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Effect of bacteriophage lysin on lysogens

Balaji Subramanyam, Vanaja Kumar*

Tuberculosis Research Centre, Chennai, India

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ABSTRACT

Objective: To study the effect of phage lysin on the growth of lysogens. **Methods:** Sputum specimens processed by modified Petroff´s method were respectively treated with phagebiotics in combination with lysin and lysin alone. The specimens were incubated at 37 ℃ for 4 days. At the end of day 1, 2, 3 and day 4, the specimens were streaked on blood agar plates and incubated at 37 ℃ for 18-24 hours. The growth of normal flora observed after day 1 was considered as lysogens. **Results:** Sputum specimens treated with phagebiotics-lysin showed the growth of lysogens. When specimens treated with lysin alone, lysogen formation was avoided and normal flora was controlled. **Conclusions:** Lysin may have no effect on the growth of lysogens.

1. Introduction

Lysin, an enzyme released by bacteriophages during the final stage of life cycle, lyses the healthy cell wall of host vegetative bacteria. The target of the lysin is murein or peptidoglycan layer of the host cell wall. This phage inducing cell wall degrading enzyme is reported to be effective against extensive range of bacteria, including the genus of *Bacillus*, *Staphylococcus*, *Lactococcus*, *Streptococcus*, *Listeria*, *Salmonella*, *Escherichia*, *Campylobacter*, *Clostridium*, etc. Lysin has shown promise for prophylaxis and treatment of bacterial infections of human and animal origins. Several patents are filed describing the use of lysin to treat human or animal bacterial infections[1]. A novel utility for the use of lysin to control normal flora in processed sputum specimens has been reported[2].

Cocktail of phages has been used to replace antibiotics in processed sputum samples to control the overgrowth of normal flora and was found to be efficient to some extent[3]. Lysin was used to supplement the phage cocktail to achieve stringent control of normal flora leading to an improved methodology[2]. However, as the phages used were temperate in nature, lysogens appeared after 24 hours of incubation. The effect of lysin on the growth of lysogens is unclear and the present work was carried out to resolve this particular issue.

2. Materials and methods

2.1. Effect of lysin on lysogens

Fifty-nine sputum samples were collected, aliquoted into two, randomized and processed by modified Petroff's method and chitin-H_2SO_4 method[4]. To the deposits, 1 mL of phagebiotics-lysin mixture was added and incubated at 37 ℃ up to 4 days. At the end of day 1, 2, 3 and day 4, one loopful of the sample was inoculated on to nutrient agar plate and incubated at 37 ℃ for 18-24 hours. The growth observed after day 1 was considered as lysogens.

2.2. Comparison of lysin alone and in combination with phagebiotics-lysin on the growth of normal flora

Fifty sputum specimens were collected and processed by modified Petroff's method. A loopful of each sample was inoculated on blood agar plates and incubated at 37 ℃ for 18-24 hours (Stage I). The deposits were aliquoted into three portions with each containing 200 μL of the sample. To the first aliquot, 400 μL of nutrient broth (Stage II) was added and used as control. To the second and third aliquots, 400 μL of phagebiotics-lysin mixture (Stage III) and 400 μL of pooled lysin at concentration 1/10 (Stage IV) were added

*Corresponding author: Dr. Vanaja Kumar, Scientist F, Department of Bacteriology, Tuberculosis Research Centre, Mayor V R Ramanathan Road, Chetput Chennai-600 031, India.
Tel: +91-44-2836 9659
Fax: +91-44-2836 2528
E-mail: vanaja_kumar51@yahoo.co.in

respectively and incubated at 37 ℃ for 4 days. At the end of day 1, 2, 3 and day 4, one loopful of the samples from stages II, III & IV was streaked on blood agar plates and incubated at 37 ℃ for 18–24 hours. All the plates were read and the results were compared.

3. Results

3.1. Effect of lysin on lysogens

The samples processed by modified Petroff's method and treated with phagebiotics–lysin mixture resulted in the growth of organisms in 53 out of 59 samples. Growth of organisms was observed at day 1 in only 2 samples denoting initial clearance in most of the samples. The number of samples showing growth of lysogens increased at day 2 (27 samples), day 3 (19 samples) and day 4 (5 samples). When the samples processed by chitin–H_2SO_4 and treated with phagebiotics–lysin mixture, 51 out of 59 samples showed growth of organisms. At day 1 normal flora was grown in only 3 samples. Reappearance of growth was seen at day 2 (24 samples), day 3 (16 samples) and day 4 (8 samples) (Table 1).

Table 1

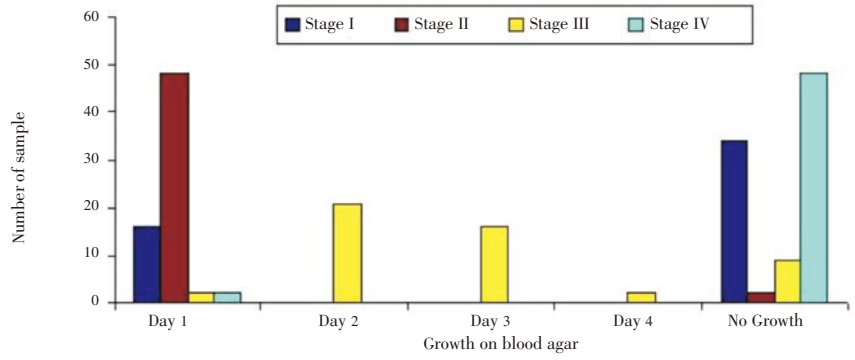
Growth of lysogens in samples after treatment with phagebiotics–lysin

	Day 1	Day 2	Day 3	Day 4	No Growth
Modified Petroff's method	2	27	19	5	6
Chitin method	3	24	16	8	8

3.2. Comparison of lysin alone and in combination with phagebiotics on the growth of normal flora and lysogens

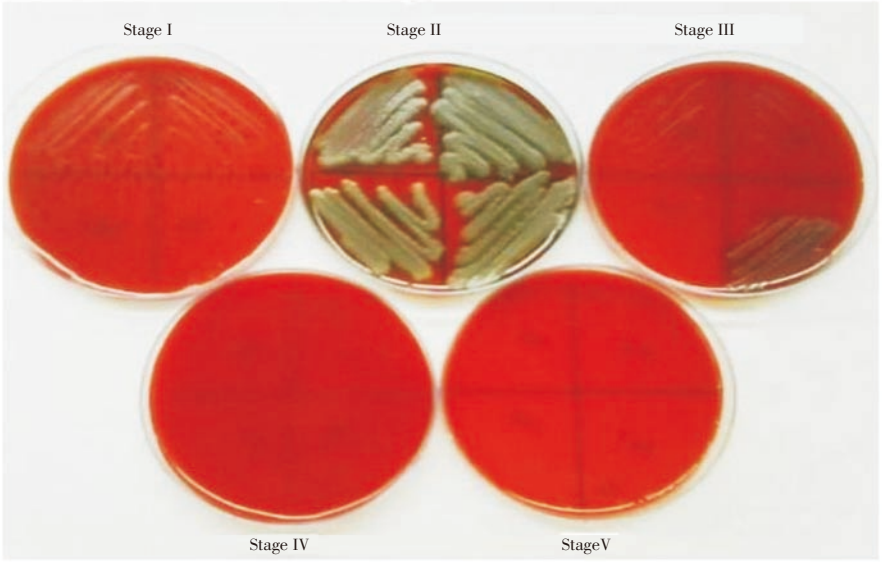
Minimal growth of normal flora was observed in 16 out of 50 samples at day 1 soon after processing the sputum samples (Stage I). Confluent and mixed growth of normal flora was observed at day 1 in 48/50 of the samples when incubated overnight with nutrient broth (Stage II). At the end of day 2, 3 and day 4 no growth of normal flora was observed in stage I and Stage II. The samples treated with phagebiotics–lysin (Stage III) showed the growth of normal flora in more number of samples at day 2 (21), day 3 (16) and day 4 (2). Only 2 samples resulted in the growth of normal flora at day 1. The

A



A. Growth of normal flora observed on different days on incubation

B



B. Growth of normal flora on blood agar

Growth of normal flora on blood agar plates soon after procession of sputum samples (StageI), grown overnight in G7H9 (Stage II), in phagebiotics (stage III), in phagebiotics supplemented with lysin (Stage IV) aand in lysin alone (Stage V).

Figure 1. Comparison of growth of normal flora after treation with phagebiotics and lysin (A & B).

samples treated with lysin (Stage IV) controlled the growth of normal flora in more number of samples (48/50) tested. Only 2 samples resulted in the growth of normal flora at day 1. No growth was observed in stage IV at the end of day 2, 3 and day 4 (Figure 1 & Table 2).

Table 2

Comparison of lysin alone and phagebiotics-lysin on the growth of normal flora and on the formation of lysogens

	Stage I	Stage II	Stage III	Stage IV
Day 1	16	48	2	2
Day 2	0	0	21	0
Day 3	0	0	16	0
Day 4	0	0	2	0
No growth	34	2	9	48
Total			50	

4. Discussion

Lysogenic conversion is a process by which a bacterial cell becomes susceptible to a temperate phage resulting in the integration of the phage genome as prophage integrates into the host genome bringing remarkable changes in the bacterial properties. For example, the structural genes encoding exotoxins, such as diphtheria toxin, botulinum toxin types C1 and D, streptococcal erythrogenic toxin, staphylococcal enterotoxin A, Shiga toxins 1 and 2 (Stx1 and Stx2), Pseudomonas cytotoxin, and cholera toxin (CT), are located in the genomes of temperate bacteriophages that confer toxinogenicity upon their respective hosts[5].

Changes in phenotypic characteristics can also be resulted from the presence of prophages in a bacterial genome. The strains of *Staphylococcus aureus* (*S. aureus*) after getting infected by two different temperate phages LS1 and LS2, differ from the parent strains by the following characters: they are coagulase, deoxyribonuclease, and lipase negative; they are untypable by the basic set of phages; they do not ferment mannitol under anaerobic conditions; and they produce only L (+) lactic acid by glucose fermentation. Interestingly, changes in the cell wall components were observed in *S. aureus* after getting infection by these phages. Moreover, lysogenising phage might induce marked changes in permeability of the lysogenic cells[6].

Jonasson *et al*[7] demonstrated that lysogenization of *Bacillus amyloliquefaciens* H with bacteriophage PK renders the bacteria resistant to superinfection with bacteriophage of PK type. Though, there were no changes in the molar ratio of cell wall components observed between normal and lysogenic cells, it was suggested that minor changes in the cross-linking of the polymers in the cell wall would significantly alter adsorption.

Lysin is a highly evolved enzyme that specifically targets peptidoglycan of healthy and vegetative forms of bacteria. Lysin so far identified contains two domains, *i.e.*, N-terminal catalytic domain and C-terminal binding domain. C-terminal binding domain is specific for particular substrate present in the bacterial cell wall. Unless C-terminal domain binds to cell wall, N-terminal domain will not cleave the bonds present in the peptidoglycan though it is found to be non-specific[8]. Certainly, changes in the composition of cell wall under certain circumstances, such as lysogenic conversion, make the cell resistant to lysin. The result obtained in this study also indicates that the lysogens cause resistance to inhibition by lysine, which may be due to changes in the composition of the cell wall.

Conversion of normal cell into lysogen brings significant changes in the composition of cell wall which make lysin kill them ineffectively. So, lysin may have no effect on the growth of lysogens. Further assays should be conducted to study the effect of lysin used alone on controling the overgrowth of normal flora and preventing the formation of lysogens in processed sputum samples.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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