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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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PHAGE-BACTERIA INTERACTIONS UNDER METAL STRESS: A STUDY OF THE NOVEL STENOTROPHOMONAS MALTOPHILIA PHAGE VB_STM18

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Abstract.

Stenotrophomonas maltophilia is a highly adaptable gram-negative bacteria, demonstrating resilience in metal-contaminated environment, which makes it a key subject for understanding microbial survival under heavy metal stress. This study investigates the effects of cadmium ions (Cd^{2+}) on the growth dynamics, cadmium uptake, and bacteriophage *vB_Stm18*-host interactions, with implications for environmental microbiology and applied biotechnology. Growth analysis revealed that *S. maltophilia* tolerates Cd^{2+} at 0.01 g/L, although exposure prolonged the lag phase by 3 hours. Despite the initial growth inhibition, the bacterium adapted and achieved control-like growth levels by 18 hours. Bioaccumulation assays showed progressive cadmium uptake, reaching 1876 µg/g at 24 hours, highlighting its potential for bioremediation. The influence of Cd^{2+} on phage *vB_Stm18*'s life cycle was assessed through adsorption efficiency and burst size measurements. Short-term exposure to Cd^{2+} caused minimal reductions in adsorption efficiency (97% vs. 98% in control) but significantly decreased the burst size to 17 particles per infected cell. Prolonged exposure exacerbated these effects, with adsorption efficiency decreasing to 58% and burst size dropping to 6 particles per infected cell, after 18 hours of pre-incubation. These findings suggest that cadmium alters bacterial surface structures, intracellular processes and disrupts phage replication and release. Therefore, this study sheds light on the molecular interplay between environmental pollutants and microbial systems providing valuable insights into microbial ecology in metal-contaminated habitats as well as informing strategies for optimizing phage therapy and bioremediation under heavy metal stress.

Key words. *Stenotrophonomas maltophilia*, *vB_Stm18*, Cd^{2+} ions.

Introduction.

Stenotrophomonas maltophilia is an opportunistic, Gram-negative bacterium known for its environmental resilience and growing significance in both clinical and ecological settings. This bacterium thrives in diverse habitats such as soil, water, and hospital environments, where it frequently exhibits resistance to multiple antibiotics [1]. As a result, *S. maltophilia* has attracted attention not only as a challenging pathogen but also as a model organism for understanding microbial survival strategies in stressed environment, particularly those containing toxic substances like heavy metals [2,3].

Cadmium (Cd^{2+}) is a pervasive heavy metal pollutant, originating from industrial discharge, mining operations, and agricultural runoff. Recognized for its detrimental impacts on environmental and human health, cadmium accumulates in soil and water, disrupting microbial communities and compromising biological functions. Exposure to Cd^{2+} ions can inhibit cellular activities, generate oxidative stress, and destabilize membrane integrity in microorganisms [4]. However, some bacteria including *S. maltophilia*, display remarkable resilience to cadmium employing mechanisms such as biosorption, efflux pumps, and the production of protective compounds to tolerate or mitigate its toxic effects [5,6]. These capabilities emphasizes *S. melophilia'*s potential role in bioremediation and makes it a compelling subject for studies on heavy metal tolerance [6]. While considerable research has explored how heavy metals impact bacterial physiology, less is known about their effects on phage-bacteria dynamics. Bacteriophages, the viruses that infect bacteria, are also influenced by their environmental conditions. Phage *vB_Stm18*, which specifically infects *S. maltophilia*, provides a unique lens for investigating how Cd^{2+} exposure affects phage-host interactions. Heavy metals can alter various stages of the phage life cycle, including adsorption to host cells, replication within the host, assembly of new viral particles, and lysis for phage release [7]. Investigating these interactions under cadmium stress can reveal how environmental pollutants shape microbial ecosystems and may offer insights into phage therapy applications in contaminated environments. In this study, we aim to examine the impact of Cd^{2+} ions on the life cycle of *S. maltophilia* phage *vB_Stm18*. By analyzing stages of phage infection under cadmium exposure, we seek to understand how metal-induced stress affects viral propagation and bacterial susceptibility. Ultimately, our findings could contribute to a broader understanding of microbial adaptation in metal-contaminated habitats and share strategies for phage-based treatments in similar conditions.

Materials and Methods.

Materials that are used in this study: *Stenotrophomonas maltophilia 18* clinical isolate (George Eliava Collection); phage *vB_Stm18* (George Eliava Collection); heavy metal- Cd $^{2+}$ ions ($CdCl_2$) (Sigma-Aldrich, Co.,3050 Spruce Street, St. Lous, MO 63103 USA) concentrated as -0.01g/l.

Phage isolation and life cycle characteristics.

Phage Isolation and Purification: The phage *vB_Stm18* was isolated from wastewater in the Mtkvari River, Tbilisi, Georgia. Standard protocols were followed for phage isolation, purification, and concentration, referring to previously established methods [8,9].

Phage particles were visualized using TEM. A 10 µL phage sample containing 3×10^{10} PFU/mL was placed on carbon-coated grids and stained with phosphotungstic acid for negative

staining. The stained grids were observed under a Jeol 100-SX transmission electron microscope at 100 Kv [8].

To determine phage adsorption, the phage-host mixture (MOI = 0.1) was incubated in a water bath at 37°C. Samples taken at 0, 5, 10, and 15 minuteswere treated with chloroform, and diluted. These dilutions were then plated on BHI agar with semi-solid overlays, and unabsorbed phages were titrated after 24 hours at 37°C [9].

The latent period and burst size of phage were determined by mixing 0.1 mL of phage with 0.9 mL of host culture to reach a MOI=0.1. After 3minutes of adsorption, the mixture was diluted and transferred to a fresh BHI broth for incubation at 37°C. Samples were taken every 10 minutes for 90 minutes, plated, and incubated for 24 hours at 37°C. Burst size was calculated by comparing viral particles after the eclipse phase with those during the latent phase [9].

DNA Sequencing and Analyses

DNA extraction from the phage lysate was carried out using the Invitrogen Genomic DNA Mini Kit (Thermo Fisher Scientific, Carlsbad, CA, USA). Whole-genome sequencing was conducted on the Illumina NovaSeq X system in paired-end mode at Macrogen Europe, Amsterdam, Netherlands, utilizing a TruSeq PCR-free library preparation with a 350 bp insert size. The NovaSeq X 10B flow cell generated 150 bp paired-end reads, with DNA fragmented mechanically and no spike-in controls included in the sequencing. Quality control of the raw fastq files was performed using FastQC [10] (v0.12.1), and the genome was assembled de novo using SPAdes v3.15.3 with default parameters [11]. GeneMarks v4.28 was employed for predicting open reading frames [12], while Artemis [13] software was used for genome annotation. Functional annotation was conducted using PHROGs v4 [14]. A circular representation of the genome was generated with Geneious software [15]. tRNAs were identified using tRNAscan-SE v1.3.1 [16]. The complete genome sequence is publicly accessible in GenBank under accession number PP554396.1.

Influence of Cadmium Ions on the Growth of the Host Strain

In the preliminary phase of this study, the maximum concentration of cadmium ions that did not exhibit inhibitory effects on the growth of the host strain was identified. Cadmium ions concentrations that were tested includes 0.1 g/L, 0.05 g/L, and 0.01 g/L. The strain was inoculated and incubated at a titre of 5×10^5 cfu/ml in each metal concentration for 24 hours after which its titre was determined via surface plating. After the selection of the non-inhibitory cadmium concentration, its influence on the strain's growth curve was evaluated. The strain was cultured in BHIB broth supplemented with 0.01 g/L cadmium ion, adjusted to a 5×10^5 cfu/ml titer, and incubated at 37°C. Growth measurements were recorded at 0, 4, 6, 10, 14, 18, and 24 hours.

Investigation of Metal Uptake by the Host Strain.

The uptake of metal by the host strain was studied during the logarithmic growth phase (A) and stationary phase (B):

A. Cd^{2+} ions (0.01g/L) were added to the strain culture at a titre of 5×10^5 cfu/ml, and after 24 hours of cultivation, the metal concentration was measured.

B. Cd^{2+} ions (0.01g/L) were added to an overnight bacterial culture with a titre of 5×10^8 CFU/mL and the metal concentration was determined at 1, 3, 5, and 24 hours.

The Cd^{2+} ions concentration in bacterial strain samples was determined as follows: the samples were centrifuged at 5000 g for 30 minutes, and the pellet was resuspended in sterile physiological saline. This washing procedure was repeated thrice. The samples were dried by lyophilization, weighed, and diluted by nitric acid after which the total Cd concentration was measured using an atomic absorption spectrometer (Analyst 800 Perkin Elmer) with an acetylene-air flame on 228.8 nm [17].

Influence of Metal on the Phage Life Cycle.

To evaluate the impact of cadmium ions (Cd^{2+}) on the life cycle of the phage *vB_Stm18*, the adsorption efficiency and burst size of the phage were assessed under varying durations of metal exposure. Three experimental conditions were designed to investigate the effects of Cd^{2+} on the bacterial host and subsequently the phage interactions:

Immediate Metal Addition (0'): Cd^{2+} ions (0.01 g/L) were introduced directly to the experimental setup at the start, with no prior incubation of the bacterial host or phage with the metal.

2-Hour Pre-Incubation with Metal (2'): The bacterial strain was cultured in a liquid medium supplemented with Cd^{2+} ions (0.01 g/L) for 2 hours before initiating the phage adsorption and burst size assays.

18-Hour Pre-Incubation with Metal (18'): The bacterial host was pre-incubated in a liquid medium containing Cd^{2+} ions (0.01 g/L) for 18 hours. Following this extended exposure, the strain was used for phage adsorption and burst size evaluations.

Results.

Phage morphology, life cycle characteristics, and genetics:

The phage *vB_Stm18*, isolated from the Mtkvari River in 2010, belongs to the *Autographiviridae* family. It is characterized by stability and rapid adsorption, with 77% adsorbed within 5 minutes and 98% within 20 minutes, and has a moderate burst size of approximately 61 phage particles (Figure 1A-C).

Genome sequencing revealed that *vB_Stm18* has a circular DNA genome of 43,504 bp with short 421 bp terminal repeats. The genome annotation identified 53 open reading frames (ORFs) out of which 26 were functionally annotated. Specifically, 8 are involved in DNA replication, repair, and metabolism; 13 encode DNA packaging and structural proteins; and 5 are associated with host lysis. The remaining 27 ORFs encode hypothetical proteins with unknown functions (Figure 1D).

The genome of *vB_Stm18* does not contain transfer RNA-encoding regions, nor does it have genes associated with antibiotic resistance, virulence, or lysogenic cycle participation.

Phage *vB_Stm18* shares significant similarity with only one known phage, Stenotrophomonas phage BUCT609 (GenBank: MW960043.1), with an average nucleotide identity of 96%.

Cadmium Ions (Cd^{2+}) and the Growth of the Host Strain:

The maximum concentration of Cd^{2+} that does not inhibit the growth of the *Stenotrophomonas maltophilia 18* strain was determined. It was found that Cd^{2+} ions at a concentration of 0.01 g/L do not have a significant adverse effect on the target strain (Figure 2A).

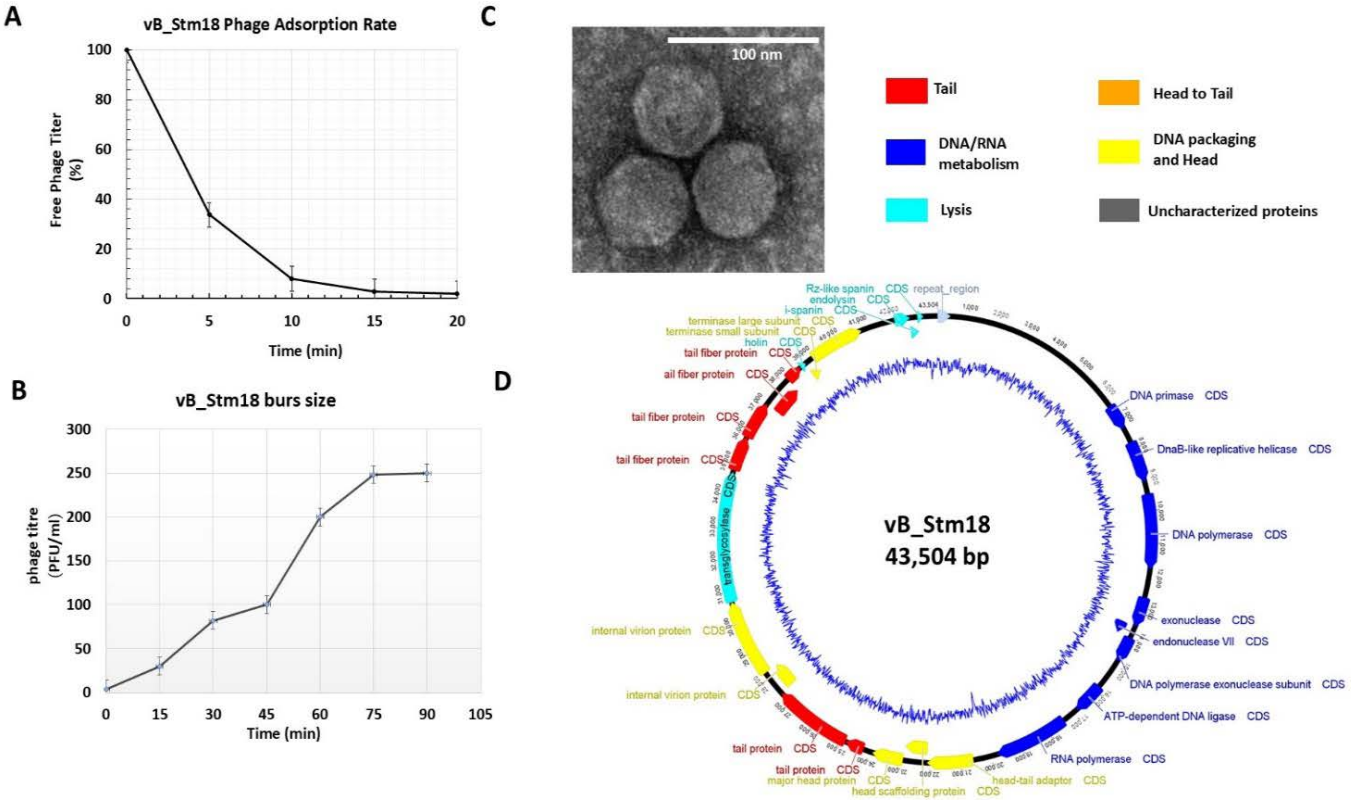

Figure 1. *vB_Stm18 phage biological and genetical characterization: Phage adsorption (A); One step growth cycle (B); TEM image vB_Stm18 virion (Magnification × 320,000) (C); vB_Stm18 genome map (D). The error bars shown in the table indicate the standard deviation derived from three independent experiments.*

As shown in the growth curve diagrams, the presence of metal caused noticeable changes in the *Stenotrophomonas maltophilia 18's* growth pattern compared to the control. The lag phase was extended by 3 hours, and growth initiation began only after 6 hours, reaching levels comparable to the control by 18 hours. At this time, the amount of Cd^{2+} ion absorbed by the strain was 5262 µg/g (Figure 2B).

We used the bacterial strain in its stationary growth phase to study the metal uptake ability of *Stenotrophomonas maltophilia 18*. Results indicated that within 3 hours, the Cd^{2+} concentration uptake was 528 µg/g, after 5 hours it was 580µg/g, and after 24 hours it reached 1876 µg/g (Figure 2C).

Influence of Metal on the Phage Life Cycle:

To examine the impact of cadmium ions (Cd^{2+}) on the phage life cycle, *Stenotrophomonas maltophilia 18* strain was pre-incubated with a fixed concentration of Cd^{2+} (0.01g/L) for varying durations: 0 hours (Experiment 0'), 2 hours (Experiment 2'), and 18 hours (Experiment 18'). Under each condition, phage adsorption and burst size were evaluated.

In Experiment 0', where cadmium was added immediately before initiating the assay, phage adsorption was only minimally affected, showing a slight reduction from 98% in the control to 97% in the test condition. However, significant changes were observed in phage burst size, with a marked reduction to only 17 virions per infected cell as compared to the control.

In Experiment 2', where the bacterial cells were pre-incubated with Cd^{2+} for 2 hours, phage adsorption efficiency dropped considerably, reaching a maximum of only 77% within 20 minutes. The burst size declined further, with infected cells releasing an average of 15 phage particles.

In Experiment 18', where bacterial cells were pre-incubated with Cd^{2+} for 18 hours, the impact on phage adsorption and burst size was even more pronounced. Phage adsorption decreased to 58% within 20 minutes, while the burst size was significantly reduced, with only 6 phage particles produced per infected cell.

Discussion.

This study provides a novel exploration of the influence of cadmium (Cd^{2+}) on the life cycle of the *Stenotrophomonas maltophilia*-specific bacteriophage *vB_Stm18*, illuminating the broader implications of heavy metal contamination on phage-host interactions. Our findings reveal that cadmium exposure disrupts critical phases of the phage life cycle, specifically impacting adsorption efficiency and burst size, with effects intensifying based on the duration of host pre-incubation with cadmium. This investigation, to our knowledge, is among the first to focus on how metal stress alters phage-bacterial dynamics in an environmental context, offering insights relevant to both microbial ecology and potential bioremediation strategies.

This study investigated the influence of cadmium ions (Cd^{2+}) on the growth and metal uptake capabilities of *Stenotrophomonas maltophilia*. The findings highlight the bacterium's ability to tolerate and accumulate significant amounts of Cd^{2+}, despite observable physiological disruptions caused by the metal.

Cd^{2+} ions at a concentration of 0.01 g/L were determined to be non-lethal to the *Stenotrophomonas maltophilia 18* strain, providing a baseline for its tolerance threshold. However,

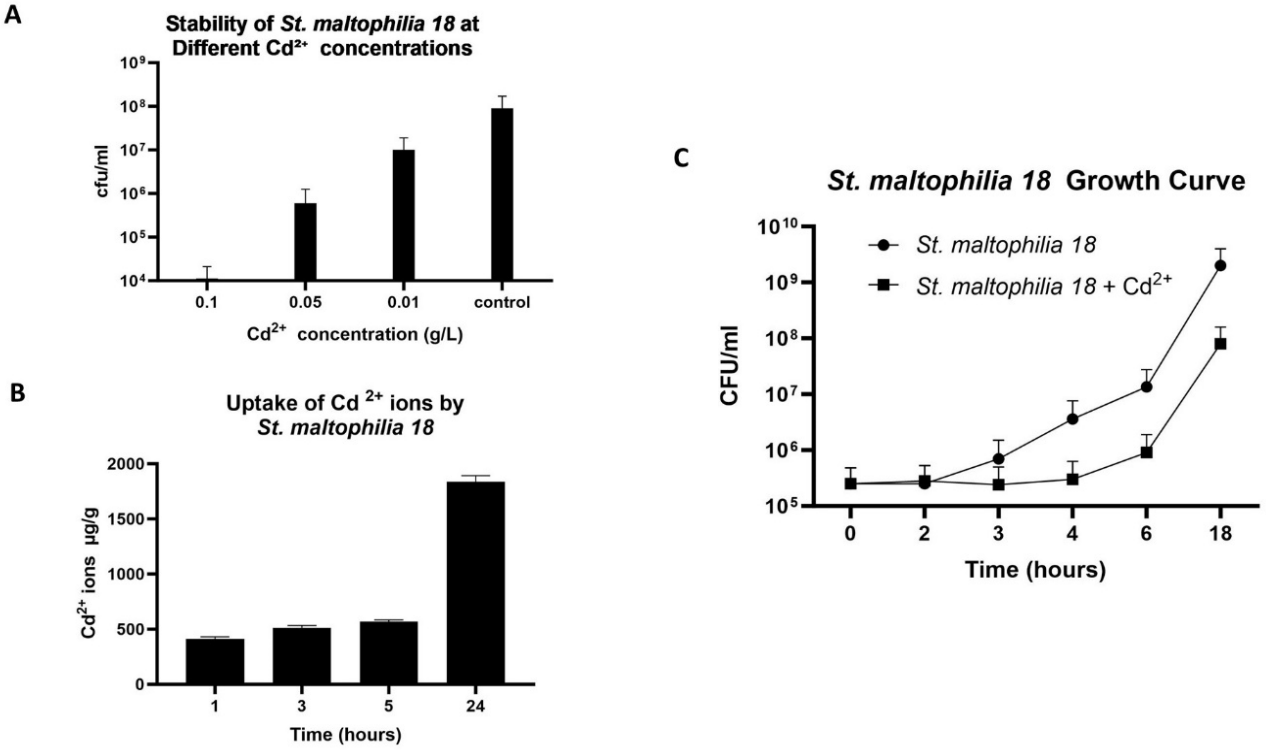

Figure 2. *Stability, Uptake, and Growth of Stenotrophomonas maltophilia 18 in the Presence of Cd^{2+} Ions: Stability of Stenotrophomonas maltophlilia 18 at different concentrations of Cd^{2+} ions (0.1g/l Cd^{2+}p=0.0041; 0.05g/l Cd^{2+} p=0.0041 and 0.01g/l Cd^{2+} p=0.0009)(A); Cd^{2+} ions uptake properties by Stenotrophomonas maltophlilia 18 (B); Influence of Cd^{2+} ions on Stenotrophomonas maltophlilia 18 growth curve (p-value: 0.092)(C). The error bars shown in the table indicate the standard deviation derived from three independent experiments.*

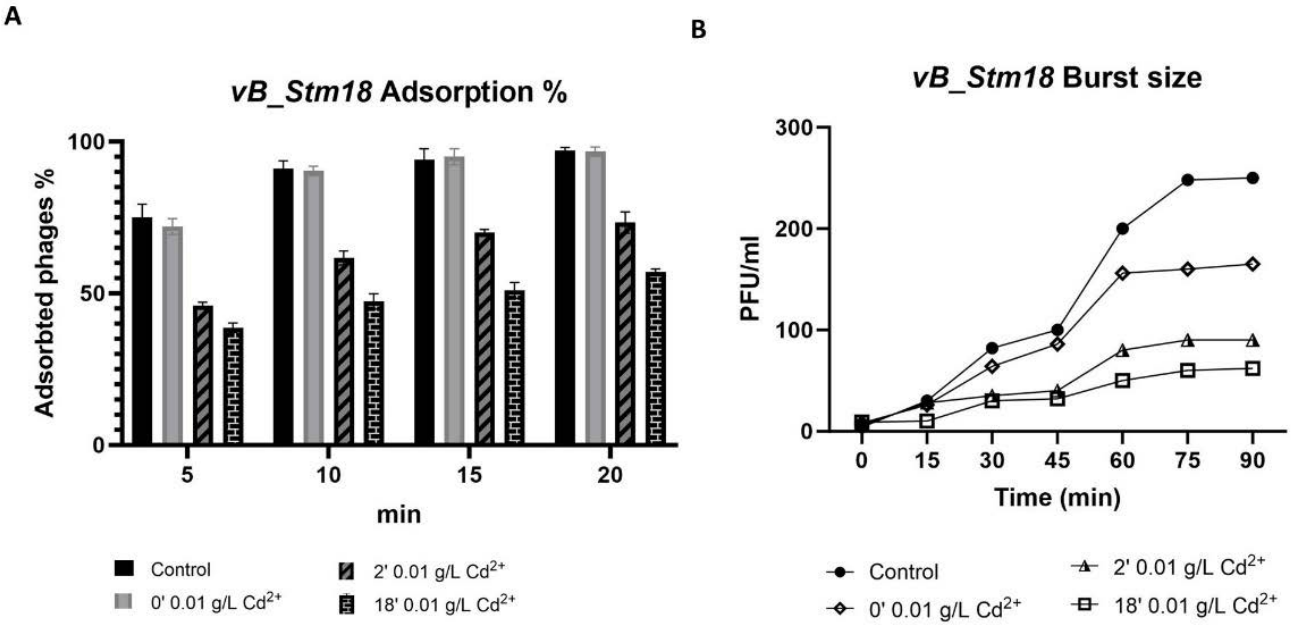

Figure 3. *Influence of Cd^{2+} ions on the Phage vB_Stm18 life cycle:* ***0'-*** *Metal was added directly before initiating the experiment,* ***2'-*** *2-hour host strain pre-incubation with Cd^{2+} ions,* ***18'-*** *18-hour host strain pre-incubation with Cd^{2+} ions. Influence of Cd^{2+} ions on the phage vB_Stm18 adsorption percentage (0' p=0.992); 2' p=0.0095; 18' p=0.0003) (A)Influence of Cd^{2+} ions on the phage vB_Stm18 burst size (0' p=0.160; 2' p=0.0012; 18' p=8.93X10-5) (B). The error bars shown in the table indicate the standard deviation derived from three independent experiments.*

the presence of Cd^{2+} altered the bacterial growth dynamics. The growth curve analysis revealed a substantial delay in the lag phase, extending by 3 hours compared to the control (Figure 2). This delay likely reflects the metabolic adjustments required for the bacterium to adapt to the presence of the heavy metal, including activation of stress response systems and detoxification pathways[18–20]. Growth resumed after 6 hours and reached levels comparable to the control by 18 hours (Figure 2), indicating that *S. maltophilia* can effectively mitigate cadmium-induced stress over time.

The metal uptake analysis further demonstrated the progressive accumulation of Cd^{2+} over time, with the highest recorded uptake of 1876 μg/g at 24 hours during the stationary growth phase (Figure 2). This suggests that *S. maltophilia* utilizes a combination of mechanisms, including biosorption, intracellular sequestration, and potentially active transport, to manage cadmium toxicity. The gradual increase in uptake over time may also reflect the interplay between cell wall interactions and intracellular detoxification processes, which together enable efficient cadmium sequestration [20-23]. The results indicate not only does *S. maltophilia* tolerate cadmium stress but also exhibit a high capacity for bioaccumulation, making it a promising candidate for bioremediation applications in cadmium-contaminated environments[6,24,25].

The results of this study demonstrate that cadmium ions (Cd^{2+}) significantly influence the interaction between *Stenotrophomonas maltophilia 18* and its phage *vB_Stm18*, specifically in terms of adsorption efficiency and burst size. These findings highlight how environmental stressors, such as heavy metals, can alter phage-host dynamics, with implications for both microbial ecology and potential biotechnological applications.

In the absence of prolonged exposure to Cd^{2+} (Experiment 0'), phage adsorption was only slightly reduced (97% compared to 98% in the control), indicating that short-term exposure to cadmium does not immediately disrupt the ability of phages to bind to bacterial cells. However, the burst size was significantly reduced to 17 virions per infected cell, suggesting that cadmium interferes with intracellular processes critical for phage replication (Figure 3). This highlights that even transient exposure to Cd^{2+} can impair phage productivity, potentially through mechanisms such as disrupted energy metabolism, oxidative stress, or compromised protein synthesis in the bacterial host.

Prolonged exposure to cadmium (Experiment 2' and Experiment 18') exacerbated the effects on both phage adsorption and burst size. After 2 hours of Cd^{2+} pre-incubation, adsorption efficiency dropped to 77%, with a further decline in burst size to 15 virions (Figure 3). These results indicate that cadmium begins to interfere with bacterial surface structures, likely altering receptor availability or membrane integrity, which impairs phage binding. Furthermore, the reduction in burst size suggests that cadmium may also inhibit cellular machinery essential for phage assembly and release.

The most pronounced effects were observed after 18 hours of cadmium exposure (Experiment 18'). Adsorption efficiency declined to 58%, and the burst size was drastically reduced to only 6 phage particles per infected cell (Figure 3). This severe reduction highlights the cumulative impact of cadmium-induced stress on the bacterial host, including compromised receptor function, disrupted metabolism, and oxidative damage, which altogether impair the phage's life cycle.

These findings align with previous studies showing that heavy metals disrupt bacterial physiological processes such as membrane stability, enzyme activity, and oxidative stress responses [18,26-28]. The decreased adsorption efficiency observed here suggests that cadmium exposure alters bacterial surface properties, potentially by modifying receptor structure or availability. Additionally, the reduced burst size across all experiments underscores the susceptibility of phage replication to host cell stress, particularly when intracellular resources and energy are diverted to mitigate metal toxicity [5,18-20,23,29,30].

The implications of this study extend to environmental and clinical contexts. In cadmium-contaminated environments, such disruptions in phage-host interactions could influence microbial community dynamics and the efficacy of phage-mediated control of bacterial populations. From a biotechnological perspective, comprehending how heavy metals impact phage replication, can devise strategies for designing phage-based therapies or bioremediation approaches in polluted areas.

Future investigations should explore the molecular mechanisms underlying these observations, including the specific effects of cadmium on bacterial receptors, phage replication machinery, and oxidative stress pathways. Such insights could provide a deeper understanding of how heavy metals shape phage-bacteria interactions and their broader ecological and practical implications.

Conclusion.

In conclusion, this study not only advances our understanding of microbial adaptation and resilience under heavy metal stress but also brings out the complex interactions between environmental factors and microbial pathogens. These insights offer valuable contributions to both the ecological study of microbial communities in contaminated environments and the development of phage-based interventions for pathogen control in challenging environmental conditions.

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Declaration of Competing Interests:

All authors declare that they have no relevant financial or non-financial interests to disclose.

Author's contribution:

All authors contributed to the study's conception and design.

The study was designed, directed, and coordinated by I.K., O.R.

All tasks and methodology were performed by I. K., R.G., L.K., N.K., M.G., S.R., O.R., A.R., A.C

The main text of the paper was written by I.K. and improved and revised by N.K., O.R, visualization and revision were done by R.G and A.C., A.R, N.M

Investigation was performed by O.R., I.K

Writing-editing was done by M.G., O.R., N.M

Sequence analysis and interpretation of data by R.G, L.K

All authors have read and agreed to the given version of the manuscript.

Ethics approval: Not applicable.

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