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Molecular cloning of a *Mycoplasma meleagridis*-specific antigenic domain endowed with a serodiagnostic potential

B. Ben Abdelmoumen Mardassi *, A. Béjaoui Khiari, L. Oussaief, A. Landoulsi, C. Brik, B. Mlik, F. Amouna

Laboratoire des Mycoplasmes, Institut Pasteur de Tunis, 13 Place Pasteur, B.P. 74, 1002 Tunis-Belvédère, Tunis, Tunisia

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Abstract

A recombinant phage library harbouring *Mycoplasma meleagridis* (MM) genomic DNA fragments was generated in the bacteriophage lambda gt11 expression vector. The library was screened for expression of MM specific antigens with a polyclonal antiserum that had been preadsorbed with antigens of the most common unrelated avian mycoplasma species. A 49-aminoacid antigenic domain unique to MM was isolated, expressed in *Escherichia coli*, and its serodiagnostic potential was demonstrated. An antiserum raised against this MM-specific antigenic domain recognized a cluster of seven membrane-associated MM proteins with molecular masses ranging from 34 to 75 kDa. Overall, this study resulted in the identification of a potent serodiagnostic tool and revealed the complex antigenic nature of MM.

Keywords: *Mycoplasma meleagridis*; Genomic library; Species-specific epitopes; Fusion protein; Serodiagnosis

1. Introduction

Mycoplasma meleagridis (MM) is widespread in turkey flocks throughout the world. It is the primary cause of poor growth and feathering, airsacculitis, osteodystrophy, and reduction in hatchability (Stipkovits and Kempf, 1996). Moreover, MM can have an immunosuppressive effect in young birds. The organism may be harboured in the cloaca and bursa of fabricius of poults, in the oviduct and the upper respiratory tract of mature birds, without causing disease in these tissues. Under such conditions the organism may survive for months (Jordan, 1990).

A number of antigens appear to be shared between MM and the more important poultry mycoplasmas, namely, *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS), resulting in cross-reactivities that complicate serological investigations (Ben Abdelmoumen and Roy, 1995a; Bradley et al., 1988; Olson et al., 1965; Taylor-Robinson et al., 1963). To circumvent this major problem, caution should be used in the establishment and the development of specific serological assays to prevent

* Corresponding author. Tel.: +216 71 845 368; fax: +216 71 791 833.
E-mail address: Boutheina.mardassi@pasteur.rns.tn (B.B.A. Mardassi).

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false-positives as it has been done with the development of a blocking ELISA using two mouse monoclonal antibodies for detection of turkey antibodies to MM (Dufour et al., 2001b). In addition, incorporation of gamma globulins of serum broth medium in the mycoplasma membrane during their culture, are worthy to consider when using serological test systems as the adsorbed globulins react with antiglobulin factors leading to false results (Bradbury and Jordan, 1971). Hence, recombinant antigens appear to be a good candidate which could be a reliable substitute for native antigens used in serodiagnostic tests and may overcome difficulties encountered in antigen preparations from mycoplasma cultures. In this respect, a significant progress has been made for the identification and characterization of genes encoding immunogenic proteins of MG and MS. With regard to diagnostics, the species-specific haemagglutinin genes of both MG and MS (the VlhA gene family) (Noormohammadi et al., 1998; Papazisi et al., 2003), contributed significantly to the set up of robust serodiagnostic and molecular assays (Ben Abdelmoumen et al., 1999, 2005; Benčina et al., 1999; Glew et al., 1995; Noormohammadi et al., 1997, 1999). However, so far, there are few molecular data concerning the MM species. The few sequences available are of the 16S ribosomal RNA (Boyle et al., 1995; Kiss et al., 1997; Moalic et al., 1997). Although some studies on MM proteins have revealed antigenic heterogeneity and variation between strains (Dufour et al., 2001a; Zhao et al., 1988), genes encoding immunodominant antigens have not yet been isolated and characterized.

This study aims at identifying MM gene products with a potential for the development of specific serodiagnostic assays. For this purpose, we used expression screening of a genomic MM library with an anti-MM polyclonal serum that had been depleted from antibodies that might recognize epitopes in other six major mycoplasma species. This strategy has previously been shown to considerably minimize cross-reactivities between avian mycoplasma species and allows for the identification of species-specific mycoplasmal antigens (Ben Abdelmoumen and Roy, 1995a,b; Ben Abdelmoumen et al., 1999). We describe the isolation of a DNA segment encoding an immunogenic site unique to MM and provide evidence of its serodiagnostic potential.

2. Materials and methods

2.1. Bacterial strains, bacteriophage, plasmids and culture conditions

The following mycoplasma reference strains were used: MM 17529 (ATCC 25294), MS WVU 1853 (ATCC 25204), MG PG 31 (ATCC 19610), *M. iners* PG 30 (ATCC 19705), *M. gallinarum* NCTC 10120 (ATCC 19708) and *M. iowae* 695 (ATCC 33552). *Acholeplasma laidlawii* (ATCC 14089), which is a mollicute capable of engendering false-positive reactions with some serological tests was also used. In addition, we included ten MM field isolates recovered from two distinct flocks located 300 km apart (Table 1). Each mycoplasma strain was cultured in Frey's medium (Frey et al., 1968) supplemented with 15% fetal calf serum. Culture and antigen preparation were performed as described elsewhere (Ben Abdelmoumen and Roy, 1995a). Antigens of the reference strains were prepared from a low passage stock and were stored at −20 °C until they were needed either for Western blots or for DNA extraction. MM field isolates were cloned thrice from separate colonies obtained after plating limiting dilutions.

For genetic manipulations and subcloning, three *Escherichia coli* strains Y1090r- (Stratagene, La Jolla, CA), HB101 (ATCC 33694) and BL21 (DE3) star (Promega, Lyon, France) were used. *E. coli* strain Y1090r- was grown in TB medium (1% Bacto Tryptone; 0.5% NaCl; 0.2% maltose; 10 mM MgSO4, pH 7.4) at 30 °C overnight with shaking. The *E. coli*

Table 1
Mycoplasma meleagridis (MM) field strains used in the dot-blot assay

Strain	Origin
MM B889	Institut Pasteur, Tunis
MM B35	Institut Pasteur, Tunis
MM B781	Institut Pasteur, Tunis
MM B870	Institut Pasteur, Tunis
MM B26B9	Institut Pasteur, Tunis
MM B25A3	Institut Pasteur, Tunis
MM B24B8	Institut Pasteur, Tunis
MM B210	Institut Pasteur, Tunis
MM B25A4	Institut Pasteur, Tunis
MM B24B3	Institut Pasteur, Tunis