Archive of SID

Investigation ctpA Gene Among Listeria Monocytogenes and Transferring it into E.coli

Jamileh Nowroozi, Ph.D. ☆ ♆

☆ Microbiology Department, Iran Medical Sciences University

♆ P.O.Box: 14155-6183, Microbiology Department, Iran Medical Sciences University, Tehran, Iran

Abstract

Introduction: Listeria monocytogenes is a Gram positive bacterium, often found in the environment and is responsible for serious food-borne diseases such as perinatal infections, septicaemia and meningoencephalitis in humans and animals. For this reason, distribution of the ctpA (copper transport protein A) among L. monocytogenes was isolated from clinical, environment, dairy, and poultry samples, and was investigated. Then, ctpA gene was transferred into E.coli DH5-α.

Materials and Methods: This investigation was carried out in two steps (ctpA was found in L. monocytogenes isolated from different sources, which was kept in culture collection of Adelaide University, Australia. Then ctpA gene was transferred into E. coli DH5-α. CtpA DNA from L. monocytogenes was amplified by PCR, identified on agarose gel, purified by phenol, and ligated into pGEM-T vector. Then, it was transferred on X-gal plate containing ampicillin. The sequencing of ctpA DNA was determined by using dye terminator kit to purify DNA, and followed by sequencing machine.

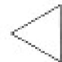
Results: By using PCR to identify the homologous DNA in 69 isolates, 38% of tested isolates contained ctpA determinant. Our results showed that: 90% of clinical and dairy isolates; 85% of environmental isolates and 7% of poultry isolates of L. monocytogenes contained ctpA in chromosome DNA. Fortunately, the transformation of ctpA from L. monocytogenes into E. coli DH5-α was successful.

Conclusion: Since, the existence of ctpA in clinical, dairy and environmental samples was 90% and in poultry was 7%, therefore, the virulence of all strains of this bacteria are not the same. Introducing such clone (ctpA gene) into suitable carrier strains, could be expected to produce a good oral immunogen against L. monocytogenes.

Key words: Listeria monocytogenes, E. coli DH5-α, transformation gene, ctpA gene.

www.SID.ir

Archive of SID



Introduction

The Gram-positive Listeria monocytogens, is a facultative, non-spore forming, rod shaped and intracellular organism. This bacterium is found in the environment. Epidemiological studies of listeriosis outbreaks have suggested butter (1), soft cheeses (2), pasteurized milk (3), raw vegetables (4), meat and production of meat (5) as probable vehicle of infections in human. Elderly patients, pregnant women and individuals with predisposing conditions that compromise their immunity, such as transplants, and malignancies such as lymphomas, are at a particular risk. Meningitis is the most commonly recognised form of human listerial infection and it demonstrates the tropism of L. monocytogenes for the central nervous system (6). In perinantal listeriosis, depending on the time of neonatal infection, two distinct forms of clinical syndromes are observed. The first is infection of the fotus which often leads to abortion or stillbirth. The second syndrome (late onset neonatal infection), which results from a later infection at or shortly after birth to third week of life, which manifests into meningitis (7).

Francis and Thomas (8) identified a gene in L. monocytogenes (ctpA), which encodes a 653 amino acid polypeptide with strong similarity to cation transporting p-type ATPases (Genbank Accession Number: U15554). Sequence and phylogenetic analysis, indicated that CtpA was most closely related to a class of p-type ATPase involved in copper transport in procartoytes and eucaryotes. Of L. monocytogenes infections (9, 10), which was considered as virulence factors known to play a role in pathogenicity. However, additional factors which affect cell viability may also play a role in allowing the organism to selectively establish an infection. For example, a significant number of L. monocytogenes strains are resistant to heavy metal cations including cadmium (11). In this case, resistance is conferred by the cadA determinant, a p-type ATPase protein involved in cadmium transport.

P-type ATPases involved in the transport of cadmium or copper ions in other bacteria was reported including Staphylococcus aureus cad A, cadC (12), Synechococcus pac S (13), E. hirae copA (14), H. pylori copA and ORF (15). These ATPases showed significant similarity to the proteins associated with copper metabolism disorders in humans, Menkes disease (16) and Wilson disease (17). In Menkes disease, the export of copper from intestinal cells is defective, and results in severe copper deficiency. In contrast, Wilson disease results from a failure in removing copper from the liver into bile, leading to copper toxicity. Interestingly, the CtpA ATPase from L. monocytogenes has significant identity to bacterial proteins involved in translocation of copper, as well as those encoding Menkes and Wilson diseasesdeterminants (10). Given L. monocytogenes is ubiquitously distributed throughout the environment, which suggests an important role for the CtpA p-type ATPase in survival of this organism in its natural habitat, and may involve maintaining copper homeostasis through the transport of copper ions. The aim of this study was concerned with the analysis and distribution of a copper transport gene (ctpA) from different sources (clinical, dairy, environment and poultry) obtained from culture collection at Adelaide University, Australia, and then to transfer this gene into E. coli DH5-α.

Materials and Methods

This investigation was carried out in 2 steps. first, ctpA was found in L. monocytogenes isolated from different sources at culture collection laboratory in the Microbiology Department of Adelaide University, Australia and then the ctpA gene was transferred into E. coli DH5-α (8, 18).

All these bacteria were kept in solution of glycerol and peptone solution at -70°C. Fresh single colonies of Listeria were prepared by streaking a loopful of glycerol stock onto Brain Heart Infusion agar and were incubated overnight at 37°C. For routine use, cultures were maintained on agar plate at 4°C.

First, chromosomal DNA from Listeria spp. was extracted by modification of Flamm et al. method (19). DNA concentration was determined by measuring the OD of the DNA solution at 260 nm using a spectrophotometer. Electrophoresis was performed at room temperature on horizontal 1% (w/v) agarose gel

www.SID.ir

Archive of SID

in a TAE buffer solution (Tris, Acetic acid and EDTA). The gel was followed by staining in ethidium bromide. DNA fragments were visualised by transillumination using a UV Transilluminator and photographed on polaroid 667 positive film.

The size of fragments obtained by restriction enzyme, was calculated by comparing their relative mobility to EcoR1 digested B. subtilis bacteriophage SPP1 DNA. L. monocytogenes. Preliminary experiments were done to determine optimum PCR modification condition. Then, ctpA in 69 samples was investigated, it was obtained from culture collection laboratory in Microbiology Department of different sources (environment, patients, dairy, meat and poultry). Plasmid pGEM-7 Z F (+) was used as vector to clone DNA fragments of L. monocytogenes (20). Escherichia coli DH5-α was used as host strain for recombinant clones, because ctpA was not detected in this bacteria. In this study, ctpA DNA from Listeria was amplified by PCR (8), then, ran on agarose gel electrophoresis and purified with phenol. Electrophoresis fragment DNA was cut without using ethidium bromide or UV. One ml phenol/gram agarose gel was vortexed and kept at 70°C for 1hr. After centrifugation, chloroform was added and DNA was obtained by 100% ethanol. Fragments containing portions of the ctpA genes were ligated into pGEM-T vectors, essentially by published procedures. Plasmids containing inserts were introduced into E. coli DH5-α by pretreating cells with CaCl2 to render their competent cells. Insert containing plasmids were identified on nutrient agar plates containing galactosidase substrate and chromophore x-gal 40 g/ml. White colonies were restreaked on ampicillin plate and one colony was picked up, then DNA chromosome was extracted, and amplified with PCR. Dye-terminator kit was used to purify ctpA DNA. Then, the DNA sequencing was performed by automatic sequencer (21).

Results

Preliminary experiments indicated that the optimum conditions were using 25mM MgCl2 and the following PCR temperature cycling: 94°C (templated denaturation) for 3 min (1cycle), 55°C (annealing) for 30 seconds, 72°C (extension) for 3 min (30 cycles), 72°C (stabilization) for 4 min (1cycle) and then holding at 4°C (1cycle) (22). Of 69 L. monocytogenes isolates, in 38% cases, 558 bp amplification products was detected. The results showed that 90% of clinical and dairy isolates, 85% of environmental isolates and 7% of poultry isolates of L. monocytogenes contained ctpA in chromosome DNA. PCR analysis indicated, that ctpA homologous DNA was restricted to some strains of L. monocytogenes.

Hpa-1 digestion of amplified DNA fragment produced two products: 176 bp and 382bp (0.558 kbp), when compared with DNA of SPP1 on agarose gel electrophoresis. After ligated chromosomal DNA fragment into pGEM-T and transforming into E. coli DH5-α competent cells, at least 200 white transformed colonies initially screened by blue/white differentiation in x-gal plates appeared. Positive clones (white colonies) were identified during growth on media containing x-gal by their ability to produce galactosidase. White colonies contained plasmid DNA were picked up and cultured on nutrient agar plates which contained ampicillin. Then, DNA from the transferred bacteria was isolated, amplified by PCR and purified by agarose gel. By using dye-terminator kit, ctpA was purified and sequencing was performed by automatic sequencer. our results indicated that the complete nucleotide sequences of ctpA in transferred E. coli was exactly the same as the ctpA in L. monocytogenes. Therefore the transformation was successful. In Fig 1, ctpA band detected on agarose gel electrophoresis is shown.

Discussion

Copper is an essential nutritional requirement for living cells, yet it is very toxic when present in excess. It is likely that the bacteria have essential copper transport systems responsible for maintaining copper ions at a relatively constant low level (23). Proteins involved in transporting copper or cadmium in some other bacteria was reported (11, 15). During infection, eukaryotic host cells are exposed to dramatic changes in trace element concentration in the serum (24). For

www.SID.ir

Archive of SID

example copper ion concentration in rat liver significantly decreased during parasitic infection (25, 26). CtpA may therefore, play a critical role in the selective adaptation of L. monocytogenes to varying environments including the intracellular location of an infected host. For survival in this environment, L. monocytogenes may have acquired the mechanism to sequester copper from infected cells by ctpA function.

CtpA is necessary for L. momocytogenes to persist in organ tissue of infected mice (18). In view of the significance of ctpA in bacterial infections, we investigated the distribution of the ctpA among L. monocytogenes isolates from environment, clinical, dairy and poultry samples, which was kept in culture collection laboratory in Microbiology Department of Adelaide University, Australia. Using PCR to identify homologous DNA in 69 isolates, 38% of the tested isolates contained the ctpA determinant. However, DNA homologous to ctpA was not detected in all strains.

Antibiotics have effectively been used to treat human listeriosis (27). Ampicillin, amoxicillin and gentamicin were considered the primary choice for treatment of human listeriosis, but antibiotics such as chloramphenicol and azlocillin had no effect. Nevertheless, it is acknowledged that up to 30% of the patients who have previously had drug therapy, will still succumb to listeria infection (28), It is likely that the intracellular habit of L. monocytogenes protects the organisms from exposure to antibiotics. Consequently, a number of researchers sought to develop a L. monocytogenes vaccine that provides a high degree of protection with minimal or no side effects, against infection. The intracellular habit is effectively utilized by L. monocytogenes against antibody mediated defense mechanisms. Therefore, a cell mediated response is required for antimicrobial protection. Protective immunity is best created by live vaccines, whereas soluble proteins, and killed vaccines are generally not sufficient (29). Presently, only two live vaccines active against intracellular pathogens are in use: Mycobacterium bovis, BCG (Bacillus Calmette- Guerin) against tuberculosis and Salmonella typhi Ty2la providing protection from typhoid fever (30).

In this investigation, transformation of ctpA from L. monocytogenes into E. coli was successful. After expression of ctpA in E.coli (produced protein), the antibody was raised against E.coli that harbor this clone, then, the mouse model system protection will be determine. Introduction of such clone into suitable carrier strains could be expected to produce good oral immunogen against L. monocytogenes.

References

1. Lyytikjainen O, Autio T, Maijala R, Ruutu Honkanen-Buzalski T, Miettinen M, Hatakka M, Mikkola J, Anttila VJ, Johansson T, Rantala L, Aalto T, Korkeala H, Siitonen SA: An Outbreak of Listeria monocytogenes serotype 3a infections from butter in Finland. J Infect Dis 2000; 181: 1838-1841

2. Larson AE, Johnson EA, Nelson JH: Survival of Listeria monocytogenes in commercial cheese brines. J Dairy Sci 1999; 82: 1860-1868

3. Teo AYL, Knabel SJ: Development of a simple recovery enrichment system for enhanced detection on heat injured Listeria monocytogenes in pasteurized milk. J Food Protection 2000; 63(4): 462-472

4. Mclauchlin JA: Listeria monocytogenes, recent advances in the taxonomy and epidemiology of listeriosis in humans. J Appl Bacteriol 1987; 63: 1-11

5. Shannon AC, Carr LE, Mallinson ET, Lamichanne C, Rice BE, Rollins DM, Joseph SW: Development and evaluation of a 24 hour method for the detection and quantification of Listeria monocytogenes in meat products. J Food Protections 2000; 63: 347-353

6. Nieman RE, Lorber B: Listeriosis in adult: a changing pattern, Report of eight cases and review of the literature, 968-1978. Rev Infect Dis 1980; 2: 207-227

7. Lorber B: Clinical listeriosis-implications for pathogenesis. In food-borne listeriosis. Miller AL, Smith J, Somkuti GA (eds). Elsevier science publications co, Amsterdam 1990; 41-49

8. Francis MS, Thomas CJ: Analysis of multiplicity of infection. J Med Microbiol 1996; 45: 323-330

9. Portnooy DA, Chakroborty T, Goebel W, Coosart P: Molecular determinants of Listeria monocytogenesis. Infect Immun 1992; 60: 1263-1267

10. Sheehan B, Kocs C, Dramsi S, Gouin E, Klarsfeld AD, Mengaud J, Coosart P: Molecular and genetic determinants of

www.SID.ir

Archive of SID

the Listeria monocytogenes infectious process Curr Top Microbiol Immunol 1994; 192: 182-216

11. Lebrun M, Audrier A, Cossart P: Plasmid-borne cadmium resistance genes in Listeria monocytogenes are to cadA of cadC of Staphylococcus aureus and are induced by cadmium. J Bacteriol 1994; 174: 3040-3048

12. Nucifore G, Chu L, Misra TK, Silver S: Cadmium resistance from Staphylococcus aureus plasmid p1258 cadA gene results from a cadmium efflux ATPase. Proc Nat Acad Sci USA 1989; 86: 3544-3548

13. Kanamaru K, Kashiwagi S, Mizuno T: The Cyanobacterium, Synechococcus sp. Pcc 7942. possess two distinct genes encoding cation-transporting P-type ATPases. FEBS Letts 1993; 330: 99-104

14. Odermatt A, Suter H, Krapf R, Solioz M: Primay structure of two P-type ATPase involved in copper homeostasis in Enterococcus hirae. J Biol Chem 1993; 268: 12775-12779

15. Ge Z, Hirastsuka K, Taylor DE: Nucleotide sequence and mutational analysis indicate that two Helicobacter pylori genes enduce a P-type ATPase and cation-binding protein associated with copper transport. Mol Microbiol 1995; 15: 97-106

16. Vulpe C, Levinson B, Whitney S, Packman S, Gitscheir J: Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper- transporting ATPase. 1993; Nature Gene 5: 7-13

17. Bull PC, Thomas GR, Rummenes JM, Forbes JR, Cox DW: The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. Nature Gene 1993; 5: 327-337

18. Francis MS, Thomas CJ: The Listeria monocytogenes gene ctpA encodes a putative P-type ATPase involved in copper transport. Mol Gen Genet 1997; 253: 484-491

19. Flamm RK, Hinrichs DJ, Thomaslow MF: Introduction of pAM b1 into Listeria monocytogenes by conjugation homology between native L. monocytogenes. Plasmids Infect Immun 1984; 44: 157-161 20. Technical M: pGEM-T and pGEM-T easy vector system: Promega: Instruction for use of products. part TM042, revised 7/79

21. The Perkin-Elmer Corporation: ABI Prism TM Dye terminator cycle sequencing ready reaction kit with Ampli Tag DNA polymerase, FS, protocol P/N 402078. revision A. Aug 1995

22. Fitter S, Heuzenroeder MN, Thomas CJ: A combined PCR and selective enrichment method for rapid detection of Lisreia monocytogenes. J Appl Bacteriol 1992; 73: 53-59

23. Brown NT, Lee BT, Silver O: In metal ions biological system. Sigel H, Sigel A (eds). Dekker NY 1994; 405-434

24. Beisel WR: Magnitude of the host nutritional responses to infection. Am J Clin Nut 1997; 30: 1236-1247

25. Matousek- be- Abel- be- la- Curz A, Burguera Burguera JL, Burguera M, Anez N: Changes in the total content of iron, copper and zinc in serum, heart, liver, spleen and skeletal muscle tissues of rats infected with Trypanosoma cruzi: Biol Tract Elem res 1993; 37: 51-70

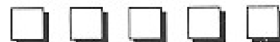
26. Crocker A, Lee C, Aboko C, Durham C: Intraction isolates of nutrition and infection: Effection of copper deficiency on resistance to Trypanosoma lewisi. J Natl Med Assoc 1992; 84: 697-706

27. Marget W, Eeliger HPR: Listeria monocytogenes infection: Therapeutic Possibilities and problems. Infection 1988; 16: 5175-5177

28. Nieman RE, Lorber B: Listeriosis in adult: a changing pattern, report of eight cases and review of the literature, 1968-1978. Rev Infect Dis 1980; 2: 207-227

29. Hess J, Kaufmann SHE: Vaccination strategies against intracellular microbes. FEMS Immunol Med Microbiol 1993; 7: 95-104

30. Germanier R: Isolation and characterization of gel mutant Ty21a of Salmonella typhi: a candidate for a live oral typhoid vaccine. J Infect Dis 1975; 141: 553-555



www.SID.ir