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Research Article

Isolation and Characterization of Lytic Bacteriophages infecting *Staphylococcus epidermidis*

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ABSTRACT

The *Staphylococcus epidermidis* isolates were obtained during a period extended between September 2014 and January 2015, depending on biochemical tests and VITEK 2 system. Several sewage water samples were assayed using a plaque assay of double agar overlay as a source of *S. Epidermidis* phages. The bacteriophages were described depending on plaques size and shapes. Phage 1 was the most predominant in the bacterial lawn and able to infect other *S*. species such as *S. aureus.* Therefore, it was decided to studythe effect of temperature on its original titer. The results revealed a gradual decrease in the phage titer with increasing dilution number.

Each temperatures at several incubation periods, significantly vary depending on phage titer. The optimum temperature was 40 ° C , while the 80 ° C was represented the inhibitor temperature. L.S.D. at level (0.05) for interaction was 39.552. The pH 6.5 – 7.5 were represented the optimal pH for the best phage activity while the phage titer beginning to decline in above and below this range of optimal pH , L.S.D. at level 0.05 was 17.898. In conclusion, our study found that Phage1 was considered as predominant phage because of their ability to infect other Staphylococci species such as *S. Aureus.*

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INTRODUCTION

Staphylococcus epidermidis predominantly colonizes the mucous membranes, groin, and axillar areas, as well as the cutaneous system of the human body, with bacterial counts of up to 10 to 10^3 CFU/cm^2 [1]. *S. epidermidis* are usually considered a harmless commensal microorganism; however, infections can occur in immunocompromised individuals and in patients with indwelling or implanted medical devices such as prosthetic heart valves and joint prostheses, where the staphylococci penetrate cutaneous and mucosal barriers [2]. With the increasing use of such devices in medical practice, several million people are affected by complications arising from *S. epidermidis*infections [3]. However, mucosal colonization by Coagulase-negative staphylococci (CoNS) is well established, suggesting that mucosal sites might be an important source of CoNS bacteriaemia [4]. The nosocomial pathogen causes infections on prosthetic valves, cerebrospinal fluid shunts, joint prosthesis vascular prostheses, valves, and in postoperative wounds and the urinary tract. It is also the most frequent organism found in the blood of bone marrow transplant patients and in central venous catheters in patients of total parnteral nutrition [5]. It is worth pointing out that neonates, the immunocompromised and hospitalized patients are considered the main infected groups. Presence of various virulence factors and resistance to β-lactam drugs such as penicillin and methicillin has increased the infections [6] and stability of this micro-organism in different parts of the human body [7]. During the last decades, it has become widely accepted that bacterial viruses or bacteriophages are extremely abundant and exert enormous influences on the biosphere. phags kill between 4-50 % of the bacteria produced everyday, are a driver of global geochemical cycles and a reservoir of the greatest genetic diversity on earth [8]. They are seen as a possible therapy against multi-drug-resistant strains of many bacteria[9,10]. The prospects of lytic phages as biocontrol agents against pathogenic bacteria are being reconsidered worldwide with the surfacing of multiple antibiotic resistances [11]. Thus, it is thought that phage therapy could be superior to antibiotic therapy in terms of the ability of the treatment to evolve in response to the development of resistance by the target bacterium. Off-target effects of antibiotic therapy can have detrimental effects on non-pathogenic normal flora, but such effects are expected to be minimal with phage therapy [11]. Bacteriophages arefound in all bacteria, so it is hoped to be able to developcontrol therapies against pathogenic bacteria such as antibiotic-resistant streptococci, *Staphylococcus aureus* and *Streptococcus pneumonia* [12].

MATERIAL AND METHODS

Effect of temperature factor in phage survivability

It was studied by a method adapted from [13] with some modification where 900 µl of D.W tubes were preheated in series temperatures 40, 50, 60,70, and 80 °C then 100 µl of phage lysate was added to each preheated tube and each tube incubated for several different periods starting from 0,

10, 15, 20, and 25 minutes then immediately chilled by placing them in ice , each temperature tube was assayed using plaque assay of over layer method in triplicates , number of survival plaques as (PFUs) were determined after incubation of the plates with the control (192 X 10^2 PFU/ml) at 37 °C for (18-24 h.) .

pH

Sufficient phage lysate were added to each 7 tubes containing aliquots of nutrient broth medium where adjusted to pH beginning (2 , 4 , 6 , 7 , 8 , 10 and 12) with (1M) HCl or (1M) NaOH to give an initial titer of (10^3 PFU/ml) all tubes were incubated at (37 °C for 1 h.), then diluted and assayed by plating in triplicates to determining surviving phages, any pH was assayed as percentage of maximum survival [14-15].

RESULT

The Thermal Effect on S. epidermidis Phage Titer

The influence of temperature on *S. epidermidis* phages was studied by subjecting the phage at titer of (280 x 10^2 PFU / ml) to different heating temperatures starting with 40 °C, 50 °C, 60 °C, 70 °C, and 80 °C, for several incubation periods (5 , 10 , 15 , 20 , and 25 min) in triplicates. After incubation all the tubes were assayed using the double agar overlay method to calculate the titer (PFU / ml) for each temperature.

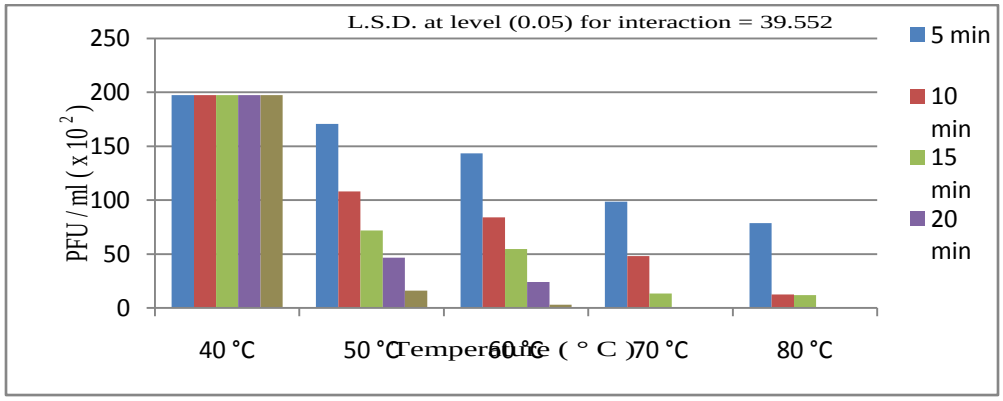

Figure 1: the effect of overlapping of temperature and incubation period on determining *S. epidermidis* phagetiter PFU / ml.

The Effect of pH onS. epidermidis Phage Titer

The importance effect of pH on the phage effectiveness was clearly appeared at ($p < 0.05$), the optimum pH for the highest titer (228 x 10^2 PFU / ml) was obtained in this study extended between 6.5 and 7.5 , all these titer were accomplished under the other suitable conditions and incubated at 37 ° C for 24 hours, these titers were significantly decreased dramatically with rising or lowing the pH until reach to the lowest observed phage titer at pH (12). Figure 2.

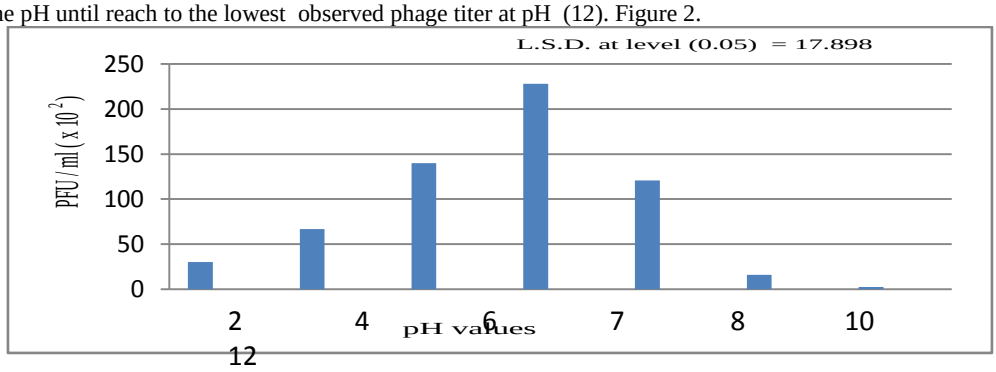

Figure 1: the effect of pH on *S. epidermidis* phage effectiveness

DISCUSSION

Thermal Effect on S. epidermidis Phage Titer

Temperature is one of the members factors such as (pH and ions) which has an important effect on the phage adsorption rate during infection [16]. Different environmental features like temperature and the chemical makeup of the phage-host ecology have a substantial influence [17-18]. In this study the influence of temperature on *S. epidermidis* phages was significant at ($P < 0.05$) when subjected the titer of (280 x 10^2 PFU / ml) to a different heating temperatures starting with 40 °C , 50 °C , 60 °C , 70 °C , and 80 °C, each temperature for several incubation periods (5 , 10 , 15 , 20 , and 25 min). The phage titers gives statisitcaly highly significant ($p<0.05$) variations for phage titers as (PFU /ml) at variable

temperature degree, we were founded that a rising in temperature, together with rising exposure period to that temperature, decreased phage activity and their reproduction rate , this study revealed the variety of overlapping to each temperature and incubation periods in figure (4-7) with L.S.D. = 39.552, the significant variance was appeared in the higher constant titer at every three incubation periods when phage was exposure to 40 ° C until it was dramatically decreased with increase temperature degree and incubation period until it reaches to the absence of any plaques at 80 °C and incubation period 25 minutes. These results were nearly similar to the results that were obtained by Zhang [19] whofound that theactivity of phage SPW remained stable in a wide range of temperature up to 40 C. Towards extreme conditions, it declined significantly after heating for 10–60 min at 60 or70 °C, and was completely inactivated when heated to 70 °C for 40 minutes. Response of phages on exposure to varying temperatures is considered as a key model for understanding the ability of the organism under question to adapt to novel environments [20-]. The plates wereovernight at each temperature and the number of PFU were calculated for each time interval and this was plotted versus time exposed to that temperature.A temperature of 40 °C, pH 8 and 0.25 M NaCl were found to be optimum for phage adsorption and it was able to survive up to a temperature of 50 °C for 3 min Augustine *et al.* [21].

The Effect of pH on S. epidermidis Phage Titer

This factor was studied by using different pH values (2, 4 , 6 , 7 , 8 , 10 , and 12) to determining the phage activity from their titer for each pH value , other cultural and environmental in optimum case, a significant difference at ($P < 0.05$) with LSD = 17.898 , this variance was observed in a high titer at pH 6.5 – 7.5 which represent the optimal pH for the best phage activity while the phage titer beginning to declining with above and below this range of optimal pH figure (4-8) these results were closed with other studies of Basdew and Laing [19, 22]. While, Other researches were exposed the phage to pH ranging from 7-10. They observed that the maximum number of phage particles survived at pH 8, with phages showing viability even at higher pH (up to 12) though in few numbers. The affinity of ΦSP-1 for the alkaline environment is easily explained, since they were isolated from intestinal contents, where the pH normally is 8 or higher in caecum [23]. Anotherfinding stated that the optimum pH for the enzyme activity is important as pH can interfere with lysozyme or protein coat, thereby preventing phage attachment to the receptor sites of the host cell [24,25].

CONCLUSION

In this study, we found that Phage1 was considered as predominant phage because of their ability to infect other Staphylococci species such as *S. Aureus.*

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