Annals of Agricultural Science (2016) **xxx**(xx), xxx–xxx

HOSTED BY

ELSEVIER

Faculty of Agriculture, Ain Shams University

Annals of Agricultural Science

www.elsevier.com/locate/aoas

Improved antibacterial efficacy of bacteriophage-cosmetic formulation for treatment of *Staphylococcus aureus in vitro*

Sabah Abo-elmaaty, Noha K. El Dougdoug *, Mahmoud M. Hazaa

Botany Dept., Faculty of Science, Benha University, Benha, Egypt

Received 4 August 2016; revised 28 August 2016; accepted 31 August 2016

KEYWORDS

Cosmetic;
Bacteriophages;
Antibacterial;
Staphylococcus aureus

Abstract Currently phages are used as alternative antibiotics for treating pathogenic bacteria causing skin disease. However, the efficacy of pure preparations of phage is greatly reduced due to its short longevity on surface of skin. supplemented cosmetic phages [0.5% phage conc./cosmetic] significantly increased phage longevity on skin surface. The phages were isolated by the single plaque assay from the infected skin showing edema and erythema symptoms. The isolated phages had plaques with 3–5 mm diameters and a distinct translucent spreading halo. The morphological phage particles were cubic nucleocapsid with 65–75 nm across with short contractile tails. The supplemented cosmetic phages reduced the bacterial growth to 95.45%, compared with free phages and non-supplemented cosmetic 86.1% and 77% respectively. The phage containing cosmetic was applied for disease treatment and increased the phage longevity from 24 to 100 h and preserved initial phage population. This work indicated the enhanced antibacterial efficacy of fortifying specific bacteriophage in cosmetics to be a promising formulation for efficient treatment of skin diseases.

Introduction

In recent years, antibiotic resistance has become one of the biggest threats to public health. Conventional antibiotics aim to kill or inhibit the growth of pathogenic bacteria leading to a strong selective advantage for pathogens to develop resistance in many cases (Jo et al., 2016). Therefore, new approaches to develop bioactive preparations as novel antimicrobial agents have been proposed that entail targeting virulence of the pathogens without inhibiting their growth therapy reducing or slowing the selection for resistance (Medellin-Pena et al., 2007). Attachment of microorganisms to skin contact surface can impact antibiotics industry economically and through associated health risks. Pathogenic bacteria have been shown to attach to a wide variety of skin contact and non-contact surfaces (Barak et al., 2005). So finding natural novel biofilm inhibitor products is of much interest (Bazargani and Rohloff, 2016). A biofilm is a functional consortium of microbial cells that adhere to a wet surface and become immobilized in a protective polysaccharide matrix that can entrap nutrients and other microbes, allowing for subsequent microbial growth.

* Corresponding author.
E-mail address: Nohaeldougdoug@gmail.com (N.K. El Dougdoug).
Peer review under responsibility of Faculty of Agriculture, Ain-Shams University.

http://dx.doi.org/10.1016/j.aoas.2016.08.002

Attached microorganisms are generally more resistant to sanitation chemicals that are their detached counterparts. This resistant is due to protection from organic materials and the extracellular polymeric substance (EPS) layer, which prevents chemicals from entering the biofilm or causes inactivation of the sanitizer (Lister and Horswill, 2014; McCarthy et al., 2015).

If weather conditions are favorable for disease development, there are no adequate control measures to manage the disease (Kucharek, 1994). Several alternative control methods have been investigated in recent years; another approach for biological control is the use of bacterial viruses to control bacterial diseases. Phages have long been proposed as human disease control agents and have been used in several human bacterial pathogens (Zaccordelli et al., 1992; Balogh et al., 2003).

However, the phages applications were in effective for controlling pathogenic bacteria and phage endurance was significantly reduced (Jones and Pernezny, 2003; Obradovic et al., 2002). Viruses are very fragile and cannot reside long on skin surface because they are quickly eliminated by harmful environmental factors such as temperature moisture and sunlight UV (Mc Guire et al., 2001). Therefore, the need arose to develop for mutations and/or change the application strategy, such as time of application in order to protect phage particles from harmful environmental factors. The accordingly enhanced residual activity of the phages could lead to increase efficacy of phage treatments and to a more convenient application schedule (Balogh et al., 2003; Jones et al., 2007). The objective of this study was to isolate *Staphylococcus aureus* from infected human skin and to investigate *in vitro* efficacy of phage supplemented cosmetic formulation for treatment of *S. aureus* causing skin disease.

Material and methods

Sampling collection

Different samples of clinical symptoms, edema and erythema (can also give rise to focal accumulation of pus or fluid) were collected from forty patients at Benha hospital and were tested for isolation *S. aureus* strains. Swabs carried with nutrient media passing up and down twice on the infected areas. The samples were incubated for 48 h at 37 °C. Swabs were prepared in triplicate for each sample.

Isolation and identification of bacteria

The samples were transferred from the swab and inoculated on the surface of nutrient agar Petri dishes by streaking method and incubated at 37 °C for 48 h for bacteria isolation. The single colony was streaked on Blood agar media and incubated at 37 °C. The pure cultures of unique colony types were obtained and saved for further analysis. The isolated bacteria were identified and classified on the basis of their morphological and biochemical properties following Bergey's Manual of Determinative Bacteriology as well as biochemical analysis (Holt et al., 1994). In addition, Vitek analysis method using VITEK® MS from bioMerieux, France, was applied after biochemical tests as confirmatory test for bacteria, aerobic and facultative bacteria identification.

Isolation and identification of bacteriophages

The swabs rich with nutrient medium and passing up and down twice on *focal accumulation of pus or fluid* were used to inoculate nutrient broth containing flasks. The flasks were shaken on a rotary shaker for 72 h at room temperature on 3000 rpm for 20 min. The flasks were inoculated with *S. aureus* isolates at log phase culture approximately 4×10^6 CFU/ml in nutrient glucose broth (2.0 g/L yeast and 2.5 g/L glucose) to achieve a multiplicity of infection varying between 0.02 and 2.0. The flask cultures were incubated and shaken continuously overnight at 37 °C in shaking incubator. Bacterial cell and debris were removed by centrifugation at 6000 rpm for 15 min. The obtained phages suspension was propagated by plaque assay method to obtain at least 10^8 PFU/ml. The phage mixtures consisted of four phage isolates and had an approximate final titer of 1×10^{10} PFU/ml. The phage mixtures were stored in 2 ml Eppendorf tube at 4 °C in complete darkness.

Phages morphology

Transmission electron microscope (TEM) was used to detected phages specific to *S. aureus* using negative staining method with 1% aqueous urinal acetate. The grids were air-dried and were examined by TEM (JEOL – JEM – 1010 Electron microscope) in The Regional Center for Mycology Al-Azhar University, Egypt, according to Heringa et al. (2010).

Phage infectivity

S. aureus was diluted in sterile distilled water to a density of 10^7 CFU/ml and inoculated on nutrient agar plates. The phage drop (20 µl) of each isolate was over layered on agar. The plates were incubated at 28 °C overnight. Clear confluent lysis, and turbid confluent lysis were recorded as positive result, while extremely faint zones were considered negative result (Heringa et al., 2010).

Formulated cosmetic

Skin cream composed of Steric acid 6%; Propylene glycol 3%; Paraffin oil 7%; Isopropyl myristate 3%; Tocopherol acetate 0.5% and Rosemary 0.2% desolated in water (Capparelli et al., 2007).

Phage treatment

S. aureus culture was harvested from nutrient agar plates 24 h post inoculation and suspended in sterile water and adjusted to A660 = 0.5 by spectrophotometer which approximately is 10^8 CFU/ml. Three Petri dishes of each treatment were inoculated with bacterial suspension using a hand-hold plastic sprayer until completely wet. The suspension phage mixtures were adjusted by spectrophotometer which approximately account for 1×10^{10} PFU/ml and is used for formulated and non-formulated biological treatment. As well as three Petri dishes were without phages as control. Inoculated Petri dishes were incubated in growth incubator at 37 °C for 48 h.

Please cite this article in press as: Abo-elmaaty, S. et al., Improved antibacterial efficacy of bacteriophage-cosmetic formulation for treatment of *Staphylococcus aureus in vitro*. Ann. Agric. Sci. (2016), http://dx.doi.org/10.1016/j.aoas.2016.08.002