Bio-Assay Test Of Antagonistic Reactions Of Bacillus Cereus Against Selected Pathogens

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ABSTRACT: *Bacillus cereus* as an endemic, soil-dwelling, rod-shaped bacteria that can cause food-borne illness is harmful to humans while it is true that other strains can be beneficial as probiotics for animals. This characteristics of *B. cereus* made way in determining its effectiveness as protectant and eradicant to selected fruits and vegetable pathogens such as *Klebsiella pneumonia, Alcaligenes spp., Xanthomonas spp.,* and *Proteus vulgaris.* Through *In-Vitro* analysis, *B. cereus* as a protectant was most effective within 12 to 24 hours as antagonist against *Alcaligenes spp.,* and *Klebsiella pneumonia* but not against *Xanthomonas spp.* As protectant for 36 to 48 hours, *B. cereus* was most effective antagonist against *Proteus vulgaris* but not against *Xanthomonas spp.Bacillus cereus* as an eradicant for 24 hours was not effective as antagonist against all pathogens but for 48 hours, it was most effective as antagonist against *Alcaligenes spp* but was not effective against *Klebsiella pneumonia.*

Keywords : *Alcaligenes spp., Bacillus cereus, Eradicant, In-Vitro Analysis, Klebsiella pneumonia, Proteus vulgaris, Protectant, Xanthomonas spp.*

1 INTRODUCTION

Fruits and vegetables are greatly demanded by all consumers worldwide regardless of social status. Deterioration of the quality of these agricultural products are due among other things to physical factors, action of their own enzymes and microbial content, improper environmental conditions during harvesting and transit, storage, packaging and marketing.[2,3] All of these leads to only one end, spoilage. On plant enzyme, their activity continue in raw plant foods after harvest. If oxygen is available, the plant cell will respire as long as they are alive, and Hydrolytic enzymes can continue their action even after death of the cells. Diseases of vegetables and fruits may result from the growth of an organism that obtain its food from the host and usually damages it or from adverse environmental conditions that causes abnormalities in functions and structures of fruits and vegetables.[4,5]. Commonly, over maturity in plants and vegetables made them inedible or spoiled. Aside from exceeding maturity, microbial spoilage caused by fungi also happens acting as plant pathogens affecting the stems, leaves, flowers or roots of the plant, on the fruits or other special part used as foods. Bacillus cereus can control mycelial growth of plant fungi and it is considered as bio-control agent to affect plant disease control, thus making B.cereuss as a possible candidate as eradicant and protectant against pathogens that cause deterioration and spoilage of fruits and vegetables. [1,3] Thus, to provide a proof as to the effectiveness of B.cereus in playing these two roles. This study was conducted.

Statement of the Problem

This study determined the antagonistic property of *Bacillus cereus* against selected bacteria pathogens of fruits and vegetables. Specifically, the study sought answers for the following questions:

1. How may the antagonistic effects of *Bacillus cereus* as eradicant against *Klebsiella pneumonia, Alcaligenes spp., Xanthomonas spp.,* and *Proteus vulgaris,* be described?
2. How may the antagonistic effects of *Bacillus cereus* as protectant against *Klebsiella pneumonia, Alcaligenes spp., Xanthomonas spp.,* and *Proteus vulgaris,* be described?

Hypothesis

There is no significant difference in the antagonistic effects of *B. cereus* as eradicant and protectant against *Klebsiella pneumonia, Alcaligenes spp., Xanthomonas spp.,* and *Proteus vulgaris.*

Scope and Delimitation

This study focused only on the In-Vitro test of four identified pathogens namely *Klebsiella pneumonia, Alcaligenes spp., Xanthomonas spp.,* and *Proteus vulgaris* against *Bacillus cereus* as an eradicant and protectant. Other pathogens were not included as they were not identified in the study.

Research Paradigm of the Study

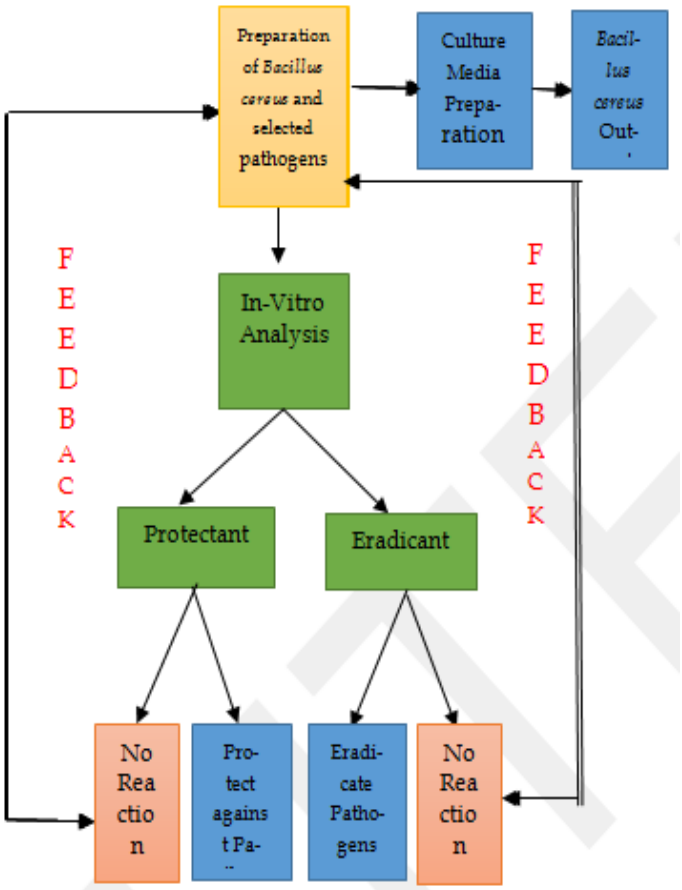

Figure 1: *The Research Paradigm*

Methods and Procedure

True experimental research was used in this study. It's the only type of research which can truly test hypothesis that involved cause and effect relationship.

A. Materials Needed

16 test tubes, 16 petri-dishes/plates, Erlenmeyer flask, Alcohol lamp, Inoculation chamber, one bottle Denatured alcohol, one bottle 70% ethyl alcohol, I kilo Cotton, one bottle Zonrox, Culture media, autoclave machine, oven, forceps

B. Disinfection of Materials

Agar Plate Method was used to disinfect and clean the petri dishes before the conduct of the experiment. Thirty-nine grams of commercially available Nutrient Agar medium with an additional 5grm of agar suspended in one liter (1000mL) of distilled water were prepared. The suspension was mixed thoroughly with constant stirring while boiling. The medium was poured into a flask and sterilized in an autoclave for 15 to 20 minutes at 121ºC at 15 psi. The sterilized Nutrient Agar medium was pour plated into sterilized petri-dishes preventing the production of moisture in the dish.

C. Antagonistic Test

Preparation of McFarland Standard. Preparation of McFarland was conducted to determine the density of the bacteria and tested the antagonistic property of *Bacillus cereus.* The four test organisms (*Xanthomas spp., Klebsiella pneumonia, Alcaligenes spp.,* and *Proteus vulgaris)* were examined against *Bacillus cereus* using culture pairing technique (combination of Agar plug technique and paper disc technique). McFarland standard number 0.5 was prepared to determine the cell density of the bacteria. This was used to test the antagonistic property of bacteria against *Bacillus cereus.*

Measurement of Bacterial Cell Density. Four test tubes with 5mL nutrient broth were prepared. A loopful of bacteria was aseptically inoculated into each test tube. Then it was incubated at room temperature until the test organisms have the same bacterial density as compared to McFarland standard. McFarland standard 0.5 was used as standard number of cell per millimeter of test organisms. The standard bacterial cells were used for the paper disc technique.

Paper Disc Technique. Paper D Technique was used to determine the effectiveness of the extracts. Five millimeter in diameter was prepared and used for the test organisms. Disc soaked with the test organism was aseptically obtained from the standardized bacterial suspension and inoculated on the center of sterile Nutrient Agar medium. Swabbing inoculations of the pathogens were made in a triangular pattern for each replicate. It was incubated for 24 hours and antagonistic property was determined.

D. Protectant and Eradicant Testing

Eradicant. This test determined the antagonistic property of *Bacillus cereus* against selected pathogens as eradicant.Filter paper disc was prepared using a paper puncher and sterilized in an autoclave for 15 minutes at 15 psi. It was oven dried for two hours at 45ºC. The pure culture of *Xanthomonas spp.* Was swabbed in a petri plates with Nutrient agar medium. Same procedures were done for other pathogens. Ten filter papers were soaked into a pure culture of *Bacillus cereus* in a Nutrient broth and aseptically planted into the prepared *Xanthomonas spp.* Swabbed into a plate. Same procedures were done for other three pathogens. The set-up was incubated at 27-30ºC in an environmental chamber for 12-48 hours.

Protectant.

This test determined the antagonistic property of *Bacillus cereus* against selected pathogens as protectant. Filter paper disc was soaked into a broth culture of *Xanthomonas spp.* and was over dried for 45ºC. The pure culture of *Bacillus cereus* was swabbed into a prepared Nutrient agar in a petri plate. Using forceps, the filter paper disc was seeded into the petri plates. Same procedures were done to *Klebsiella pneumonia, Alcaligenes spp.* and *Proteus vulgaris*

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E. Making of Bacillus Output

Nutrient Broth was the medium needed for making the *Bacillus* output where 8 grams of Nutrient broth was weighed and was dissolved in 1000mL of distilled water. The dissolved Nutrient broth was sterilized in an autoclave and was cooled afterwards. The dissolved Nutrient Broth was used in the inoculation of *Bacillus cereus.*

F. Inoculation of *Bacillus cereus*

Bacillus cereus was contained in a test tube and was poured with the prepared Nutrient broth in an Inoculation Chamber. The mixed of *bacillus cereus* and Nutrient Broth takes 2 days to form the *Bacillus* Output

Results and Discussion

The following results were obtained based from the output of all the procedures described above.

***Table 1** shows the rate of inhibition of Bacillus cereus as protectant against Xanthomonas spp., Klebsiella pneumonia, Alcaligenes spp. and Proteus vulgaris.*

Table 1
Bacillus cereus as Protectant for 12 to 24 hours

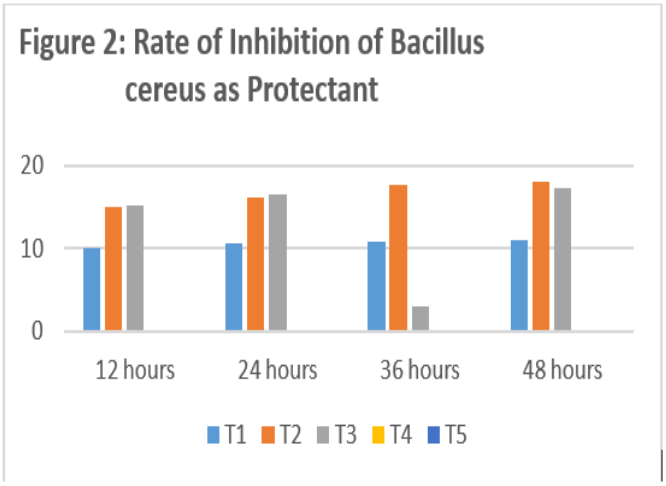
Treatments	12 Hours		24 Hours	
	Trial 1	Trial 2	Trial 1	Trial 2
T1 (*Klebsiella pneumonia)*	10.1 mm	10.0 mm	10.5 mm	10.7 mm
T2 (*Proteus vulgaris)*	15.0 mm	15.0 mm	16.1 mm	16.1 mm
T3 (*Alcaligenes spp.)*	15.0 mm	15.2 mm	16.4 mm	16.6 mm
T4 (*Xanthomonas spp.)*	0	0	0	0
T5 (control)	0	0	0	0

As revealed on Table 1, the four pathogens were identified as Treatment 1, Treatment 2, Treatment 3 and Treatment 4. Treatment 5 was distilled water served as control treatment. Two replicates were prepared for two trials. As a result, for 12 hours, the growth of *Bacillus cereus* was highest in *Alcaligenes spp.* with 15.0 to 15.2 mm, followed by *Proteus vulgaris* with growth of 15.0mm and *Kledsiella pneumonia* with 10.0 – 10.1 mm growth. *Bacillus cereus* did not grow in *Xanthomonas spp.* as well as in the control treatment. For 24 hours, the growth of *Bacillus cereus* was highest in in *Alcaligenes spp.* with growth measurement of 16.4 to 16.6 mm, followed by *Proteus vulgaris* with 16.1 mm growth, and *Klebsiella pneumonia* with 10..5 to 10.7 mm growth. Again, at 24 hours, observations revealed that Bacillus cereus did not grow in *Xanthomonas spp*.

Table 2
Bacillus cereus as Protectant for 36 to 48 hours

Treatments	36 Hours		48 Hours	
	Trial 1	Trial 2	Trial 1	Trial 2
T1 (*Klebsiella pneumonia)*	10.8 mm	10.8 mm	11.1 mm	11.0 mm
T2 (*Proteus vulgaris)*	17.8 mm	17.5 mm	18.0 mm	18.0 mm
T3 (*Alcaligenes spp.)*	16.9 mm	16.9 mm	17.1 mm	17.4 mm
T4 (*Xanthomonas spp.)*	0	0	0	0
T5 (control)	0	0	0	0

Table 2 shows the rate of inhibition of *Bacillus cereus* as protectant against the four subject pathogens from 36 hours to 48 hours of observation. As can be seen from the gathered data, the growth of *Bacillus cereus* was highest in *Proteus vulgaris* with 17.5-17.8 mm, followed by *Alcaligenes spp.* with 16.4mm to 16.6 mm growth, and *Klebsiella pneumonia* with 10.5-10.5mm growth. *Bacillus cereus* did not grow in *Xanthomonas spp* as well as in the control. For 48 hours, the growth of *Bacillus cereus* was highest in *Proteus vulgaris* with 18.0mm measurement, followed by *Alcaligenes spp.* with 17.1 to 17.4 mm growth, and *Klebsiella pneumonia* with 11.0 to 11.1mm growth. *Bacillus cereus* did not grow in *anthomonas spp* as well as in the control treatment. Figure 2 shows the graphical presentation of the rate of inhibition of *Bacillus cereus* as protectant for 12 hours, 24 hours, 36 hours and 48 hours periods.

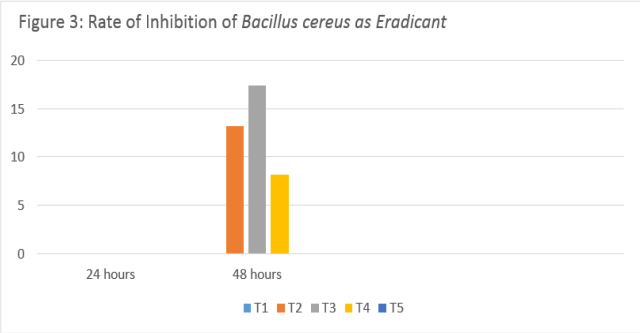


As graphically shown, *Bacillus cereus* did not perform as protectant on Treatment 4 (*Xanthomonas spp.)* and Treatment 5 (the control).

Table 3
Bacillus cereus as Eradicant for 24 to 48 hours

Treatments	24 Hours		48 Hours	
	Trial 1	Trial 2	Trial 1	Trial 2
T1 (*Klebsiella pneumonia)*	0	0	0	0
T2 (*Proteus vulgaris)*	0	0	13.4 mm	13.0 mm
T3 (*Alcaligenes spp.)*	0	0	0	17.4 mm
T4 (*Xanthomonas spp.)*	0	0	8.3 mm	8.0 mm
T5 (control)	0	0	0	0

As an eradicant, the rate of inhibition of *Bacillus cereus* after the test on two replicates against the four identified fruit and vegetable pathogens were presented on Table 3. As revealed on the table, for 24 hours, *Bacillus cereus* did not grow in all the four pathogens, even on the control treatment. For 48 hour period, the growth of *Bacillus cereus* was highest in *Alcaligenes spp.* with 17.4 mm, followed by *Proteus vulgaris* with 13.0 to 13.4 mm growth, and *anthomonas spp.* with 8.0 to 8.3 mm growth. *Bacillus cereus* did not grown in *Klebsiella pneumonia* as well as the control treatment.



On Figure 3 shows the graphical presentation of the rate of inhibition of *Bacillus cereus* as eradicant for 24 hours and 48 hours period.

Conclusions

1. *Bacillus cereus* can serve as eradicant by inhibiting the growth of *Alcaligenes spp., Proteus vulagris,* and *Xanthomonas spp.* but not of *Klebsiella pneumonia.*

2. *Bacillus cereus* can serve as protectant in inhibiting the growth of *Klebsiella pneumonia, Alcaligenes spp.,* and *Proteus vulgaris* but not of *Xanthomonas spp.*

3. There was a significant difference between and among the selected pathogens in terms of rate of inhibition.

Recommendations

1. If the results of this study is to be verified in the future, it is recommended the use of different methods in proving and determining the efficiency of *Bacillus cereus* as an eradicant and a protectant.

2. The need to quantify the characteristics and qualities of *Bacillus cereus* is also needed for future use.

3. Since *Bacillus cereus* is scarce in nature, the production of its stocks by means of alternative medium is suggested.

4. An in-depth analysis utilizing other techniques in determining the best ways to protect the quality of fruit and vegetables is highly suggested.

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