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RESEARCH ARTICLE

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NON-SPECIFICITY OF PHAGE ENZYMES

W. N. PAUNIKAR*, S. G. SANMUKH AND T. K. GHOSH

Applied Aquatic Ecosystem Division, National Environmental Engineering Research Institute, Nehru Marg, Nagpur- 440 020, India

W. N. PAUNIKAR
Applied Aquatic Ecosystem Division, National Environmental Engineering Research Institute, Nehru Marg, Nagpur- 440 020, India

**Corresponding author*

ABSTRACT

The phage enzymes can lyse the host in which they resides, but it is not yet known whether they show any effect on the non-susceptible hosts on solid media (Consortia). It is observed during our studies that the consortial lawn on the solid medium shows clear plaques irrespective of their specificity to the inoculated phages. The consortial studies indicate the combined application of phage enzymes for controlling pathogenic organisms. Our experiment thus proves the non-specificity of phage enzymes towards host cell components and states that the activity of phage enzymes is totally substrate (cell wall components) specific and is independent of host type.

KEYWORDS

enzymes; non-susceptible; Consortia; plaques; pathogenic; substrate

1. INTRODUCTION

The majority of endolysins show narrow range of lytic activity against bacterial cells when applied exogenously (Loessner, et. al, 1997) and the spectrum of enzyme activity is restricted to the host bacterial species from which it is derived (Zimmer et. al 2002, Loeffler, et. al, 2001) and sometimes it is genus specific (Loessner, et. al, 1997 & Loessner, et. al, 2002). Amidases is reported to have broad range of antibacterial activity (Navarre, et. al, 1999). The broad spectrum lysins like PlyV12 (amidase) of Enterococcus faecalis phage Φ1 which exhibit lytic activity against streptococcus pyrogenes, group B and C streptococci, and S. aureus (Yoong, 2004) and Mur-LH (muramidase) of Lactobacillus helviticus phage Φ 303 shows lytic activity against 10 different bacterial species (Deutsch, et. al. 2004). The other example of lysins includes S. agalactiae B30 phage lysin (Pritchard et al. 2004) and clostridium perfringens phage Φ3626 Ply3626 amidase (Zimmer et al. 2002). The antibacterial activity of endolysins is to cleave the covalent bonds in peptidoglycan of bacterial cell wall (Loessner 2005, Fischetti 2004 , Navarre et al. 1999).

Polysaccharide depolymerases is produced in phage infected bacteria. The enzyme produced during the lytic activity of the phage, releases the phage progeny outside the host destroying the host cell membrane. Lysins can be endo-beta-N acetyl-glucosaminidases or N-acetylmuramidases (lysozymes), which act on the sugar moiety, endopeptidases, which act on the peptide cross bridge, or more commonly, an N acetylmuramoyl-L-alanine amidase (or amidase), which hydrolyzes the amide bond connecting the sugar and peptide moieties. Typically, the holin is expressed in the late stages of phage infection, forming a pore in the cell membrane, allowing the preformed lysin(s) to gain access to the cell wall peptidoglycan, resulting in release of progeny phage (Vincent 2005).

Phages encode murein-degrading enzymes that hydrolyse either the glycosidic linkages between the amino sugars of the peptidoglycan (glucosaminidases, lysozymes), the N acetylmuramoyl–L-alanine amide bond between the glycan strand and the cross-linking peptide (amidases), or the inter-peptide bridge linkages (endopeptidases) (Lopez et al.

1997). Phage lytic enzymes have recently been used successfully as tools to destroy the cell wall of pathogenic bacteria such as Streptococcus pyogenes (GAS), S. pneumonia and Bacillus anthracis, which has led to the designation of these muralytic proteins as enzybiotics (Nelson et al. 2001 , Schuch et al. 2002). Phages influence the evolution of bacterial genomes (in the development of bacterial Pathogenicity) (Boyd & Brussow 2002.), and they might provide potential tools to face the antibiotic resistance crisis in medicine. These enzymes are highly evolved molecules designed for a specific purpose, to quickly destroy the bacterial cell wall (Vincent 2005).The lytic activity of phage enzymes is also observed in growth media, phosphate buffer, and even human blood (Sulakvelidze et al. 2001). The present work interprets that; phage enzymes can be useful in lysing phage non-specific hosts in consortia of different hosts and their phages.

2. MATERIALS AND METHODS

To prove our hypothesis, we first studied the consortial effect of host and phage limited to a particular host showing variation in phage susceptibility. For this *E.coli* was selected as a control organism.

2.1. Enrichment of Host

The organisms used as a control host were *E.coli* (ATCC 15597), *E.coli* (ATCC 13706), *E.coli* (MTCC 1585), *E.coli* (MTCC 1588), *E.coli* (MTCC 521) & *E.coli* (MTCC 1650). The enrichment of different *E.coli* hosts was done in EC media (Standard methods 1998).

2.2. Enrichment of Phage

The bacteriophage ATCC 13706-B1 and MTCC 1742 was enriched in EC broth (Standard methods 1998).

2.3. Procedure

To study the effect of phage enzymes on the hosts the experiment was designed to facilitate the effective activity of lytic enzymes released from lysed cells. The concentrations of hosts were so adjusted during plating that the plaque formation due to surrounding cell lysis can be seen.

We first assayed all six hosts for phage susceptibility by plaque assay method. Each host was individually inoculated by selected phage and pour plated (Standard methods 1998). The plates were observed for plaque formation. *E.coli* (ATCC 15597) and *E.coli* (ATCC 13706) were susceptibility for ATCC 13706-B1 whereas, *E.coli* (MTCC 1585) and

E.coli (MTCC 1650) were susceptibility for MTCC 1742.

To study the effect of phage on consortia of different hosts among same and different genus, we inoculated different hosts in equal volume so as to meet the requirement of the standard methods and other components were kept constant and pour plated. The plates were observed for plaque formation (Standard methods 1998).

3. RESULTS

The results obtained for the hosts inoculated with phage separately showed that only the four hosts susceptible for phage showed plaques formation after incubation, but other two hosts showed no formation of any plaques due to their insusceptibility to phage. In the other set of experiment, where the consortia of six hosts were inoculated with phage separately showed plaque formation with some deformities in shape of plaque characteristics (Fig.1).

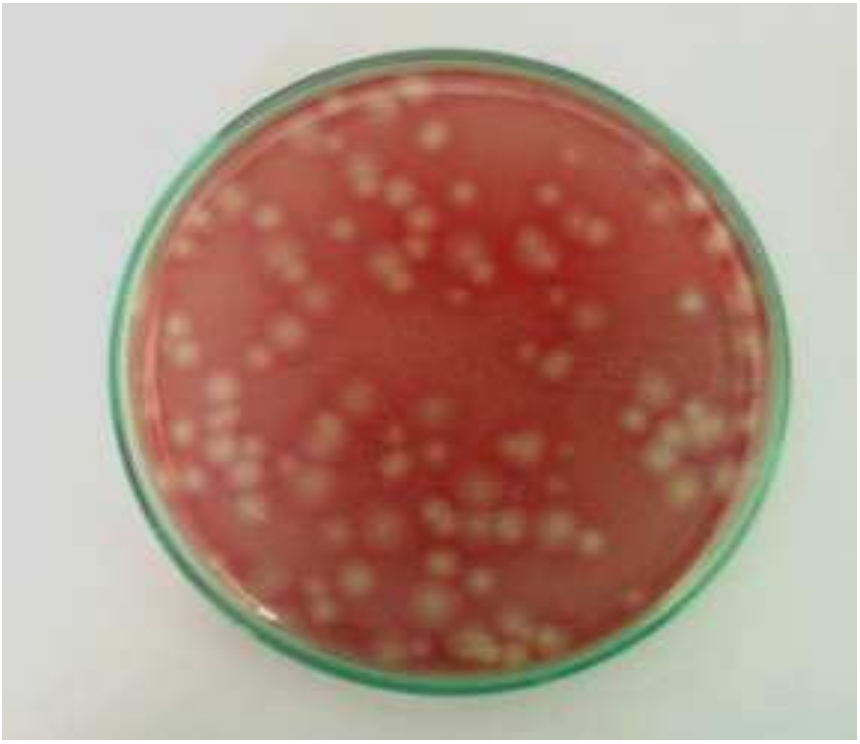

Figure 1: Consortia of six E.coli strains showing non-specificity of phage enzymes

4. DISCUSSION

The host cells in consortia were of *E.coli* but, all were not susceptible for the phages taken under consideration. The solid media formed after the pour plating provides solid surface area for the growth and development of the host and facilitate the phage replication. The susceptible host gets attacked by the phages in the media and gets lysed due to the lytic activity of the phage enzymes; they also lyse the neighboring host cells which are not susceptible for the phage. The experiments conducted on consortia of different and diverse host and their phages showed the same results with delayed incubation time. Later on the same experiment was conducted with different host and phages of different genus like *Citrobacter spp, Rhizobium spp.* and *Salmonella spp*. with their phages and the results obtained were the same. This experiment thus proves the universal activity of phage enzymes against specific and non-specific hosts.

As per the present work carried out in this area, we know that the endolysins are classified in to 5 classes depending upon enzymatic specificity towards cell wall components (Young et al. 2000, Loessner et al. 2005, Fischetti 2004) which is helpful in combined application of phage enzymes against pathogenic hosts. Experiments suggests that repeated exposure to low concentrations of lysin to bacteria grown on agar plates did not lead to the recovery of resistant strains, no identified resistant bacteria was found (Loeffler et al. 2001). It is also proposed that during a phage's association with bacteria to avoid becoming trapped inside the host cell, the binding domain of their lytic enzymes has evolved to target a unique and essential molecule in the cell wall, making resistance to these enzymes a rare event (Vincent 2005).

5. CONCLUSIONS

This experiment showed that how phage enzymes are helpful in destroying non-specific hosts which do not have susceptibility to that phage. This also proves that different phage enzymes can be used for preventing pathogenic bacteria in natural environment and living organisms like humans, animals, etc. The different phage enzymes varying in enzyme activity against various hosts can be applied in combination to lyse any pathogenic bacterial cell and thereby prevent outbreak of disease. The consortial studies indicate the combined application of phage enzymes for controlling pathogenic organisms. Our experiment thus proves the non-specificity of phage enzymes

towards host cell components and states that the activity of phage enzymes is totally substrate (cell wall components) specific and is independent of host type.

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