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 lysophosphatidyl 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 yolk 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/potasliosis, foodborne 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 gastrointestinal 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.

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 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.

Recent 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 lysophosphatidyl serine and lysophosphatidyl ethanolamine. The ability of the wild type strain to only hydrolyze lysophospholipids 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 phospholipids 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 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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References

8. Finkelstein, J. R., Garcia, D. M., and Ortmann, J. 1996. A special thanks to Dr. Timothy Scott for his unconditional support in making this research project a success. You are truly an inspiration to me and those that know you, and words cannot express how grateful I am for all you’ve done. God bless you all!