

Characterization of *Listeria monocytogenes* isolates by CAMP test

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Abstract

In this study, a total 28 *Listeria monocytogenes* field isolates obtained from different animal species were characterized phenotypically by CAMP test. Characterization by CAMP test on all the 28 field isolates revealed positive reaction, of which twenty-three isolates showed characteristics enhancement of haemolytic zone with *S. aureus* on 5 % Sheep Blood Agar (SBA) and five isolates showed weak haemolytic zone.

Key words: *Listeria monocytogenes*, CAMP test, Haemolytic, Sheep Blood Agar

Introduction

The genus *Listeria* represents a group of closely related, Gram-positive, facultative anaerobic, non-spore-forming, rod-shaped bacteria 0.5 µm in width and 1–1.5 µm in length, and with a low G+C content. Taxonomically, it is divided into six species viz. *Listeria monocytogenes*, *L. ivanovii*, *L. seeligeri*, *L. innocua*, *L. welshimeri* and *L. grayi*, of which only *L. monocytogenes* and *L. ivanovii* are pathogenic (Robinson *et al.*, 2000). While *L. monocytogenes* infects both man and animals, *L. ivanovii* (previously known as *L. monocytogenes* serotype 5) is principally an animal pathogen that rarely occurs in man (Low and Donachie, 1997). Being tolerant to extreme pH, temperature, salt conditions (Sleator *et al.*, 2003; Liu *et al.*, 2005), and unique ability to cross three barriers during infection: the intestinal barrier (Lecuit *et al.*, 2001), the blood-brain barrier (Huang *et al.*, 2000), and/or the placental barrier (Lecuit *et al.*, 2004; Vazquez-Boland *et al.*, 2001). *L. monocytogenes* is responsible for severe food-borne infections in humans and can also cause invasive disease in many different animal species, including farm ruminants cattle, sheep, and goats.

In human listeriosis during the early stages of infection it often displays non-specific flu-like symptoms (e.g. chills, fatigue, headache, and muscular and joint pain) and gastroenteritis. However, without appropriate antibiotic treatment, it can develop into septicaemia, meningitis, encephalitis, abortion and, in some cases, death (Vazquez-Boland *et al.*, 2001) and mortality rates on average approaching 30%. The Listeric infection in animals causes clinical

syndromes of abortion or neonatal septicemia, encephalitis localized to the brain stem or spinal cord is the most common illness of adult animals (Blood *et al.*, 1989). Listeric mastitis is the most stubborn and difficult to treat and results in culling of the infected animals from a herd (Stewart, 1998).

Materials and methods

The present work is comprised of characterization of 28 *L. monocytogenes* isolates by CAMP test. The present work was conducted at the Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand.

Isolates: All the 28 *Listeria monocytogenes* isolates were obtained from the Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand.

Reference bacterial strains: The reference strains of *Listeria monocytogenes* 4b (MTCC 1143), *Rhodococcus equi* (MTCC 1135) and *Staphylococcus aureus* (MTCC 1144) were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. All the strains were maintained by periodically subculturing in brain heart infusion (BHI) agar slant. These strains were used as reference bacterial strains during the present study.

Characterization of *Listeria monocytogenes* isolates by Christie, Atkins and Munch-Petersen (CAMP) test: All the 28 *L. monocytogenes* isolates were tested by CAMP test as per the method of

Anonymous (1994) with some modifications. Briefly, the standard strains of *Staphylococcus aureus* and *Rhodococcus equi* were grown overnight on 5% sheep blood agar (SBA) plates at 37°C and their colonies were again streaked onto freshly prepared SBA plates having 5% sheep blood in a manner that these were wide apart and parallel to each other. In between the parallel streaks of *S. aureus* and *R. equi*, the *Listeria monocytogenes* isolates were streaked at 90° angle and 3 mm apart before incubating them at 37°C for 24 h. The plates were examined for enhancement of haemolytic zone from partial haemolysis to wider zone of complete haemolysis, if any, between a *Listeria* strain and the *S. aureus* or *R. equi* strain owing to the synergistic effect of their haemolysins in case of a CAMP-positive reaction. The *L. monocytogenes* isolates showed CAMP-positivity only with *S. aureus* were characterized as *L. monocytogenes* but not with *R. equi*.

Results and Discussion

A total of 28 *Listeria monocytogenes* isolates and the reference strain *Listeria monocytogenes* 4b (MTCC 1143) were subjected to CAMP test with *Staphylococcus aureus* (MTCC 1144) and *Rhodococcus equi* (MTCC 1145). Twenty-three isolates exhibited characteristic enhancement of haemolytic zone with *S. aureus* on SBA (Plate:1), five isolates showed weak haemolytic zone. The five isolates produced weak haemolytic zone with *S. aureus*. Then isolates were further confirmed by PCR (Not shown).

The factors involved in the synergistic lysis of RBC and use of CAMP test for identification of *L. monocytogenes* isolates have been reviewed by McKellar (1994). A 58-kDa protein (LLO) secreted by *L. monocytogenes*, which was strongly haemolytic against sheep erythrocytes when combined with culture supernatants from *S. aureus*, and not with supernatants from *R. equi*, has been suggested to represent the *L. monocytogenes* CAMP factor. The synergistic hemolytic reaction of *L. monocytogenes* is due to a haemolytically active exosubstance, which has the character of the sulfhydryl (SH)-activated bacterial cytotoxin similar to streptolysin O (Mengaud *et al.*, 1987), and it has been directly implicated in virulence (Cossart *et al.*, 1989). Thus, LLO is required in order to lyse the host vacuole, allowing *L. monocytogenes* to grow in the host cytoplasm. The characteristic enhancement of the -hemolytic zone towards *S. aureus* was due to the synergism between -toxin produced by *S. aureus* and LLO, confirming *L.*

monocytogenes.

Seeliger and Jones, (1986) also reported that *L. monocytogenes* isolates were positive for CAMP test. Jaradat and Bhunia, (2003) found similar results in all twenty-five *L. monocytogenes* strains belonging to 13 serotypes. They had tested all twenty-five isolates for haemolysin production with the CAMP test on sheep blood agar plates and were found to be hemolytic for all twenty-five isolates.

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