Role of the ApeE Esterase in the Growth of *Salmonella* on Phospholipids as Phosphate Sources

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Abstract

Salmonella enterica serovar Typhimurium, a bacterium frequently implicated in outbreaks of food poisoning, is able to survive in limiting phosphate environments by inducing a number of proteins that allow it to use a variety of compounds as phosphate sources. The gene *apeE* is induced when the cells are starved for phosphate. This gene encodes an outer membrane esterase that is not found in *E. coli*, and has been shown to be necessary for the growth of *Salmonella* Typhimurium in phosphate-limiting environments, such as those that may be encountered in an egg. To demonstrate the role of *apeE* in these environments, wild type and *apeE* mutant strains of *Salmonella* Typhimurium were separately tested for growth on minimal medium containing either phosphatidyl choline or lisophosphatidy choline as phosphate sources. Growth was observed for the wild type strain in the presence of both phosphate donors, but no growth was observed for the *apeE* mutant. To further support the role of *apeE* in the growth of *Salmonella* Typhimurium under phosphate-limiting conditions, other purified phospholipids as well as egg yolks were tested for their ability to support the growth of both strains.

Introduction

Salmonella is a genus of Gram-negative, rod-shaped bacteria, closely related to *Escherichia coli* (*E. coli*). It is associated with food poisoning, typhoid/paratyphoid fevers, diarrhea, and abdominal cramps which develop within 12 to 72 hours after infection (3). It can be transmitted between human and nonhuman animals, making it a zoonotic pathogen, and is found in the intestinal tracts of humans and other animals. Many infections caused by the bacteria are due to ingestion of contaminated food. It is found in foods like eggs, poultry, meat, cheese, unpasteurized milk/juice, spices, nuts, and contaminated vegetables (6).

Due to the antibacterial action of the bile in the mammalian gastro-intestinal tract, most pathogens are not able to colonize it (1). *Salmonella enterica* serovar Typhimurium is found to thrive in bile-containing environments like the liver and gallbladder. Bile is rich in phospholipids, and *Salmonella* uses these phospholipids as carbon sources during its survival in bile (1, 2).

Salmonella is able to survive in limiting phosphate environments by inducing a number of proteins that allow it to use a variety of compounds as phosphate sources (4). Studies in our lab have shown that the *apeE* gene is induced when the cells are starved for phosphate (5). This gene encodes an outer membrane esterase that is not found in *E. coli* and may be responsible for the pathogen's growth in limiting phosphate environments and environments rich in phospholipids like bile and chicken eggs (2). Studies have shown that phosphatidyl serine and phosphatidyl choline in particular can serve as sole sources of carbon for *S. enterica* (4). Our lab has also shown that *apeE* is required for the utilization of the model lipid substrate Tween 80 (5).

The research questions for this investigation were:
1) Which phospholipids can be used by *Salmonella* as phosphate sources?
2) Does *apeE* play an important role in the use of phospholipids as phosphate sources by *Salmonella*?
3) Do strains of *Salmonella* that have this gene grow better in limiting phosphate environments than those without the gene?

Methods

❖The wild type strain and the *apeE* mutant strain were both tested for growth via culturing on minimal media containing inorganic phosphate as positive controls.

❖ The wild type was spot tested for growth on minimal bacteriological media with the addition of phosphatidyl choline, lysophosphatidyl choline, lysophosphatidyl serine, lysophosphatidyl ethanolamine, and lysolecithin (egg yolk) as phosphate sources via a soft agar overlay.

❖ The *apeE* mutant strain was also spot tested for growth on same phospholipids as phosphate sources used for the wild type strain via soft agar overlay.

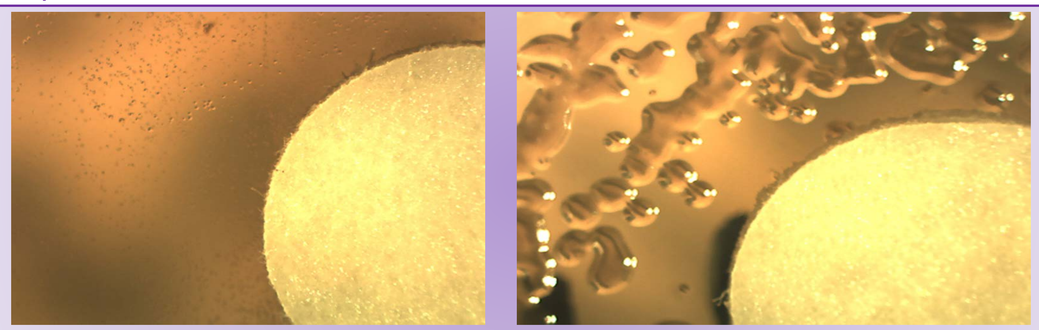

Figure 1 – Interpretation of ApeE activity in vitro. Wild type or *apeE* mutant bacteria were inoculated into soft agar and overlayed onto minimal bottom agar without phosphate. Paper disks were impregnated with inorganic phosphate or phospholipids and placed on top of the soft agar. Plates were then incubated at 37°C for up to 48 hours. Left panel, negative. Right panel, positive

Table 1 – Growth of wild type and apeE mutant *Salmonella enterica* serovar Typhimurium in the presence of different phosphate sources

SUBSTRATES TESTED	OBSERVED RESULTS	
	Wild type strain	Mutant strain
Inorganic Phosphates:		
Sodium Phosphate	Growth	Growth
Disodium Hydrogen Phosphate	Growth	Growth
DL-α-Glycerophosphate	Growth	Growth
Phospholipids:		
Phosphatidyl choline	No growth	No growth
Lysohosphatidyl choline	Growth	No growth
Lysophosphatidyl ethanolamine	No growth	No growth
Lysophosphatidyl serine	No growth	No growth
Lysolecithine (egg yolk)	Growth	No growth

Results

Growth was observed from both strains on the minimal bacteriological media containing inorganic phosphate sources. The wild type strain formed large halos around the spot disc containing lysophosphatidyl choline and lysolecithin as phosphate sources, but no halos were observed for the other tested phospholipids (Table 1). No halos were formed around any of the phospholipid disks placed in the mutant strain culture after 48 hours of incubation.

Discussion

Observation of growth from both strains when cultured on the minimal bacteriological media containing inorganic phosphate sources indicated that they could both grow using regular inorganic phosphates. Formation of halos around spot disc containing the various tested phospholipids corresponds to growth, and as such, no halos were observed for the mutant strain on all the spot tested phospholipids used. The halos formed around spot disc containing lysophosphatidyl choline and lysolecithin by the wild type strain indicated growth. Thus, this confirmed that the *apeE* gene does play a role in *Salmonella's* use of these two phospholipids as phosphate sources. The ability of the wild type strain to grow in lysolecithin – an egg yolk phospholipid – also suggested that *apeE* is responsible for the ability of *S. enterica* to grow in phospholipid-rich foods such as chicken eggs, leading to their contamination.

Previous studies have reported that *apeE* cannot hydrolyze peptide bonds, and will not be able to use amino acid-containing phospholipids as phosphate sources (2). This was confirmed by the inability of the wild type strain to form halos around spot discs containing lisophosphatidyl serine and lisophosphatidyl ethanolamine. The ability of the wild type strain to only hydrolyze lyso-phospholipid types indicated that the *apeE* gene is only activated in limiting phosphate environments containing phospholipids with only one fatty acid. This leaves room for further testing with other lysophosphatides not used in this research.

Overall, our experiment was successful because we were able to confirm that *apeE* is induced during limited phosphate conditions as proposed from previous studies done in and outside of our lab. In addition to this, we were also able to prove that it is responsible for the ability of *S. enterica* to grow in limiting phosphate conditions, which indicates why it is responsible for the contamination of phospholipid-rich foods such as eggs and peanut butter.

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