate (continued)

Type of bacteria tested	Time (Minute)	Number of microorganisms (log CFU/ml)			
		Control (TSB)	Concentration of Biowish		
			1%	0.5%	0.25%
<i>L. monocytogenes</i> **	10	3.02	4.03	4.25	4.14
	30	3.27	3.74	4.41	4.26
	60	3.38	3.60	4.28	4.23
	120	3.34	0	4.33	4.34
	24 hours	9.07	0	0	0
<i>C. perfringens</i> **	120	0	0	0	ND
	24 hours	0	0	0	ND

Remarks TNTC = cannot count the colonies of *B. cereus* due to Biowish that grew to the full plate.

ND = It is not experimented.

+ = Cannot count the number of *C. perfringens*, but remove the germs from where they grew.

* = Biowish Powder soaking in water for 1 night.

** = Biowish Powder soaking in TSB for 1 night

Table 2 Efficacy of Biowish in inhibiting bacteria that causes digestive diseases of negative gram by the Agar diffusion, measuring from the inhibiting ability in diameter of the clear loop (mm).

Type of bacteria tested	Diameter (mm)		
	Concentration of Biowish		
	1%	0.5%	0.25%
<i>E. coli</i>	10	7	3
<i>Vibrio parahaemolyticus</i>	16	10	8
<i>Salmonella Typhimurium</i>	14	10	6

Remark: 0 is no clear zone or cannot inhibit the growth of the tested bacteria.



Table 3 Summary of the efficacy of Biowish powder at 1% in inhibiting pathogenic bacteria

Type of microorganisms tested	Agar diffusion	Spiral Plate					
	24 hours	10 minutes	30 minutes	60 minutes	90 minutes	120 minutes	24 hours
<i>S. Typhimurium</i>	-	-	-	-	-	ND	ND
<i>E.coli</i>	-	-	-	-	-	ND	ND
<i>V.parahaemolyticus</i>	-	-	-	-	-	ND	ND
<i>S. aureus</i>	ND	+	+	-	-	-	-
<i>B. cereus</i>	ND	+	+	+	+	-	-
<i>L. monocytogenes</i>	ND	+	+	+	+	-	-
<i>C. perfringens</i>	ND	+	+	+	+	-	-

Remarks + = Growth microorganisms
 - = No growth microorganisms
 ND = No experimentation

Table 1 Experiment of efficacy of Biowish powder at the concentration of 1%, 0.5% and 0.25% in inhibiting and destroying pathogenic bacteria causing digestive diseases of negative gram, i.e. *S. Typhimurium*, *E.coli* and *V. parahaemolyticus* found the solution prepared from Biowish powder at 3 levels of concentration can inhibit these microorganisms at 10, 30, 60 and 90 minutes. The experiment on efficacy of Biowish powder at the concentrations of 1%, 0.5% and 0.25% in inhibiting and destroying pathogenic bacteria causing digestive diseases of the positive gram are *S. aureus*, *B. cereus*, *C. perfringens* and *L. monocytogenes* found the solution prepared from Biowish powder at the concentration of 1% can inhibit *S.aureus*, *C. perfringens* and *L. monocytogenes* at 120 minutes and 24 hours. Moreover, the powder Biowish at the concentration of 0.5 and 0.25% can inhibit *S. aureus*, *C. perfringens* and *L. monocytogenes* at 24 hours as well.

Table 2 Shows the experimental results of efficacy of the solution prepared from Biowish powder at the concentrations of 1%, 0.5% and 0.25% in inhibiting and destroying pathogenic bacteria causing digestive diseases of the negative gram in 3 types are *E. coli*, *S. Typhimurium* and *V. parahaemolyticus* by the Agar dilution at 24 hours found the solution prepared from Biowish powder at the concentration of 1% can inhibit *S. Typhimurium*, *E. coli* and *V. parahaemolyticus* with higher efficacy than using the solution prepared from powder Biowish at the concentrations of 0.5% and 0.25%. But in the case of the pathogenic bacteria of the positive gram was not tested by this method, as the remaining microorganism cells left in the Biowish extract (after throwing the cells) grew in competition with the germs appear as colonies around the pit of the dropped extract, so the inhibition around the pits cannot be noticed clearly.



Table 3 summarizes the efficacy of Biowish powder at 1% in inhibiting pathogenic bacteria by the solution prepared from Biowish powder at this concentration. The test by the spiral plate technique can inhibit *S. Typhimurium*, *E.coli* and *V. parahaemolyticus* at 10, 30, 60 and 90 minutes, and can inhibit *S. aureus*, *B. cereus*, *C. perfringens* and *L. monocytogenes* at 120 minutes and 24 hours, while the test by Agar diffusion found the 1-% Biowish extract can inhibit *S. Typhimurium*, *E. coli* and *V. parahaemolyticus* in 24 hours.

Summary of the experiment

Results of the inhibition of pathogenic bacteria causing digestive diseases in seven types are *Salmonella*, *Typhimurium*, *Escherichia coli*, *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes* and *Clostridium perfringens* of Biowish powder appear that the Biowish powder soaked in water overnight before experimenting at the concentration of 1%, 0.5% and 0.25% can inhibit *S. Typhimurium*, *E. coli* and *V. parahaemolyticus* within 90 minutes. The Biowish powder that soaks TSB one night before experimenting at three levels of concentration can inhibit *S. aureus*, *C. perfringens* and *L. monocytogenes*, when the antagonistic germs grew in competition with the pathogenic microorganisms 24 hours. Moreover, the powder Biowish at 1% concentration can inhibit *S. aureus*, *B. cereus*, *C. perfringens* and *L. monocytogenes* at 120 minutes as well. It is possible to bring the 1% Biowish to apply as it has the efficacy in inhibiting the microorganisms best.

References:

- Monthakan Boonyakan. 2002. Reduction of contamination of *Salmonella Typhimurium* during preparation of salad vegetables by germ-killing chlorine, thesis of master's degree, Kasetsart University, Bangkok.
- Parish, M.E. and P.M. Davidson. 1993. Methods for Evaluation, pp. 597-615 In P.M. Davidson and A.L. Branen, eds. Antimicrobials in Foods. 2nd ed. Marcel Dekker, Inc. New York.

Appendix

Selective media of each microorganisms

Microorganisms	Selective Media
<i>S Typhimurium</i>	Xylose Lysine Deoxycholate aga (XLD)
<i>E. coli</i>	Chromocult Coliform agar
<i>V. parahaemolyticu</i>	Thiosulfate Citrate Bile Salt agar (TCBS)



Certified True and Correct Translation

Aree Saphuntaramee
 INTERLANGUAGE TRANSLATION CENTER
 Tel. 243-2018, 243-2109 Fax. 243-5086

<i>S. aureus</i>	Baird-Parker medium (BP)
<i>B. cereus</i>	<i>B. cereus</i> agar (BC)
<i>L. monocytogenes</i>	Modified Oxford Listeria Selective agar (MOX)
<i>C. perfringens</i>	SFP agar



Certified True and Correct Translation

Arce Suphathamant

Arce Suphathamant
 INTERLANGUAGE TRANSLATION CENTER
 Tel. 243-2018, 243-2109 Fax. 243-5686

TSB+3% NaCl and *L. monocytogenes* cultured in TSB+0.6% yeast extract, while *C. perfringens* cultured in the Brain Heart Infusion broth and cured at the temperature $35 \pm 2^\circ\text{C}$ (except *B. cereus* cured at 30°C) for 24 hours and transfer the TSB or medium that is suitable once more. Then pipette 1 ml in TSB or liquid culture food that is suitable with each type for 50 ml, cured at $35 \pm 2^\circ\text{C}$ for 16 hours (except *B. cereus* cured for 18 hours) then dilute with 0.1% peptone water until the solution start at 5 log CFU/ml.

3. Study of efficacy of Biowish in inhibiting bacteria growth

3.1 Preparation of solution from Biowish powder.

Preparation of solution from Biowish powder at three concentrations as follows:

- 3.1.1 Soak Biowish powder 1 gram in tap water or TSB (depending on testing the bacteria) 100 ml 1 night at the room temperature ($30 \pm 2^\circ\text{C}$) to get Biowish solution at 1% concentration.
- 3.1.2 Dilute the solution in No. 3.1.1 with TSB at the ratio of 1:1 to derive at 0.5% Biowish.
- 3.1.3 Dilute the solution in No. 3.1.1 with TSB at the ratio of 1:3 to derive at 0.25% Biowish.

3.2 Test of Biowish by the Petri dis (Spiral plate)

- 3.2.1 Mix the solution of bacteria in No. 2 the volume of 10 ml solution from powder Biowish 90 ml (from No. 3.1.1 or 3.1.2 or 3.1.3) to have the last concentration as 4 log CFU/ml.
- 3.2.2 Make a random sample out of No. 3.2.1 at 10, 30, 60, 90 and 120 minutes and at 24 hours by the spiral plate technique from the Autoplate 4000, Advance Instruments, USA, by culturing pathogenic bacteria of each type on the selective media (Appendix) and count the number in colony per milliliter.

3.3 Test of Biowish by the Agar diffusion (Parish and Davidson, 1993)

Since the test of growth and survival of the pathogenic microorganisms with the negative gram, when mixed the microorganisms with Biowish solution for 24 hours found no inhibition of germs, so it was confirmed by the agar dilution method.

- 3.3.1 Prepare Biowish solution the same with No. 3.1.1 then bring the solution to process by a Biofuge primo, Heraeus Instruments, Germany, at 7,000 rpm to separate the cells for 8 minutes. While the supernatant derived called 1% Biowish extract, then dilutes the supernatant with 0.85% NaCl (NSS) at the ratio 1:1 and 1:3 to derive at 0.5% and 0.25% Biowish extract respectively.



Certified True and Correct Translation

Arce Suphontlanant
INTERLANGUAGE TRANSLATION CENTER
Tel. 243-2018, 243-2109 Fax. 243-3686