Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/jopr

Original Article

Identification and characterization of foodborne pathogen *Listeria monocytogenes* strain *Pyde1* and *Pyde2* using 16S rRNA gene sequencing

Acharya Nagarjun Pyde [a], *P. Nagaraja Rao* [b], *Aditya Jain* [d], *Divya Soni* [d], *Shailesh Saket* [d], *Sheaza Ahmed* [d], *Sugunakar Vuree* [c], *Anuraj Nayarisseri* [e,*]

[a] *Associate Professor & Head, Department of Microbiology, University College of Science, Osmania University, Hyderabad 500007, Andhra Pradesh, India*
[b] *Professor, Department of Zoology, Osmania University, Hyderabad 500007, Andhra Pradesh, India*
[c] *Assistant Professor, Joginapally Engineering College, R.R. District, Hyderabad 500075, Andhra Pradesh, India*
[d] *Research Associate, In Silico Research Laboratory, Eminent Biosciences, Vijyanagar, Indore 452010, India*
[e] *Bioinformatics Scientist, In Silico Research Laboratory, Eminent Biosciences, Vijyanagar, Indore 452010, India*

ARTICLE INFO

Article history:
Received 24 April 2013
Accepted 19 May 2013
Available online 27 July 2013

Keywords:
Listeria monocytogenes strain *Pyde1*
Listeria monocytogenes strain *Pyde2*
16s rRNA gene sequencing

ABSTRACT

Aim: *Listeria monocytogenes* acts as a pathogen for humans and animals, mainly causing, neonatal sepsis, abortions in pregnant females and severe infections such as septicemia and meningoencephalitis in susceptible hosts. Current study was aimed to identify novel strains of *L. monocytogenes* from retail chicken, beef meat and seafood samples.

Methods: In order to identify the strain, extraction and amplification of genomic DNA, 16S rRNA sequence analysis was carried out. Phylogenetic trees were constructed using dnapars and dnaml available in Phylip. The secondary structures of 16S rRNA gene sequence were predicted using UNAFOLD, a Linux based software.

Results: The results obtained were found to be a novel foodborne pathogens, which was further named *L. monocytogenes* strain *Pyde1* and *L. monocytogenes* strain *Pyde2*, after characterization the sequence of isolate was deposited in GenBank with accession numbers 'KC852899' and 'KC852900' respectively. The Gibb's free energy of the secondary structures of *L. monocytogenes* strain *Pyde1* and *Pyde2* were −275.60 and −282.20 kcal/mol seems to be more stable in the present investigation.

Conclusion: The described results of phylogenetic distinctiveness and phenotypic disparities indicate that strain 2b represents a novel strain of foodborne pathogens within *L. monocytogenes* species, for which the name *L. monocytogenes* strain *Pyde1* and *L. monocytogenes* strain *Pyde2* is proposed.

* *Corresponding author*. Tel.: +91 9752295342.
E-mail address: anuraj@eminentbio.com (A. Nayarisseri).

0974-6943/$ – see front matter
http://dx.doi.org/10.1016/j.jopr.2013.07.009

1. Introduction

Listeria monocytogenes is an intracellular pathogen that can cause obtrusive disease in *Homo sapiens* and other animals.[1] Most *Listeria* infections are sub clinical they may go unnoticed. However, in some cases, a listeria infection can lead to life-threatening complications such as septicemia and meningitis. Foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5000 deaths in all over the world each year.[2] *Listeria* infections are caused by eating food contaminated with the bacteria *L. monocytogenes*, which can also be found in water, soil etc. Humans are often afflicted to listeria by consuming: Unpasteurized milk or foods made with unpasteurized milk, soft cheeses, hot dogs and deli meats that have been contaminated after processing, raw vegetables that have been contaminated from the soil or from contaminated manure used as fertilizer and infected animal meat etc.[3] Therefore the present study describes the isolation of two novel strains of *L. monocytogenes* from retail chicken, beef meat and seafood samples.

2. Methods

2.1. Isolation of L. monocytogenes from retail chicken, beef meat and seafoods

Samples were collected from various supermarkets and open markets in and around Andhra Pradesh. The samples were transported in clean plastic bags chilled on ice to the laboratory within 1 h after sampling. Twenty-five g of each sample was placed into a bag containing 225 mL of Half Fraser's broth. 100 μL of each sample were inoculated into 10 mL of Fraser's broth (FB) in a culture tube and incubated at 37 °C with shaking (250 rpm) for 48 h. Aliquots (60 μL) of positive FB cultures, i.e. dark color caused by esculin hydrolysis, were plated individually on BBL CHROM agar and PALCAM agar (Oxoid), and the plates were incubated at 37 °C for 48 h. The greenish-black colonies on the PALCAM agar and the blue colonies with a white halo on the BBL CHROM agar were separately subcultured onto tryptone soy agar (TSA) (Oxoid) supplemented with 2% of soy yeast extract (TSYEA) (Oxoid) and incubated at 37 °C overnight.

2.2. PCR amplification of the 16S rRNA

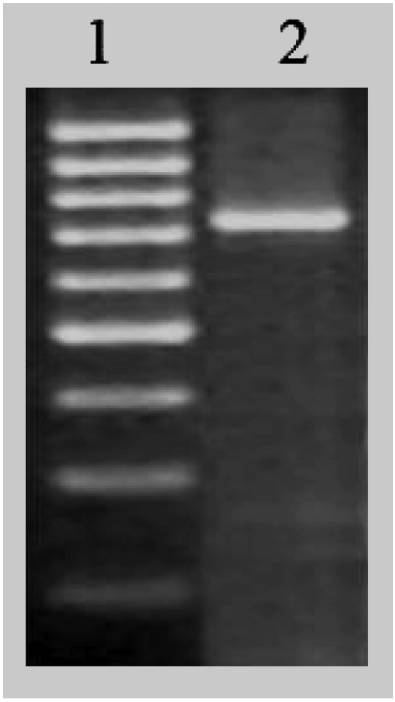
Genomic DNA was extracted from the bacterial cells grown at 37 °C overnight in tryptic soy broth (TSB) using a DNA extraction kit. The PCR mixture (25 μL) consisted of: 1 μM of each primer, 100 ng of DNA template, 2.5 μL of 10× *Taq* PCR buffer, 0.2 mM dNTP, 2 mM $MgCl_2$, and 1 unit of *Taq* DNA polymerase (Fermentas, St. Leon-Rot, Germany). The PCR mixture was subjected to the following thermal cycle conditions using the Lifecycler (Bio-Rad, California, USA): 5 min of 95 °C before 30 cycles of amplification at 95 °C for 45 s, 60 °C for 45 s, and 72 °C for 45 s.

2.3. Purification of the amplified product

After the amplification of the DNA in PCR we took the PCR sample in a fresh vial and added 5 μL of 3 M sodium acetate solution (pH = 4.6) and 100 μL of absolute ethanol in it and mixed it thoroughly. Then we vortexed the vial and left it at −20 °C for 30–40 min to precipitate the PCR products. Then it was subjected to centrifugation for 5 min at 10,000 rpm. To the pellet we added 300 μL of 70% ethanol, without mixing, it was again subjected to centrifugation for 5 min at 10,000 rpm. The produced pellet was air dried until the ethanol effervescence is removed. And lastly the pellet is suspended in 10 μL of sterile distilled water.

2.4. Sequencing of PCR product

Primers (5′- GTGGGGAGCAAACAGGATTA- 3′ and 5′- TAAGGTTCTTCGCGTTGCTT- 3′) of the 16S rRNA gene of Listeria were used to amplify from the isolated DNA sample. The amplified product from three independent PCRs was gel-purified, ligated into pCR2.1 (Invitrogen Life Technologies) and transformed into *Escherichia coli* INVáF' (Invitrogen), as recommended by the manufacturer. Plasmid DNA was isolated using a plasmid isolation kit (Bio-Rad), digested with EcoRI and resolved by agarose gel electrophoresis [Fig. 1]. Plasmids containing appropriately sized inserts were sequenced using Sanger dideoxy sequencing. The novel isolated sequence was deposited in GenBank with Accession number KC852899 and KC852900 respectively, maintained by the National Centre for Biotechnology Information (NCBI), at the National Institute of Health (NIH), Rockville, Maryland, USA.



Lane 1: Marker, Lane 2: PCR Product

Fig. 1 – 16S rRNA PCR product on 2% agarose gel.