Available online at www.sciencedirect.com
ScienceDirect
Comparative Immunology, Microbiology & Infectious Diseases 31 (2008) 47–62
COMPARATIVE IMMUNOLOGY MICROBIOLOGY & INFECTIOUS DISEASES
www.elsevier.com/locate/cimid

Molecular characterization of Avian strains of *Pasteurella multocida* serogroup-A:1 based on amplification of repetitive regions by PCR

S.B. Shivachandra*, A.A. Kumar, P. Chaudhuri

Division of Bacteriology and Mycology, Indian Veterinary Research Institute, Izatnagar 243122, UP, India

Accepted 17 April 2007

Abstract

Repetitive extragenic palindromic (REP)-PCR (polymerase chain reaction), enterobacterial repetitive intergenic consensus (ERIC)-PCR, and single primer PCR assays were employed to characterize 66 strains of *Pasteurella multocida* serogroup A:1 isolated from avian species belonging to different regions of India. REP-PCR resulted in amplification of REP sequences from the genome which were in the range of ~200 to ~3000 bp and accounted for a total of 54 distinguishing profiles ($D = 0.99$). ERIC-PCR analysis also generated amplified products in the range of ~200 to ~3200 bp categorizing strains into a total of 50 different profiles ($D = 0.98$). Amplification of repetitive regions using a microsatellite primer $(GTG)_5$, resulted in clear distinctive bands ranging from ~200 to ~2400 bp. Strains were assigned to 43 profiles ($D = 0.96$). No correlation could be drawn between genotypic profiles and avian hosts with their geographical area of origin. Avian strains of *P. multocida* serogroup A:1 were found to be highly heterogeneous with diverse profiles. REP-PCR was found to be highly discriminatory and simple method for differentiation of phenotypically similar strains. The present study also indicated that PCR based amplification of repetitive regions of *P. multocida* is a rapid

*Corresponding author. Present address: Department of Biology, The Catholic University of America, 103 McCort Ward Hall, 620 Michigan Ave, NE, Washington, DC 20064, USA. Tel.: +1 301 755 3311, +1 202 319 5271; fax: +1 202 319 5721.

E-mail addresses: sbshivachandra@rediffmail.com, shivachandra@cua.edu (S.B. Shivachandra).

doi:10.1016/j.cimid.2007.04.001

technique with good discrimination and could be employed directly for routine typing of field isolates from fowl cholera outbreaks.

Keywords: Avian; *Pasteurella multocida*; PCR; Repetitive region; Typing

Résumé

Les méthodes PCR: repetitive extragenic palindromic (REP)-PCR, enterobacterial repetitive intergenic consensus (ERIC)-PCR, et les PCR simples ont été utilisées pour caractériser 66 souches de *Pasteurella multocida* serogroup A:1 isolées à partir d'espèces d'oiseaux de différentes régions de l'Inde. La (REP)-PCR a eu pour résultat le développement de séquences de REP du génome dans la gamme de ~200 à ~3000 bp et a permis de distinguer cinquante quatre profils différents. La méthode (ERIC)-PCR a permis d'amplifier des produits dans la gamme de ~200 à ~3200 bp caractérisant cinquante profils différents. L'amplification de régions répétitives utilisant un microsatellite primaire $(GTG)_5$, a permis de distinguer des bandes entre ~200 à ~2400 bp. Les 66 souches ont été classées dans 43 profils. Il n'a pas pu être établi de corrélation entre les profils génotypiques et les secteurs géographiques dans lesquels ont été prélevées les souches. Les souches aviaires de *P. multocida* serogroup A:1 ont montré des profils hétérogènes divers. La méthode REP-PCR s'est montrée comme étant la plus discriminatoire et la plus simple pour la différenciation de phénotypique. Cette étude a indiqué que la PCR basée sur une amplification des régions répétitives de *P. multocida* est une méthode rapide permettant une bonne discrimination et pourrait être utilisée directement pour le typage de routine d'enzooties de Pasteurellose aviaire.

Mots clés: Pasteurella multocida aviaire; PCR; (REP)-PCR; (ERIC)-PCR

1. Introduction

Pasteurella multocida, a gram-negative bacteria which resides as a commensal in upper respiratory tract of many mammals and birds, is also associated with human infections resulting from cat and dog bites [1–3]. Apart from being a causative agent for many economically important diseases such as Haemorrhagic septicemia, atropic rhinitis, pneumonia and snuffles among domesticated animals throughout the world [1], it is also known to cause fowl cholera in domestic as well as wild birds [4]. Although, five capsular serogroups (A, B, D, E & F) based on indirect haemagglutination assay (IHA) and 16 somatic serogroups (1–16) based on agar gel precipitation test have been identified, the strains belonging to *P. multocida* serogroup A:1 are predominantly associated with most severe form of fowl cholera [4]. The diagnosis and differentiation of strains still largely rely on conventional methods of isolation and identification followed by serotyping, pathotyping, antibiogram and biotyping despite the implication of various DNA based assays for rapid detection [5,6] and differentiation of phenotypically similar strains [7–10].