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BACTERIOPHAGE-MEDIATED MODULATION OF MULTIDRUG-RESISTANT PATHOGENS AND THEIR SUBSEQUENT ENCAPSULATION IN SODIUM ALGINATE MICROSPHERES

Sutripto Ghosh [*1], Tamalika Chakraborty [2], Quazi Sayeed Ahmad [1] and Sharmistha Bhandari [3]

Department of Biotechnology [1], Department of Life Science [2], Department of Genetics [3], Guru Nanak Institute of Pharmaceutical Science and Technology, Kolkata - 700114, West Bengal, India.

Keywords:

Multidrug resistance, Phage therapy, Bacteriophage, Microsphere, Simulated gastric juice

Correspondence to Author:
Mr. Sutripto Ghosh

Master of Science (Biotechnology), Department of Biotechnology, Guru Nanak Institute of Pharmaceutical Science and Technology, Kolkata - 700114, West Bengal, India.

E-mail: mbt21.0127@gnipst.ac.in

ABSTRACT: Antibiotic resistance is one of the gravest threats to human health today. Antibiotic resistance has emerged among hazardous bacteria due to the widespread use of drugs for human health, marine life, and agricultural animals. In contrast, bacteriophages are viruses that parasitize other bacteria for sustenance. A host cell is necessary for phage development and may be found practically everywhere in this ecosystem. The emphasis of the research is on the therapeutic application of bacteriophages in the treatment of a Multidrug resistant potential bacterial pathogens isolated from hospital waste and identified as *Staphylococcus saprophyticus* ATCC 15305, *Staphylococcus aureus, Escherichia coli, Pseudomonas* sp. populations, as well as determining their MIC_{90} value against four different antibiotics, are controlled with the use of bacteriophages recovered from waste as well as river water. In addition, the isolated bacteriophages are coated and packed into prepared sodium alginate microsomes. Furthermore, the stability of the microspheres is analyzed artificially under different simulated gastrointestinal conditions (phage titers of 4.7 Log_{10} PFU/ml to 5.04 Log_{10} PFU/ml were obtained after an incubation period of 1 hour and further increasing to 5.07Log_{10} PFU/ml to 5.11 Log_{10} PFU/ml after an incubation for 5 hours), pH (at pH 5.0, titers of 3.3 Log_{10} PFU/ml to 3.6 Log_{10} PFU/ml was obtained, whereas the phages were completely impotent when pH was lowered to 2.0) as well as their storage capacity at 4°C, with the goal of assessing the release as well as stability of bacteriophages for using it as a potential therapeutic intervention.

INTRODUCTION: Antibiotic resistance is one of the gravest threats to human health today. Antibiotic resistance has emerged among hazardous bacteria due to the widespread use of drugs for human health, marine life and agricultural animals [1]. Furthermore, factors including rising infection rates and chronic disorders requiring antimicrobial therapy have contributed to a rise in antibiotic consumption.

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Since, antimicrobial resistance is growing, measures must be taken to prevent the spread of illness and protect the health of humans and animals [2]. Several efforts are being made to combat this, including developing new, cutting-edge methods like phage treatment.

Also, the development of 16s RNA sequencing and DNA sequencing has opened up new frontiers in the study of the microbiome [3]. While DNA sequencing has led to the sequencing of the genomes of many different kinds of microorganisms, 16s RNA sequencing has helped us get a deeper knowledge of many bacterial communities. As established, bacteriophages are viruses that can exclusively infect bacteria.

The initial step in the infection process involves the phages attaching to the bacterial cells with the help of specific receptors on the host cells [4]. Host cell-specific receptors, including sugars, polysaccharides and proteins, facilitate the contact between bacteriophages and their host cells [5, 6]. Phage infectivity is constrained by a specific recognition sequence in the receptors of the infected host cells [5, 6]. We can now classify bacteriophages as virulent or temperate based on their life cycles. For viral particle cloning, lytic phages hijack a host cell's machinery and subsequently release the resulting viral particles. The lytic cycle is maintained as the viral progeny infect more bacteria [7]. While temperate phages may transmit genetic information to the host cells, virulent phages are more well-adapted for phage treatment [8]. New phages are generated significantly faster by lytic phages than by temperate phages.

Because of the pervasiveness of antibiotic resistance in bacteria, phage treatment should now be prioritised. Additionally, although the breakdown of pharmaceutical drugs leads to the generation of dangerous metabolites, the breakdown of phages does not [9]. But a major drawback of phage treatment is that many phages are preferential for more than one bacterium. This method may infect otherwise healthy bacteria with a phage that is specific to many bacterial species. This might cause a variety of other gastrointestinal issues. Another major drawback of phage treatment is that numerous endotoxins are produced from bacterial cells during lysis due to phage infection. It is believed that endotoxins can trigger allergic reactions and immune system responses [9].

Alginate being acid-resistant and able to regulate the sustained release of living things like probiotic bacteria and bacteriophages into the digestive tract, is a promising method for phage encapsulation [10, 11]. The gel-forming polysaccharide alginate is derived from algae and bacteria and crosslinks with calcium to yield a gel-like structure [11]. The inner matrix must have an appropriate aqueous viscosity to retain the bead structure as well as preserve the loaded phage. Gelatine was tested to enhance the beads' functionality, and honey was tested to sustain the bacteriophage load [12]. Consequently, our research focuses on packaging phage viruses inside sodium alginate microspheres utilizing gelatine to preserve the structure of the bead and honey to prevent the phage load from becoming ineffective.

MATERIALS AND METHODS:

Isolation of Pathogenic Microorganisms: Sewage water was isolated from Guru Nanak Institute of Dental Science and Research, Panihati, Sodpur. The sewage water was diluted using 0.9% Sodium chloride from 10^{-1} through 10^{-5}. 1 ml of 10^{-3} dilution was streaked separatelyinto plates containing EMB agar, Mannitol Salt agar (MSA) and Pseudomonas agar and incubated for 24 hours at 37°C. Two well-distinguished golden colonies were selected and isolated from MSA plate (turned yellow); hence, pure cultures of *Staphylococcus aureus* were prepared in nutrient broth. Further two colonies giving green metallic sheen were isolated from EMB agar plates; thus, pure cultures of Escherichia coli were prepared in nutrient broth. Similarly, pure cultures of *Pseudomonas* sp. were also prepared.

Selection of Multidrug-Resistant Pathogenic Microorganism: We previously established the phenomenon of antibiotic resistance against Amoxicillin, Tetracycline, Cefixime and Azithromycin in *Staphylococcus saprophyticus* ATCC 15305 isolated from the sewage water of Guru Nanak Institute of Dental Science and Research, Panihati, Sodpur. Further, *Staphylococcus saprophyticus* ATCC 15305 was validated using 16s rRNA sequencing [13]. The same Staphylococcus saprophyticus ATCC 15305 sample was used further for bacteriophage isolation. Further, this phenomenon of antibiotic resistance was tested with the same antibiotics against isolated strains of *Staphylococcus aureus, Pseudomonas* sp., *Escherichia coli*and further used for phage isolation. Antibiotic susceptibility testing was performed in Mueller Hinton agar plates. Antibiotic concentrations of 4 µg/ml, 8 µg/ml, 12 µg/ml, 16 µg/ml, and 20 µg/ml were prepared, with attention given to the MIC_{90} values established by the 2020 Clinical and Laboratory Standards Institute recommendations. Mueller Hinton agar plates were used for the antibiotic susceptibility testing. All the experiments were performed in triplets [13].

Preparation of Hard Agar: 2.5gof Peptone, 1.25g of Sodium Chloride, 0.02M Calcium Chloride, 3.75g of agar is dissolved in 250ml of water. The solution is heated such that the constituents dissolve uniformly. The solution is then sterilized by autoclaving at 121°C and 15psi pressure for 15 minutes

Preparation of Soft Agar: 2.5g of Peptone, 1.25ml of Potassium Chloride and 1.5g of agar is dissolved in 250 ml of distilled water. The solution is heated such that the constituents dissolve uniformly. The solution is then sterilized by autoclaving at 121°C and 15psi pressure for 15 minutes.

Water Samples: Total four water samples were collected from four different places. They included river water from two different cities [from banks of Howrah, West Bengal, India (R1) and Kolkata, West Bengal, India (R2)]. The other two specimens included sewage water collected from the same cities (S1 and S2). Water was filtered through a pore size filter of 0.22µm to a sterile screw cap bottle.

Isolation of Bacteriophages using Plaque Assay Method: 1ml of obtained bacterial culture of *Staphylococcus saprophyticus* ATCC 15305 (SS), *Staphylococcus aureus* (SA), *Pseudomonas* sp. (PSU) and *E. coli* (ECO) was mixed separately with 0.5ml of 10^{-3} serially diluted phage filtrate (bacteriophage are diluted by preparing serial dilution from 10^{-1} through 10^{-3}. The phage-host cell suspension was left at ambient temperature for 15 minutes so as to facilitate the attachment of the phages to the host bacterial cell. After 15 minutes, the phage-host cell suspension was mixed with prepared molten soft agar (50°C) and poured over the previously prepared hard agar plate. The molten soft agar was mixed uniformly by gently rotating the Petri plates. The plates were kept in an incubator at 37 degrees Celsius for a whole day. If any viral plaques were found during analysis, the plates were labelled as positive [14]. The plaque forming units per millilitre (PFU/ml) were recorded.

Purification of Bacteriophages: In order to perform this experiment, clear plaques, were located on the petri plate and are very carefully plugged off and added to the pure culture of respective bacterial cultures and incubated for 24 hours at 37°C. The solution is then centrifuged at 3000rpm for 30 minutes to remove cell debris along with agar. The supernatant was collected carefully and stored at 4°C until further processing [14].

Assessment of Phage Dosage for Packaging into Microspheres: Phage dosage was calculated based on measuring PFU/ml using double layer agar plate methods. Isolated phages of each type were incubated with 1 ml of respective host bacterial culture and then incubated for 15 minutes. They were then laid on to double layer agar plates and incubated for 24 hours at 37°C. The number of plaques were calculated following incubation.

Preparing Phage Encapsulation in Microsphere: Phages were loaded into prepared microspheres. 0.6 ml of SA specific phage and 0.8 ml of ECO specific phage and 0.8 ml of PSU specific phage are suspended separately with 0.4% commercialized honey, 1% gelatine, and 1.5% sodium alginate. The suspension was then extruded dropwise into 100mM calcium chloride solution using a syringe. The prepared beads were washed with distilled water after 30 minutes [14].

Preparation of Simulated Intestinal Juice to Assess Phage Stability and Phage Release: In order to study the survivability of encapsulated phages in conditions resembling the intestines, synthetic intestinal juice was utilised, which was made by dissolving bile salt (0.1%) and pancreatin (0.4%) in potassium dihydrogen phosphate (50 mM, pH 7.0). Three different phage strains, phage SA (14 x10^4 PFU/ml), phage PSU (18 x 10^4 PFU/ml), and phage ECO (14 x 10^4 PFU/ml), were encapsulated in Na-alginate beads and then incubated in the prepared synthetic intestinal juice at 37^o Celsius with agitation at 110 RPM. The free phage titer for three distinct phages were evaluated by double layer agar plate method [14, 15].

Assessment of Phage Leakage on Storage: The prepared sodium-alginate beads were immersed in distilled water at 4 °C for a period of 7 days, and then after 7 weeks of storage, samples were extracted daily to test the retention as well as stability of the encapsulated phages [14].

Assessment of Microspheres Under Acidic Conditions: The microspherecapacity to withstand the stomach acid found in the digestive tract was evaluated. The phage-encapsulated beads were suspended in 0.5% NaCl, and the pH was adjusted to 2.0 and 5.0 by adding 1.0 molar Hydrochloric acid; the mixture was then incubated at 37°C for an additional hour. After incubating the beads for an initial hour at 37°C, they were dissolved in a buffer containing sodium citrate (50mM), sodium bicarbonate (0.6M), and tris-HCl (50mM), and the pH was adjusted to 7. The mixture was again incubated at 37°C for an hour. The titre of bacteriophage release was measured using double layer agar plates [14].

RESULTS:

Average Zone of Inhibition Obtained after Charging Antibiotics against the *Pseudomonas* sp., *Staphylococcus aureus, Escherichia coli, Staphylococcus saprophyticus* ATCC 15305:

TABLE 1: ZONE OF INHIBITION OF *PSEUDOMONAS* SP., *STAPHYLOCOCCUS AUREUS, ESCHERICHIA COLI, STAPHYLOCOCCUS SAPROPHYTICUS* ATCC 15305 AGAINST DIFFERENT ANTIBIOTICS

Name of Microorganism	Concentration of antibiotics (µg/ml)	Zone of inhibition of Amoxicillin (mm)	Zone of inhibition of Azithromycin (mm)	Zone of inhibition of Cefixime (mm)	Zone of inhibition of Tetracycline (mm)
Pseudomonas sp.	4	-	0	5	7
	8	-	6	9	26
	12	-	14	12	36
	16	-	22	17	49
	20	-	27	26	58
Escherichia coli	4	0	12	11	5
	8	0	16	26	5
	12	0	20	36	8
	16	0	28	44	11
	20	14	32	60	26
Staphylococcus aureus	4	-	-	1	6
	8	-	-	5	12
	12	-	-	8	22
	16	-	-	14	30
	20	-	-	21	39
Staphylococcus saprophyticus ATCC 15305	4	1	14	0	3
	8	7	16	4	7
	12	12	23	6	11
	16	28	42	11	13
	20	36	45	17	19

TABLE 2: ANTIBIOTIC RESISTANCE PATTERN OF *PSEUDOMONAS* SP., *STAPHYLOCOCCUS AUREUS, ESCHERICHIA COLI, STAPHYLOCOCCUS SAPROPHYTICUS* ATCC 15305IN ACCORDANCE WITH CLSI 2020

Name of Microorganism	Name of Antibiotic	Minimum Inhibitory Concentration (Experimental) [µg/ml]	Minimum Inhibitory Concentration (Standard) [µg/ml]	Outcome
Pseudomonas sp.	Amoxicillin	-	-	-
	Azithromycin	-	-	-
	Cefixime	12	≥32	Intermediate Resistant
	Tetracycline	-	≥16	-
Escherichia coli	Amoxicillin	20	≥16	Resistant
	Azithromycin	4	≥32	Susceptible
	Cefixime	4	≥4	Resistant
	Tetracycline	16	≥16	Resistant
Staphylococcus aureus	Amoxicillin	-	≥4	-
	Azithromycin	-	≥8	-
	Cefixime	8	≥8	Resistant
	Tetracycline	8	≥16	Intermediate Resistant
Staphylococcus	Amoxicillin	16	≥4	Resistant

saprophyticus ATCC	Azithromycin	4	≥8	Intermediate Resistant
15305	Cefixime	20	≥8	Resistant
	Tetracycline	16	≥16	Resistant

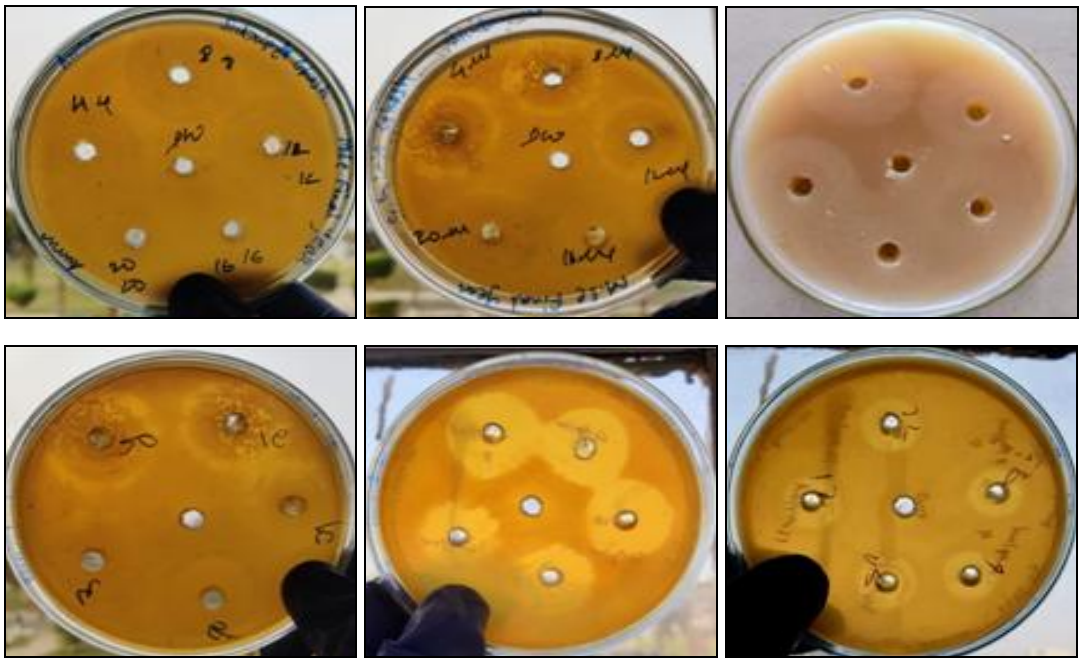

FIG. 1: EVALUATION OF MIC_{90} OF DIFFERENT ANTIBIOTICS USING WELL DIFFUSION ASSAY

Bacteriophages Isolated from Different Sources by Plaque Assay Method:

TABLE 3: PHAGE ISOLATED FROM DIFFERENT SOURCES

Site	SS	SA	PSU	ECO
S1	-	+	-	-
S2	-	-	+	+
R1	-	-	-	+
R2	-	-	-	-

*(-): Negative result; (+): Positive result.

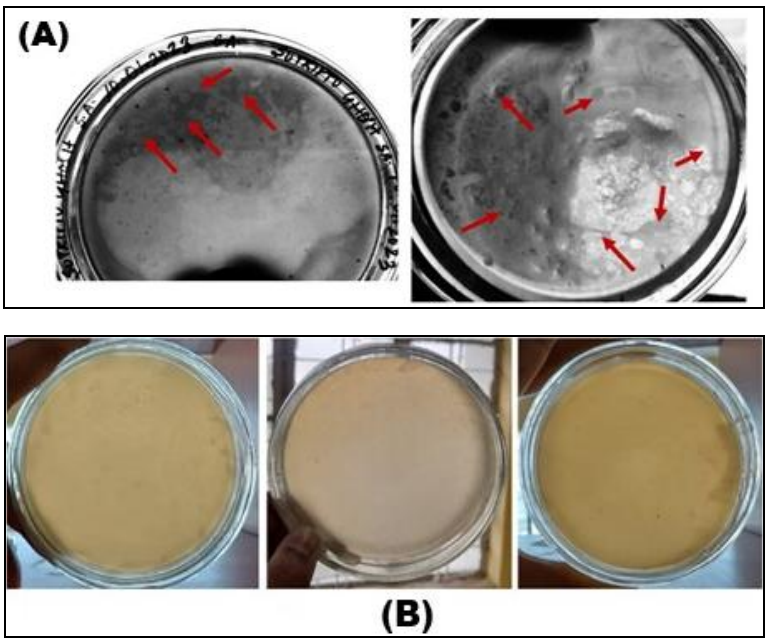

FIG. 2(A): PLATES WITH BACTERIOPHAGE PLAQUES (B) PLATES WITH NO PLAQUES DUE TO THE PRESENCE OF PHAGE-RESISTANT BACTERIUM

Assessing Phage Dosage for Packing into Microspheres: The number of plaques from each plate containing phage was calculated and tabulated in the form of PFU/ml.1ml of bacterial cultures ranging from 5 $x10^4$ to 6 $x10^4$ CFU/ml were each inoculated with specific phage at the concentrations shown below **Table 4.**

TABLE 4: DETERMINATION OF BACTERIOPHAGE DOSAGE FOR PACKAGING INTO MICROSPHERES

Phage Amount	SA Phage Plaque Count (PFU/ml)	PSU Phage Plaque Count (PFU/ml)	ECO Phage Plaque Count (PFU/ml)
0.4 ml	9 x 10^4	6 $x10^4$	7 $x10^4$
0.6 ml	14 x 10^4	11 $x10^4$	10 $x10^4$
0.8 ml	TNC	18 x 10^4	14 10^4

*TNC: Too numerous to count.

Preparation of Phage Encapsulated Microsphere: Sodium-alginate microspheres were successfully prepared. The microspheres exhibited smooth round edges on observation under Binocular light microscope [Magnus Mlx-B plus Led] (Magnification: 4x) **Fig. 4(A).**

Evaluation of Release of Bacteriophages: The produced sodium-alginate beads were submerged in distilled water at 4 °C for seven days, and then seven weeks of storage. Nonetheless, no bacteriophage discharge was seen during the duration of the experiment **Fig. 3.**

FIG. 3: NO VISIBLE PLAQUE DUE TO THE NON-RELEASE OF PHAGE ON STORING THE BEADS IN DISTILLED WATER AT 4°C

Stability of Microspheres and Bacteriophages after Incubation in Gastric Juice: The three distinct phage strains were encapsulated separately in alginate microsphere. The microspheres were incubated in prepared simulated gastric juice. Titers of 4.7±0.2 Log_{10} PFU/ml for phage SA, 4.9±0.1Log_{10} PFU/ml for phage PSU, and 5.04±0.5 Log_{10} PFU/ml for phage ECO were all generated by the beads after an incubation period of one hour. Further on incubation for five hours, titers of 5.07±0.03 Log_{10} PFU/ml for phage SA, 5.17±0.07 Log_{10} PFU/ml for phage PSU and 5.11±0.6 Log_{10} PFU/ml for phage ECO were yielded ($P<0.0001$) **Fig. 4B, E, Fig. 5(A).**

Assessment of Stability of Microspheres and Phage Release under Acidic Conditions: The microsphere beads' capacity to withstand the stomach acid found in the digestive tract was evaluated. All the three distinct phages (Phage SA, Phage PSU and Phage ECO) were encapsulated in sodium alginate beads. The encapsulation of the phages performed significantly well in protecting the phages against the strong acidic environment of the stomach. At pH 5.0, we saw phage SA titers of 3.6±0.3 Log_{10} PFU/ml, Phage PSU titers of 3.3±0.4 Log_{10} PFU/ml, and phage ECO titers of 3.5±0.2 Log_{10} PFU/ml, whereas non-encapsulated phages showed a very significant reduction in titers, by 2±0.3 Log_{10} PFU/ml compared to encapsulated phages ($P<0.0001$) **Fig. 4(C, F), Fig. 5 (B).** However, all three types of phages were rendered inert in an acidic environment (pH 2.0) **Fig. 4(D).**

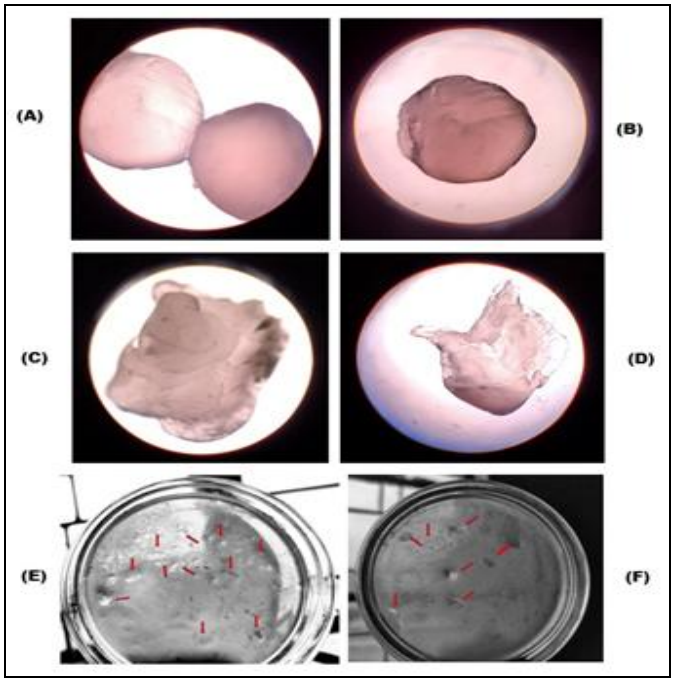

FIG. 4: (A) PREPARED SODIUM ALGINATE BEADS (MAGNIFICATION OF 4X) (B) MICROSPHERE AFTER INCUBATION IN SIMULATED GASTRIC JUICE FOR 5 HOURS (MAGNIFICATION: 4X) (C) MICROSPHERE AFTER INCUBATION IN PH5.0 (D) MICROSPHERE AFTER INCUBATION IN PH 2.0 (E) PLAQUE FORMED DUE TO PHAGE RELEASED FROM MICROSPHERES IN SIMULATED GASTRIC ACID JUICE (F) PLAQUE FORMED DUE TO PHAGE RELEASED FROM MICROSPHERES KEPT IN PH 5.0

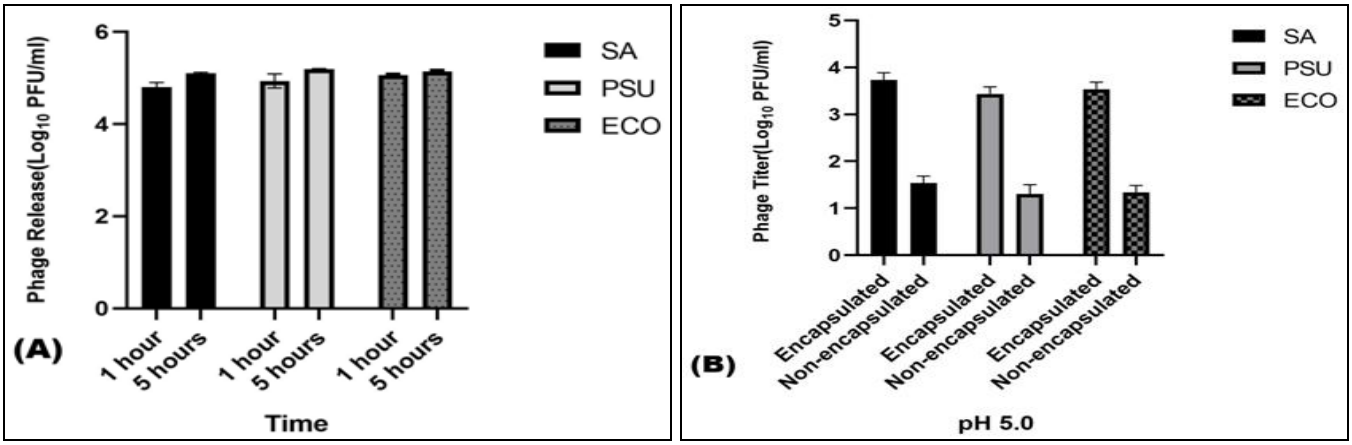

FIG. 5: (A) RELEASE OF THREE DISTINCT BACTERIOPHAGES (PFU/ML) FROM ALGINATE BEADS DURING THE PROCESS OF INCUBATION FOR 5 HOURS (B) COMPARISON OF STABILITY OF MICROSPHERES AND PHAGE TITERS UNDER ACIDIC CONDITIONS

DISCUSSION: Antibiotic resistance has recently become a big problem that threatens humans, animals, and plants. Antibiotic resistance in microorganisms is a huge public health problem, and it is partly due to the inappropriate use of these drugs. Through our research, we have discovered that the aforementioned bacterial strains are resistant to the medications Amoxicillin, Azithromycin, Cefixime and Tetracycline by measuring the diameter (in mm) of the zone of inhibition after being doused with each antibiotic. In our research, isolated *Staphylococcus aureus* has shown resistance against amoxicillin and azithromycin. Previously, *Staphylococcus aureus* has shown resistance against amoxicillin [16]. *S. aureus* has also shown resistance against azithromycin in a previous study [17]. Further, our results agreed with the results established by a previous study, where *Pseudomonas* sp. was found resistant against amoxicillin [18]. The CLSI 2020 recommendations state that the disc diffusion approach for obtaining the minimum inhibitory concentration has not been comprehensively tested for *Pseudomonas* sp.

Viruses that infect bacteria are highly selective for the bacteria they rely on. Of the sixteen water and sewage samples we isolated, only four had phages that were active against the bacteria we were studying. This allowed us to show that phages are extremely selective for their host bacteria. Bacteriophage stability must be considered in the development of any phage-based treatment delivery strategy. Encapsulation of phages is an exciting new method that uses feed-compatible substances without compromising the virulence of the phages. We show that therapeutic administration in case of diseases caused due to MDR pathogens is possible using bead-encapsulation of bacteriophage in artificial gastrointestinal fluid while protecting the phage from severe conditions experienced in the human gastrointestinal system. Encapsulating the three distinct bacteriophages separately in sodium-alginate microspheres showed no reduction in phage titers when stored at 4°C for seven weeks. In this study, the concentration of sodium alginate was reduced from 2%-2.2% to 1.5%, in contrast to previously published scientific literatures [19–21].

Sodium alginate is a natural polysaccharide extracted from brown seaweed and is widely used as a thickener and gelling agent in food and pharmaceutical applications. Its gel-forming properties and the ability to form strong ionic crosslinks with divalent cations such as calcium make it an ideal material for the controlled release of bacteriophages [22]. The sodium-alginate bead system has several advantages over other systems for bacteriophage delivery. For example, it provides a pH-responsive release mechanism, with the release rate of bacteriophages increasing as the pH of the environment decreases. In this work, the bacteriophages were stable and underwent a sustained release at pH 5.0. Yet, when the pH was lowered to 2.0, near-complete dissolution of the microspheres was observed, and the phages were rendered impotent.

Additionally, the sodium-alginate bead system has high mechanical stability, making it suitable for use in harsh environments [21]. The controlled release of bacteriophages SA, PSU, and ECO using the sodium-alginate bead system can thus be said to have potential applications in the treatment of bacterial infections, particularly in the case of antibiotic-resistant bacteria. Bacteriophages are a natural and safe alternative to antibiotics, and the controlled release of bacteriophages using this system could provide a continuous and effective treatment for bacterial infections [7]. Furthermore, this system could also be used for the delivery of other types of phages, as well as other biological entities, for various applications. Our work further highlighted that bacteriophage may be safely delivered to humans as well as animals by sodium-alginate bead encapsulation, which allows for precise dosing in simulated gastrointestinal fluid and protects the phage from the severe conditions of the stomach and intestines.

Future Aspects: The stability of bacteriophages at extreme pH is important in phage-based therapy. It's important to find ways to maintain the activity of phages in different pH conditions, as the acidic or basic environment can greatly impact their effectiveness. We found that at pH 2.0, phages were completely dormant. Therefore, further studies are needed to ensure the stability of bacteriophages at extremely low or high pH to improve the efficacy of phage-based therapy in treating diseases caused by bacterial infections. In addition, combining phages with other nutritional or medicinal components could potentially enhance the healing process and improve the efficiency of phage-based therapy. This can be achieved by using phages as delivery vehicles for these components, allowing for targeted treatment of bacterial infections. It's also important to note that using phages as a therapeutic option is still in the early stages of development. Much more research is needed to understand this approach's potential benefits and limitations fully. However, the results from future studies should be focused on easing our understanding of how to optimize phage-based therapy and make it a more effective treatment option for bacterial infections.

CONCLUSION: The use of bead-encapsulated bacteriophages to treat bacterial infections in humans and animals is an exciting area of research with great potential. The ability of the alginate bead to protect the phages against inactivation by low pH and to sustain their release and activity over time is a significant step forward in the development of effective phage-based therapies. Additionally, investigating the potential of this approach for controlling the intestinal colonization

of zoonotic and pathogenic bacteria could lead to the development of new strategies for managing bacterial infections and preventing the spread of disease. Further research in this area will be critical for advancing our understanding of phage-based therapy's potential benefits and limitations and optimizing its use in treating bacterial infections.

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REFERENCES:

1. Patra B, Chakraborty T and Ghosh S: Prevalence and Transmission of Multi Drug Resistance Gene in *Staphylococcus aureus*. Curr Biotech 2022; 11(3): 196–11.
2. Ghosh S, Patra B and Chakraborty T: Gut Microbiome Drives the Development of Colorectal Cancers and Potential Therapeutic Interventions. IJPSRR 2023; 78(2).
3. Durrant MG and Bhatt AS: Microbiome genome structure drives function. Nat Microbiol 2019; 4(6): 912–3.
4. Kumari P, Chakraborty T and Ghosh S: Advancement of Phage Therapy Approaches in the Battle of Multi-Drug Resistance: A Review. Int J Life Sci Pharma Res 2023 1.
5. Marks T and Sharp R: Bacteriophages and biotechnology: a review. J of Chem Techn & Biotech 2000; 75(1): 6–17.
6. Hyman P and Abedon ST: Bacteriophage Host Range and Bacterial Resistance. In 2010; 217–48.
7. Ghosh S and Chakraborty T: Bacteriophage Mediated Modulation of Gut Microbiome Responsible For Colorectal Cancer. IJPSR 2023; 14(2): 852–9.
8. Monteiro R, Pires DP, Costa AR and Azeredo J: Phage Therapy: Going Temperate. Trends Microbiol 2019; 27(4): 368–78.
9. Abedon S and Thomas-Abedon C: Phage Therapy Pharmacology. Curr Pharm Biotechn 2010; 11(1): 28–47.
10. Gbassi G, Vandamme T, Ennahar S and Marchioni E: Microencapsulation of *Lactobacillus plantarum* spp in an alginate matrix coated with whey proteins. Int J Food Microbiol 2009; 129(1): 103–5.
11. Lee KY and Heo TR: Survival of *Bifidobacterium longum* Immobilized in Calcium Alginate Beads in Simulated Gastric Juices and Bile Salt Solution. Appl Environ Microbiol 2000; 66(2): 869–73.
12. Oliveira A, Ribeiro HG, Silva AC, Silva MD, Sousa JC and Rodrigues CF: Synergistic Antimicrobial Interaction between Honey and Phage against Escherichia coli Biofilms. Front Microbiol 2017; 8.
13. Chakraborty T, Chatterjee S, Datta L and Sengupta A: Antimicrobial activity of *Terminalia bellerica* (Gaertn.) roxb. against multidrug-resistant *Staphylococcus saprophyticus*. Int J Pharm Sci Res 2021; 12(10): 5353–63.
14. Abdelsattar AS, Abdelrahman F, Dawoud A, Connerton IF and El-Shibiny A: Encapsulation of *E. coli* phage ZCEC5 in chitosan–alginate beads as a delivery system in phage therapy. AMB Express 2019; 9(1): 87.
15. Gupta R, Prajapati SK, Pattnaik S and Bhardwaj P: Formulation and evaluation of novel stomach specific floating microspheres bearing famotidine for treatment of gastric ulcer and their radiographic study. Asian Pac J Trop Biomed 2014; 4(9): 729–35.
16. Bantawa K, Sah SN, Subba Limbu D, Subba P and Ghimire A: Antibiotic resistance patterns of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella*, *Shigella* and *Vibrio* isolated from chicken, pork, buffalo and goat meat in eastern Nepal. BMC Res Notes 2019; 12(1): 766.
17. Poiată A, Tuchiluș C, Ambăruș A, Teodor A, Teodorescu I and Luca V: Antimicrobial susceptibility of *Staphylococcus aureus* isolated from colonized hospital personnel. Rev Med Chir Soc Med Nat Iasi 2006; 110(3): 723–6.
18. Thomassen GMB, Reiche T, Tennfjord CE and Mehli L: Antibiotic Resistance Properties among Pseudomonas spp. Associated with Salmon Processing Environments. Microorganisms 2022; 10(7): 1420.
19. Ma Y, Pacan JC, Wang Q, Xu Y, Huang X and Korenevsky A: Microencapsulation of Bacteriophage Felix O1 into Chitosan-Alginate Microspheres for Oral Delivery. Appl Environ Microbi 2008; 74(15): 4799–805.
20. Dini C, Islan GA, de Urraza PJ and Castro GR: Novel Biopolymer Matrices for Microencapsulation of Phages: Enhanced Protection against Acidity and Protease Activity. Macromol Biosci 2012; 12(9): 1200–8.
21. Colom J, Cano-Sarabia M, Otero J, Aríñez-Soriano J, Cortés P and Maspoch D: Microencapsulation with alginate/$CaCO_3$: A strategy for improved phage therapy. Sci Rep 2017; 7(1): 41441.
22. Anal AK and Stevens WF: Chitosan–alginate multilayer beads for controlled release of ampicillin. Int J Pharm 2005; 290(1–2): 45–54.

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