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Original Research Article

Preparation of Bacterial Ghosts from *Campylobacter jejuni* Strain PP3894181

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Abstract: In the industrialized world, *campylobacter jejuni* is a prevalent foodborne bacterial infection. And is zoontic disease and causing gastroenteritis and others disease in the study using this bacteria as vaccine by preparing of bacterial ghost from C. jejuni This is the aim of the study for vaccine was prepared by determining the MIC, MGC of the bacteria that were grown on N.B medium after that a group of chemicals compound SDS, NaOH, $CaCO_3$ and H_2O_2 were added at specific concentration and according to the Sponglike Protocol and using Plackett-Burman Experiment design and conducting a viability test for the bacteria, the concentration of MIC and MGC were determined.

Keywords: Ghosts, *Campylobacter jejuni*, Sponglike protocol, MIC, MGC.

INTRODUCTION

The microbial species has been linked to hundreds of hospital admissions, thousands of food-borne infections, and thousands of fatalities globally each year (Atiyah, Degaimand, Al-yassari, & Abady, 2019). *Campylobacter* is one of pathogensis bacteria is frequently linked to enterocolitis and gastroenteritis in people all over the world (Igwaran & Okoh, 2019). In the industrialized world, *campylobacter jejuni* is a prevalent foodborne bacterial infection. The majority of Campylobacter infections cause a mild gastroenteritis that goes away on its own (Kim *et al*., 2021).

The bacterium's intestinal origin is the source of most of gram-negative bacteria, which travel from the intestines to anther places (Ali, Khudhur, Hasan, & Microbiology, 2021). Gram-negative bacteria form BG, or empty envelopes, by the regulated expression of Lys gene of bacteriophage phiX174 Hutchison and Sinsheimer were the first to describe the involvement of gene E in the lysis of Escherichia coli, a Gram-negative bacterium. Apart from lysis mediated by gene E (Abtin, 2010) creating a structure akin to a lysis tunnel inside the live bacteria's envelope. BGs have been employed as adjuvants and delivery methods for vaccines all over the world. (Chen *et al*., 2021b) nanomaterials (NMs) have become a unique tool for combating multidrug resistant (MDR) pathogen (Al-Hilli, Aldujaili, & Toxicology, 2020) and used the nanomaterials as delivery system gene E-mediated lysis, which removes the genetic material while preserving the original cell shape (Zhu *et al*., 2022). For the manufacture of BGs, the E lysis gene is well-established. The E lysis gene, which is really one of the Φx174 phage genes, merely carries out the Bacteriophage approach for cell lysis. However, there may be certain danger issues when employing these genes in human applications (Amara *et al*., 2013) have introduced a strategy for manufacturing BGs only utilizing chemical substances in order to address this. In order to manufacture the BG, low amounts of chemicals such as NaOH, SDS, $CaCo_3$ and H_2O_2 were used; this produced structures resembling sponges. Both Gram-positive and Gram-negative bacteria can be treated with this technique (Hajam, Dar, Won, & Lee, 2017). This process is known as Sponglike(SL). A technique based on determining the essential concentration of certain chemical compounds that affect bacteria's cell walls and genetic material was originally proposed in 2013 (Jaleta, 2015). (Amro A Amara, *et al*.,). The primary innovation is the effectiveness of utilizing the necessary concentration of a few readily accessible, reasonably priced chemical chemicals to create holes in the bacterial cell wall and H2O2 are substances that

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have an active effect on cell macromolecules. CaCO3 makes the cell wall more stiff, which permits these substances to enter the cell.

MATERIALS AND METHODS

Requirements of the Bacterial Culture and Strain

The *Campylobacter jejune* strain pp3894181 was cultivated in Nutrient Broth (NB) and absorbed the turbidity.

Generating Ghosts of *C. jejnui*

According to Amara, "SLRP," a sponge-like simplified technique, was used to create *C. jejnui* ghosts for BG preparation (Amara, 2013). The "sponge-like" method is a widely utilized chemical process for BG production. With this technique, chemical chemicals are used to create holes in the cell walls of bacteria. Centrifugation is then used to extract the contents of the cells (Chen *et al.*, 2021a).

Finding the Minimum Growth Concentration (MGC) and Minimum Ionic Strength (MIC) for H2O2, CaCO3, SDS, and NaOH

The MIC and MGC were determined by using the broth microdilution technique.(AL-Roomi & Ajeel, 2024) the bacterial sample was refreshed in N.b before the day of preparation of the dilution and cultivation the Campylobacter in flask contain 500ml of N.B for 72 hr. 24 tubes were taken and divided into four groups of 6 tubes each. 4.5 ml of N.B was added to each tube. The first group, 500 ml of NaoH was added to the first tube, then half a ml was taken from it and added to tube No. 2, and from tube No. 2 we took half a ml to the tube. The third and so on this process was repeated in the remaining groups, differing only in the chemical and with the same concentrations after that added 100 µl from bacteria which was reactivated in a solution of N.B the group of tubes was incubated in the incubator at 37 º for one night to determine the individual MIC for NaOH, SDS, and H2O2, a conventional experiment was conducted (Andrews, 2001). The concentration at which the initial bacterial growth happens after the minimum inhibitory concentration (MIC) of each substance was found, as well as its maximal growth concentration (MGC) (A. A. Amara, M. M. Salem-Bekhit, & F. K. Alanazi, 2013).

Cleaning, Collecting, and Processing of Bacterial Biomass:

Under static circumstances, the Campylobacter was grown in a one-liter flask with 500 mL of NB. The bacterial cells were left to develop at 37oC for 72 hours. Next, the biomass was collected at 4000 rpm/min using centrifugation, and it was then cleaned using 0.5% saline. After that, the cell biomass was collected and again suspended in distilled water to provide a final solution for every chemical combination that were utilized, which are NaOH, H2O2, and SDS. CaCO3 has been produced five times with the amounts described in Table (1).

Plackett-Burman Experiment:

When evaluating the impact of process factors on performance, the Plackett-Burman model is a crucial tool that may drastically cut down on the amount of repeated trials needed for a follow-up optimization research that use response surface methods (Mehdaoui *et al.*, 2022). Two physical characteristics were represented as Temperature- shacking rate (X5) is the only variable, while SDS (X1), H2O2 (X2), CaCO3 (X3), and NaOH (X4) are the four chemical components.

Represented the other variables as shown in Table 1, There was randomization in the five variables using the Plackett-Burman design. To maximize the five variables, two Plackett-Burman design trials were carried out. As seen in Table 1, each variable was given +1 and −1 to represent its two levels (high and low). For SDS, H_2O_2, and NaOH separately, the +1 value indicates the MIC, while the −1 number indicates the computed MGC. For ten minutes, the bacterial broth was centrifuged at 4000 rpm in order to extract the biomass from the 72-hour-cultivated Campylobacter culture. Following a gentle 0.5% saline wash, the cells were recentrifuged for 10 minutes at 4000 rpm. After then, the supernatant was thrown out. After being gathered as a cell pellet, the Campylobacter cells were once again cleaned. Five times as much NaOH, SDS, and H_2O_2 stock was made from each of the two, of the values +1 and –1, which were ascertained previously from the MIC and the MGC. two tests were carried out using the Plackett-Burman design shown in Table (1). Depending on the design, each variable's value was either +1 or –1. Two experiment was carried out in three stages.

1. First step contains all variables except H2O2
2. Second step contain only H2O2
3. Third step Pellets were reconstituted in 60% Ethanol and allowed to stand at room temperature for half an hour, gently swirling them every five minutes for 30 seconds. The collecting and cleaning of the cell pellets were carried out once again.

Table 1: Experiments I and II for Bacterial ghost preparation

Experiment variables (X)					
Experiment.No.	NaOH	$CaCO_3$	H_2O_2	SDS	Shaking /temperature
I	-1	1	1	1	1
II	-1	-1	-1	1	1

Viability Test:

Samples were obtained from the preparation and grown on nutrient broth to assess if any still-viable cells were in the prepared *C. jejnui* ghosts.

Quantification of Protein and DNA Concentrations:

Based on the spectrophotometer measurement, the quantities of DNA and protein for each supernatant from the three stages of the experiment were calculated in accordance with (Amara *et al*., 2013).

RESULT AND DISCUSSIONS

Viability Test: No growth in the prepared *C. jejuni* ghost cell absorbed as turbidity in the N.B.

Determination of MIC and MGC: The numbers of tubes in which MIC and MGC occurred were as follows after 24 hr. incuboter.

Chemical compound	Number of Tube	
	MIC	MGC
SDS	3	4
H_2O_2	2	3
NaOH	3	4
$CaCO_3$	all tube growth $CaCO_3$ didn't any effect	

In the case of NaOH and SDS, the MIC and the MGC were 0.1 mg \L and 0.01mg\L. In case of H_2O_2 the MIC and the MGC were 0.03% and 0.003% In case of $CaCO_3$ the used amount of +1 value was 1.05 µg\ml while -1 value was o.35µg\ml.

Table 2: DNA and protein concentration during each step of preparing bacterial ghost

Basic Experiment			H_2O_2 Step		Ethanol step		
Experiment No.	Protein % Mg ml^{-1}	DNA Mg ml^{-1}	Protein Mg ml^{-1}	DNA Mg ml^{-1}	Protein Mg ml^{-1}	DNA Mg ml^{-1}	(BGQ)
1	1984.78	353.45	2314.11	389.11	2963.24	176.7	100
2	2657.32	289.43	3621.85	398.83	2784.78	288.6	97

It is commonly recognized that SDS, CaCO3, and NaOH may damage the cell wall. But the only substance that significantly degrades is NaOH.

H_2O_2 having a positive effect on the quality of the cells. Elevated levels of both DNA and protein release signify that the cells have lost their H_2O_2 damage DNA (Nagao *et al*., 2018) contents and have transformed into BGs. Complete cell lysis, however, ought to be avoided (A. A. Amara, M. M. Salem-Bekhit, & F. K. J. T. S. W. J. Alanazi, 2013). It has been documented that SDS and NaOH can disrupt the bacterial cell wall. It is commonly recognized that H2O2 is an oxidant that breaks down DNA (Amara *et al*., 2013).

The first experiment's usage of H2O2(+1) yielded less DNA than the second experiment (-1), with 389.11 µg ml in experiment (1) and 398.83 µg ml in experiment (2). Increased H2O2(1) causes greater DNA degradation, which reduces the reading that a spectrophotometer can measure. Table 1 shows that all experiments were conducted under the same first step circumstances, with the exception that experiment one utilized CaCO3 at a value of (+1) while experiment two used a value of (-1). In this way, CaCO3 can be responsible for both boosting and lowering protein release as well as DNA release. These actions prepared the bacterial ghosts.

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