Cell wall lytic enzymes and their role in bacteriophages infection
(Literature Review)

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ABSTRACT

Use of chemical pesticides has been shown to have many negative side effects, such as insecticide resistance and resurgence, outbreak of secondary pests and diseases, disappearance of parasitoid and predator species as well as residual effects on food crops and on the environment. Over the past 60 years both the number of agricultural toxins in the environment and incidence rates of toxin-related diseases has increased dramatically. The most effective way to combat this problem is through the use of natural predators. One of the best examples of this is the use of host specific bacteriophages to control bacterial diseases. The mechanism of infection is a very interesting one. To break through the bacterial cell wall the bacteriopages must produce a range of lytic enzymes. This review will examine and discuss studies of these site specific cell wall lytic enzymes and their roles in the infection of bacteriophages.

Key words: Cell wall lytic enzymes, biological control, bacteriophages

INTRODUCTION

As global populations and food consumption grow rapidly, the amount of productive land available for agriculture is decreasing. As a solution to this problem, in 1992 UNCED (United Nations Conference on Environmental and Development) recommended the adoption of the concept of sustainable development (Djuniadi, 2003). Based on this recommendation, sustainable development must address social, economic, and ecological factors at the local, regional, and global level. Unfortunately, food production in the decades since the 1940s has increasingly been conducted using methods involving heavy application of chemical pesticides and fertilizers (Erickson 2009). Despite the pest control benefits they provide, chemical pesticides have many negative effects such as insecticide resistance and resurgence, outbreak of secondary pests and diseases, disappearance of parasitoid and predator species, and residual environmental effects. Over the past 60 years both the number of agricultural toxins in the environment and incidence rates of toxin-related diseases have increased dramatically. These 'toxicants'

are widely distributed in nature and many lack an established NOAEL (no observed adverse effect level) or consensus about their long term health effects (Oates and Cohen, 2009).

One possible way to reduce the volume and frequency of chemical pesticide use in agriculture areas is through the use of natural pest control methods. In natural ecosystems, pests and diseases have enemies, competitors and antagonists such as parasitoids, predators, pathogens and viruses (bacteriophages) (Lacey *et al*., 2001; Loessner 2005; Susila *et al*, 2005; Supartha *et al*, 2005; Sumiartha *et al*, 2006;). Through organic farming techniques it is possible to harness these naturally existing relationships to balance ecosystem factors and make farming more sustainable.

One way to control diseases caused by bacteria is to utilize bacteriophages, whose mechanisms of infection have been previously studied. Bacteriophage T7 was reported to infect the *Escherichia coli* cells, with cell wall lytic enzyme gp16 playing a beneficial role during that infection (Moak and Molineux, 2000). Loessner (2005) reviewed the application of bacteriophage endolysins (cell wall lytic enzymes). Endolysins have previously been used in food and in biotechnology for their target specificity. Therefore they offer a unique possibility for the biological control of unwanted bacteria without having effects on other organisms such as natural flora. Medically, purified preparations of cell wall lytic enzymes could also be used as therapeutic agents either alone or in combination with classical antibiotics, particularly in external applications (Loeffler *et al*, 2001; Loessner, 2005). However, in the field of agriculture, research on the application of bacteriophage cell wall lytic enzymes for the control of bacteria is still limited. This review will examine the possible roles of cell wall lytic enzymes and will summarize and discuss the mechanism of infection of a bacteriophage.

DISCUSSION
Cell wall lytic enzymes

Cell wall lytic enzymes reportedly play many important roles in the life cycle of bacteria, as well as in vegetative growth, in sporulation and in germination. The roles of individual enzymes in each process have been studied by constructing mutants. One of the most cited is a study about cell wall lytic enzymes conducted on *Bacillus subtilis*. *B. subtilis* is the best characterized member of the Gram-positive bacteria and has been studied for more than 50 years as a model microorganism to understand its biochemistry, physiology and genetics. Its genome of 4,215,810 base pairs comprises about 4,100 protein-coding genes, with the origin of replication coinciding with the base numbering start point and terminus, about

2,017 kilo bases. The genome contains at least ten prophages or remnants of prophages. *B. subtilis* is an aerobic, non-pathogenic, endospore-forming, rod-shaped bacterium commonly found in soil, in water, and in association with plants. (Kunst *et al.*, 1997).

The roles of cell wall degradation enzymes include peptidoglycan maturation, cell separation, motility, competence, cell expansion, cell wall turnover, protein secretion, differentiation, mother-cell lysis, and pathogenicity (Fig. 1) (Foster 1992; Blackman *et al.,* 1998; Smith *et al.,* 2000)

B. subtilis has more than 30 cell wall lytic enzymes. The cell wall lytic enzymes in *B. subtilis* are classified according to their hydrolytic bond specificity as muramidases, lytic transglycosylases, glucosaminidases, amidases, and endopeptidases (Fig. 2)

(Shida and Sekiguchi 2005, Sudiarta *et al.*, 2010a, Sudiarta *et al.*, 2010b). In applied science, cell wall lytic enzymes as well as muramidase (lysozymes) have been utilized in a wide range of fields (biochemistry, agriculture, textiles as well as the food industry). In addition some cell wall lytic enzymes have been utilized in the field of medicine, for the control of *Staphylococcus aureus,* for example. The advent of *S. aureus* strains that are resistant to virtually all antibiotics has increased the need for new antistaphylococcal agents. An example of such a potential therapeutic agent is lysostaphin, an enzyme that specifically cleaves the *S. aureus* peptidoglycan, thereby lysing the bacteria (Kumar, 2008; Francius *et al.,* 2008).
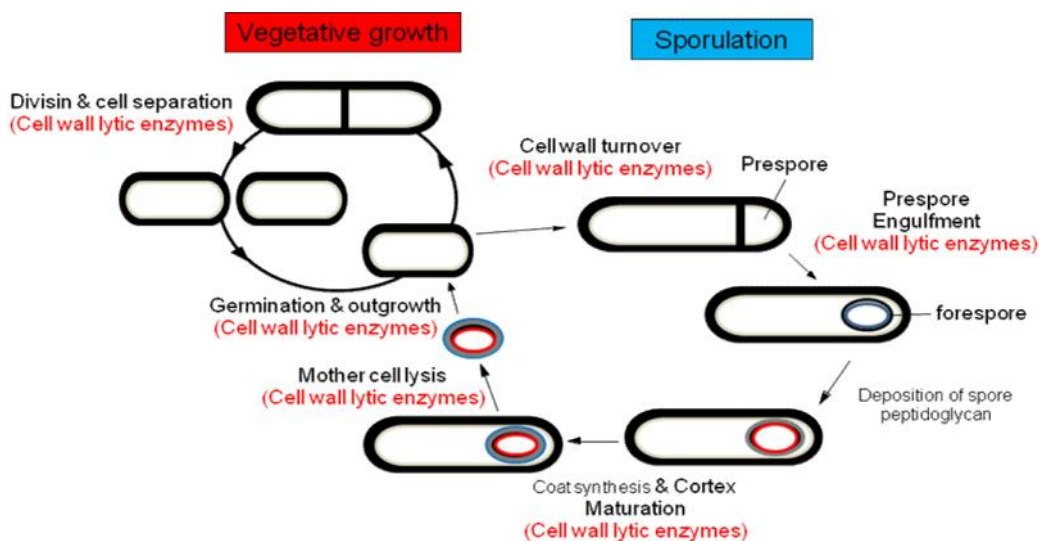

Figure 1. Important roles of cell wall lytic enzymes in the life cycle of *Bacillus subtilis*.
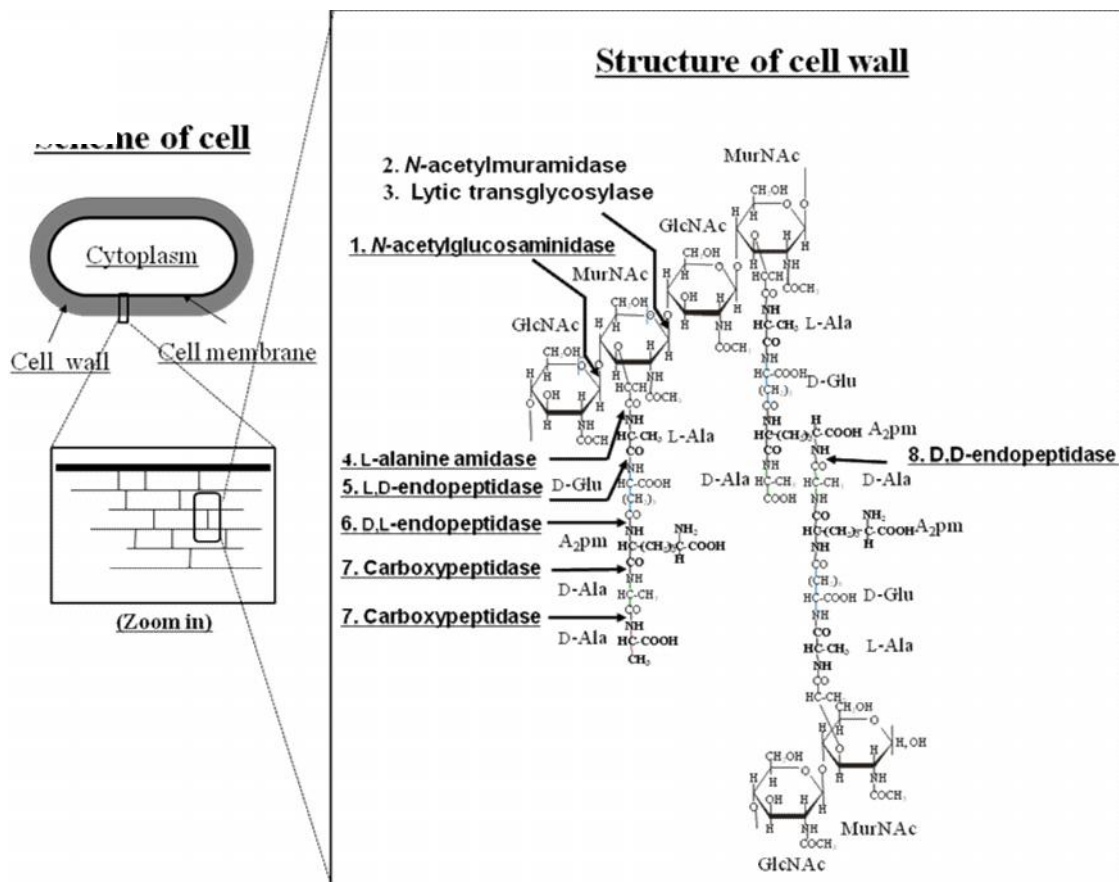
Figure 2. Structure of typical *Bacillus subtilis* peptidoglycan of vegetative cells. The *arrows* indicate hydrolytic bonds attacked by cell wall hydrolases.

The role of cell wall lytic enzymes in bacteriophages infection

On the other hand, the cell wall lytic enzymes are also reported to play an important role in phage infection (Fig. 3). Cell wall lytic enzymes (endolysins) are phage-encoded enzymes that degrade the peptidoglycan of their hosts at the terminal stage of phage production (Loessner, 2005). The gp 13 of bacteriophage Φ29 plays an active role in cell wall lysis and is crucial in bacterial virus entry (Cohen *et al*., 2009).

The cycle of infection of microorganisms by bacteriophages seems simple: adsorption, insertion of nucleic acids, production of nucleic acids and proteins of bacteriophages, and host cell lysis, sequentially. However, the infection of suitable target microorganisms is very specific. Cell wall hydrolases encoded in bacteriophage genomes are involved in host cell lysis (final infection cycle) and in adsorption (facilitation of infection), the initial stage of infection (see Fig. 3) (Piuri and Hatfull, 2006).

Recently, we reported the possible role of cell wall lytic enzymes (CwlP) in

phage infection in *B. subtilis* (Sudiarta *et al.*, 2010b). The target protein, CwlP, is a phage-related protein whose gene is located in the SP-beta prophage (Regamey and Karamata, 1998). CwlP is the largest protein, comprising 2,285 a.a. (252 kDa), in the prophage region. Since CwlP has a phage-related minor tail domain, it is possible that the protein acts as a tail protein. Recently, Piuri and Hatfull (2006) described that gp17 of the so-called "Tape measure protein" (*Tmp*) is the mycobacteriophage TM4 tail protein (1,229 a.a.). The protein contains a cell wall lytic enzyme, and hydrolysis by the hydrolase facilitates efficient infection of stationary phase cells. Interestingly, Kenny et al. (2004) showed that Orf50 of bacteriophage Tuc2009 (906 a.a.) encodes tail-associated cell wall-degradation activity and is involved in infection through cell wall hydrolysis. To deliver the DNA and to break the cell wall of their hosts completely at the end of their life cycle, the bacteriophages reportedly produce cell wall lytic enzymes (Fig. 3).
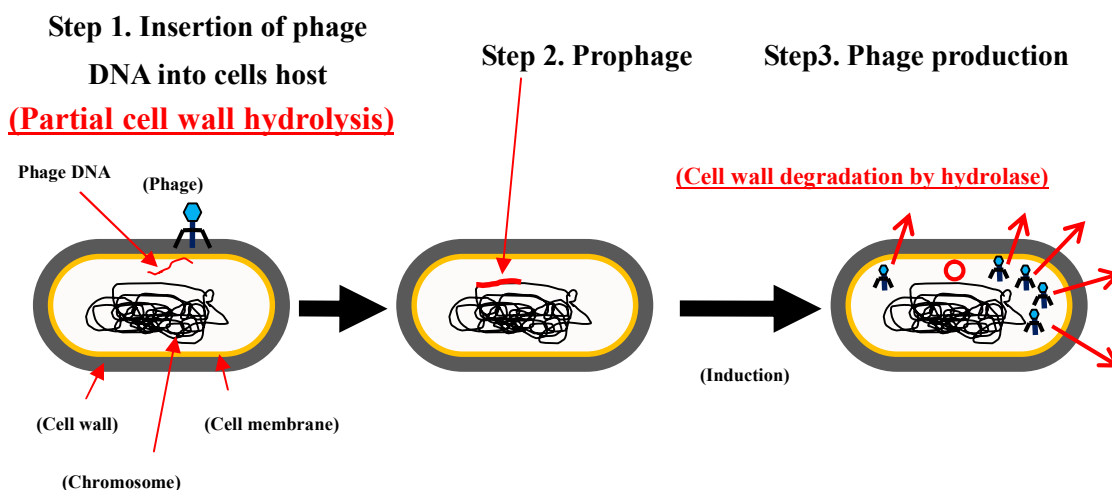
Step 1. Insertion of phage DNA into cells host
(Partial cell wall hydrolysis)

Phage DNA
(Phage)

Step 2. Prophage

Step3. Phage production

(Cell wall degradation by hydrolase)

(Cell wall) **(Cell membrane)**

(Chromosome)

(Induction)

Figure 3. Life cycle of a lysogenic phage. Cell wall lytic enzymes play important roles in life cycle of lysogenic phages, especially in the periods of phage DNA insertion into the host cell (step 1) and host cell lysis after phage production (Step 2).

Concluding remarks

To support organic farming systems and sustainable agriculture the biological control of pests and diseases is an essential approach. Biological control through the harnessing of natural predator-prey relationships is an important step in reliance on environmentally damaging chemical inputs. Bacteriophages represent a viable alternative approach to the control of diseases caused by bacteria. Although

the proper use of bacteriophages requires significant access to technology and an understanding of the specific steps of bacteriophage infection, techniques themselves are simple if one understands the role of cell wall lytic enzymes. This information will be necessary for both basic and applied study, particularly for investigation into the use of bacteriophages in agriculture areas.

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