The 32 kDa major outer-membrane protein of *Pasteurella multocida* capsular serotype D

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The major outer-membrane protein (MOMP) of *Pasteurella multocida* serotype D strain P210, with an apparent molecular mass of 32 kDa, was purified and characterized. The purification method involved selective extraction of MOMP with *N*-lauroylsarcosine and SDS, followed by immunoaffinity chromatography using a murine monoclonal antibody (mAb). The N-terminal sequence and amino acid composition of the MOMP showed considerable similarity to other Gram-negative bacterial porins, notably to the 37 kDa MOMP (porin H) of *P. multocida*. Immunoelectron microscopy and colony blotting assays were used to demonstrate the surface localization of the 32 kDa MOMP on bacterial cells. The colony blotting assay provided a simple, sensitive and rapid screening method for visualizing accessibility of the antibody on the cells. In a Western blot assay, murine polyclonal hyperimmune serum against the purified 32 kDa MOMP recognized both serotype B and D strains bearing either a 32 kDa or a 37 kDa MOMP, whereas the mAb recognized only serotype D strains bearing a 32 kDa but not a 37 kDa MOMP. The present data indicate that the 32 kDa MOMPs of *P. multocida* are antigenically heterogeneous and possess both specific and cross-reacting epitopes. Detection of type-specific epitopes on the 32 kDa MOMP using an mAb may have potential implications regarding the feasibility of developing a serotyping system for *P. multocida*.

Keywords: *Pasteurella multocida*, porin, monoclonal antibody, purification

INTRODUCTION

Pasteurella multocida, a Gram-negative organism, is widely distributed throughout the world and is known to cause a variety of diseases in animals and humans (Rimler, 1994; Fajfar-Whetstone *et al.*, 1995). The outer membrane of Gram-negative bacteria contains lipopolysaccharides, several minor proteins and a limited number of major proteins present in very high copy number (Hancock, 1991). Some of the major outer-membrane proteins (MOMPs) are called porins because of their role in cellular permeability (Benz, 1988; Lugtenberg & van Alphen, 1983). Immunological (Roy *et al.*, 1994; Lupi *et al.*, 1989; Takada *et al.*, 1988), biochemical (Lugtenberg & van Alphen, 1983; Rosenbusch, 1974) and physiological (Hancock, 1987) properties of Gram-negative bacterial porins have been extensively studied. The interest in the potential use of porins in developing serotyping systems (Tagawa *et al.*, 1993; Hamel *et al.*, 1987; Barenkamp *et al.*, 1981; Buchana & Hildebrandt, 1981) and effective vac-

cines (Isibasi *et al.*, 1992; Gilleland *et al.*, 1984) is rapidly increasing.

Several studies have been reported to identify the potentially important outer-membrane proteins (OMPs) of *P. multocida* (Dubreuil *et al.*, 1992; Lu *et al.*, 1991a, b; Abdullahi *et al.*, 1990) and to classify *P. multocida* strains based on the electrophoretic mobility of MOMPs (Vasfi Marandi & Mittal, 1995; Ramdani & Adler, 1994; Johnson *et al.*, 1991), but only a few clarify the basic characteristics of the OMPs of *P. multocida*. Kasten *et al.* (1995) cloned and sequenced a gene encoding a 16 kDa OMP of *P. multocida* and designated it P6-like protein of *Haemophilus influenzae*. Chevalier *et al.* (1993) purified a 37 kDa MOMP (H protein) of *P. multocida* and characterized it as a pore-forming protein related to the superfamily of non-specific porins of Gram-negative bacteria. We have recently classified *P. multocida* serotype D strains into two types (I and II) based on the electrophoretic mobility of MOMPs with a molecular mass of 32 or 37 kDa (Vasfi Marandi & Mittal, 1995). The purpose of the present study was to purify and characterize a 32 kDa MOMP of *P. multocida* serotype D and to compare it with the other MOMPs.

Abbreviations: KDO, 2-keto-3-deoxyoctonate; (M)OMP, (major) outer-membrane protein.

METHODS

Bacterial strains. Reference strains 656 (serotype B) and P210 (serotype D) of *P. multocida* were obtained from G. R. Carter of Virginia-Maryland Regional College of Veterinary Medicine, USA. Strains 5120 and 4992 (serotype B) and 5152, 3881, 4065, 3018, 2153, 4275 and 4275 B (serotype D) of *P. multocida*, isolated from bison, cattle, chicken, rabbit, swine or turkey, were obtained from R. B. Rimler of the National Animal Disease Center, Ames, IA, USA.

Preparation of OMP fractions. *P. multocida* serotype D strain P210 was grown on blood agar base containing 5% (v/v) calf blood at 37 °C for 24 h. The growth was harvested and washed three times with 0.01 M PBS (0.145 M NaCl; 0.15 M sodium phosphate), pH 7.2. The bacterial suspension was centrifuged at 10000 *g* for 20 min. The pellet was suspended in 10 ml 50 mM Tris/HCl (pH 7.5) containing 5 mM EDTA and used for preparation of OMPs. The bacterial cells were disrupted in a French press at 20000 p.s.i. (138000 kPa). OMP fractions were prepared as described by Chevalier *et al.* (1993) with some modifications. Briefly, an aliquot of 10 ml disrupted cells containing 30 mg protein was treated with 1% (w/v) *N*-lauroylsarcosine (Sarkosyl, Sigma) at 4 °C overnight to solubilize the inner-membrane proteins. The mixture was centrifuged at 120000 *g* for 1 h. This step was repeated once. The insoluble material was incubated with 10 ml 2% (w/v) SDS in 50 mM Tris/HCl containing 5 mM EDTA and 0.5 M NaCl and the suspension was gently stirred for 1 h at 37 °C. The supernatant recovered after centrifugation at 120000 *g* for 1 h was dialysed at room temperature and subjected to affinity chromatography.

Antibody production

(i) **Murine antiserum.** Polyclonal hyperimmune serum to the 32 kDa MOMP of *P. multocida* serotype D strain P210 was produced in four 6-week-old female BALB/c mice. The mice were inoculated intraperitoneally with 30 µg MOMP emulsified in an equal volume of Freund's incomplete adjuvant on day 0, 14, 21 and 28 and blood samples were collected 3 d after the last injection by puncturing the supraorbital plexus with a capillary pipette. Sera were separated and stored at -20 °C.

(ii) **mAb.** Murine mAb against the 32 kDa MOMP of *P. multocida* serotype D strain P210 (mAb MT1) was produced as described by Vasfi Marandi & Mittal (1995). mAb MT1 was purified from 20 ml ascite fluid with caprylic acid and ammonium sulphate as described by Ogden & Leung (1988). The precipitate was resuspended in PBS and dialysed extensively against PBS and coupling buffer (0.1 M sodium bicarbonate buffer containing 0.5 M NaCl; pH 8.5).

Affinity chromatography. mAb MT1 (27 mg) was covalently coupled to 7 ml acid-washed (1 mM HCl) cyanogen-bromide-activated Sepharose 4B according to the manufacturer's instructions (Pharmacia). The unreacted sites were blocked with 0.2 M glycine (pH 8.0) at 4 °C for 12 h. The gel was treated with four alternating washes of 0.1 M acetate buffer (pH 4.0) containing 0.5 M NaCl and coupling buffer and later stored at 4 °C. Affinity chromatography was carried out by the method of Serhir *et al.* (1995). Briefly, the gel with immobilized mAb was washed with 50 mM Tris/HCl containing 5 mM EDTA and 0.5 M NaCl, pH 7.5, followed by elution buffer (0.1 M citrate buffer, pH 4.0), and re-equilibrated in starting buffer. The OMP-enriched fraction was loaded onto the column, then incubated for 1 h at room temperature and washed with 25 ml starting buffer. The bound material was eluted with citrate buffer, and the peak was monitored at 280 nm with a UV-M detector (Pharmacia). The

eluted material was dialysed against triple distilled water, lyophilized and stored at -20 °C.

Determination of protein and 2-keto-3-deoxyoctonate (KDO). The protein concentration was determined by the method of Bradford (1976). KDO was determined as described by Hanson & Phillips (1981), using commercial KDO (Sigma) as a standard.

SDS-PAGE. SDS-PAGE was performed by the method of Laemmli (1970). The antigens were solubilized in sample buffer containing 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, 1 M Tris base (pH 8.3) and 0.025% bromophenol blue, and the solution was heated at 100 °C for 5 min. Proteins were separated on a 4% (w/v) polyacrylamide stacking gel and a 12% (w/v) separating gel. The protein bands were visualized by staining with 0.1% (w/v) Coomassie brilliant blue R-250 or silver nitrate (Morrisey, 1981).

Western blotting. This was performed by the method of Towbin *et al.* (1979). Briefly, the samples separated by electrophoresis were transferred onto nitrocellulose membranes. The membranes were blocked with 2.5% (w/v) skimmed milk in PBS for 90 min and incubated with murine antiserum diluted 1 in 100 or hybridoma culture supernatant containing mAb MT1 diluted 1 in 4 in the blocking solution for 90 min at room temperature. The membranes were then washed in PBS containing 0.05% Tween 20 (PBS-T), and bound antibodies were detected with horseradish-peroxidase-conjugated goat anti-mouse IgG (Bio-Rad) using 4-chloro-1-naphthol as the chromogenic substrate.

Amino acid composition analysis. The 32 kDa MOMP was electroblotted onto Immobilon-P transfer membrane (Millipore) using the method of LeGendre & Matsudaira (1988). The protein was stained with Coomassie brilliant blue R-250, then the sample was subjected to automatic hydrolysis. The amino acid composition was determined with a model 420A derivatizer-analyser system which included a model 920A analysis module and a model 130A separation system (Applied Biosystems). The analysis was performed with the standard program.

N-terminal amino acid sequencing. Automated Edman degradation was performed with a model 470A gas phase sequencer (Applied Biosystems) equipped with a model 120A on-line phenylthiohydantoin analyser (Applied Biosystems) using the general protocol of Hewick *et al.* (1981). Samples were applied to trifluoroacetic-acid-treated cartridge filters coated with 1.5 mg Polybren and 0.1 mg NaCl (Biobrene Plus; Applied Biosystems). The standard 03RPTH program was used for sequencing. The identities of the phenylthiohydantoin amino acid derivatives were determined by comparison with standards (phenylthiohydantoin analyser standards; Applied Biosystems) that were analysed on-line prior to the sequence analysis.

Immunoelectron microscopy. Immunogold labelling of whole cells of *P. multocida* capsulate strain 4275 and its non-capsulate variant 4275 B was performed as described earlier by Li *et al.* (1992). Single drops of sample were placed on Formvar-coated grids and blocked for 5 min with 1% (w/v) egg albumin. The grids were incubated for 30 min with optimally diluted murine hyperimmune serum or normal mouse serum as control and rinsed five times with distilled water. The grids were then incubated for 30 min with goat anti-mouse IgG conjugated with 10 nm gold particles (Sigma), rinsed and negatively stained with 1% (w/v) phosphotungstate for 10 s. The grids were observed with an electron microscope (Philips 201) at an accelerating voltage of 60 kV.

Colony blotting assay. A colony blotting assay was performed using the modified method of Mutharia & Hancock (1985). Bacterial colonies were transferred from agar plates onto nitrocellulose membranes by contact at 37 °C for 30 min. The membranes were then carefully removed and dried at room temperature for 20 min. The colony blots were blocked with PBS containing 2.5% skimmed milk and successively incubated with hybridoma culture supernatant containing mAb MT1. The membranes were then washed in PBS-T and bound antibodies were detected with horseradish-peroxidase-conjugated goat anti-mouse IgG (Bio-Rad) by using 4-chloro-1-naphthol as the chromogenic substrate.

RESULTS AND DISCUSSION

Purification of the 32 kDa MOMP

OMP fractions were obtained by treatment of disrupted bacterial cells with *N*-lauroylsarcosine and SDS as described by Chevalier *et al.* (1993) for protein H of *P. multocida*. Although the last fraction obtained by this procedure was highly enriched in 32 kDa MOMP (Fig. 1, lanes 1–4), further purification was accomplished by affinity chromatography using mAb MT1 (Fig. 1, lane 5). Six millilitres of the OMP fraction containing 0.85 mg protein ml⁻¹ was applied to the immunoabsorbent column prepared with mAb MT1. Although MOMP was also detected in abundance in the unbound fractions (data not shown), 425 µg purified MOMP was eluted from the immunoabsorbent column. MOMP purified by this procedure still contained a small amount of LPS as indicated by detection of 0.48 µg KDO (mg protein)⁻¹. The results suggested that *P. multocida* MOMP also tends to associate with LPS as has been described for other porins by Gulig

Fig. 1. SDS-PAGE analysis of MOMP purification steps of *P. multocida* P210. Lanes: 1, cell envelope preparation fraction (22 µg protein); 2, first *N*-lauroylsarcosine-insoluble fraction (18 µg); 3, second *N*-lauroylsarcosine-insoluble fraction (12 µg); 4, supernatant of SDS fraction in the presence of 0.5 M NaCl (10 µg); 5, MOMP purified by affinity chromatography (10 µg). The gel was stained with Coomassie brilliant blue. Molecular mass markers are indicated on the left.

Fig. 2. SDS-PAGE and Western blot analysis of a whole-cell suspension (lane 1) and affinity-purified MOMP (lane 2) of *P. multocida* P210. Bands were stained with silver nitrate (a) or the proteins were transferred onto a nitrocellulose membrane and incubated with a hybridoma culture supernatant of mAb MT1 (b). Molecular mass markers are indicated on the left.

& Hansen (1985) and Nikaido & Vaara (1985). Thus, the present purification method failed to purify the 32 kDa MOMP to homogeneity. Besides a major band of 32 kDa, a few faint bands of low and high molecular mass were also observed on staining of SDS-PAGE-separated proteins by Coomassie brilliant blue (Fig. 1, lanes 4 and 5) and silver nitrate (Fig. 2a, lane 2). In a Western blot, mAb MT1 recognized a major band in both whole cell (Fig. 2b, lane 1) and MOMP (Fig. 2b, lane 2) antigens, as well as a few faint bands present in purified MOMP (Fig. 2b, lane 2). Although purification was carried out in the presence of 0.5 M NaCl, in order to avoid the spontaneous dissociation of MOMP, it is speculated that the low molecular mass faint bands still present in the affinity-purified 32 kDa MOMP were possibly degraded products of MOMP. Although it is difficult to explain the appearance of the faint high molecular mass bands in the purified protein, it may possibly be due to insufficient solubilization of purified MOMP in the sample buffer.

Amino acid composition and sequence analysis

N-terminal amino acid sequence analysis was carried out until the first 30 residues of the 32 kDa MOMP from *P. multocida* P210 were determined (Fig. 3). A search for homology revealed that it shared 93.75%, 75%, 56.25% and 50% identity in a 16-amino-acid overlapping region with protein H of *P. multocida* (Chevalier *et al.*, 1993),

32 kDa MOMP	ATVYNQDGTKVDVNGSVXLI LKKEKNEXGD
H	ATVYNQDGTKVDGNGSVXRLXXXI LKKEKD
40 kDa MOMP	TTVYNQNGTKVDVGGRXXXXXXXXXXXXXXXXXX
OmpC	AEVYNKDGKLDLYGKVDGLHXXYFSDNKD
P2	AVVYNNEGTVNVEI XGGXXRLSXXI I AEQSN

Fig. 3. Comparison of the N-terminal sequence of the 32 kDa MOMP of *P. multocida* P210 with protein H of *P. multocida* 9222, the 40 kDa MOMP of *H. somnus*, OmpC of *E. coli* and porin P2 of *H. influenzae*. The N-terminal sequences of porin H of *P. multocida* and the 40 kDa MOMP of *H. somnus* have been determined by Chevalier *et al.* (1993) and Tagawa *et al.* (1993), respectively. The N-terminal sequences of the other bacterial porins were determined from the DNA sequences of the cloned porin genes for *H. influenzae* type b P2 (Hansen *et al.*, 1989) and *E. coli* OmpC (Mizuno *et al.*, 1983). X, Not identified.

MOMP of *Haemophilus somnus* (Tagawa *et al.*, 1993), OmpC of *Escherichia coli* (Mizuno *et al.*, 1983) and P2 of *H. influenzae* (Hansen *et al.*, 1989), respectively. Comparison of 30 amino acid residues of the N-terminal sequence of the 32 kDa MOMP of *P. multocida* with those of protein H of *P. multocida* showed 60% similarity. Results indicate that the 32 kDa MOMP of *P. multocida* exhibits considerable N-terminal sequence homology with the porin proteins of other Gram-negative bacteria, notably with protein H of *P. multocida*.

The amino acid composition of the 32 kDa MOMP is shown in Table 1. The molecule comprised 30.41% hydrophobic, 27.17% acidic and 21.84% basic residues. On the basis of amino acid composition, the structure/function ratio 3 calculated by the algorithm of Barrantes (1975) was 1.61, suggesting that the protein is characteristically a peripheral membrane protein. Conclusive evidence for this came from N-terminal sequence homology of MOMP with other membrane proteins of Gram-negative bacteria.

The overall amino acid composition of the 32 kDa MOMP of *P. multocida* was compared with that of protein H of *P. multocida*, P2 of *H. influenzae* and OmpC of *E. coli* (Table 1). It shared several properties with the porins as illustrated in Table 1, such as a low proline and high glycine content. The amino acid composition of MOMP of *P. multocida* P210 also exhibited considerable differences from that of protein H of *P. multocida*, such as a higher aspartic acid, arginine and lysine residue content.

Comparison of MOMPs of *P. multocida* strains

The OMP profile of *P. multocida* strains of serotypes B and D isolated from different animal species was compared by SDS-PAGE using whole-cell antigens (Fig. 4). All the strains tested contained MOMP with a molecular mass of 32 kDa (Fig. 4a, lanes 1–4 and lanes 6–8), except strain 2153, which possessed a 37 kDa MOMP instead of a

Table 1. Comparison of the amino acid composition of the 32 kDa MOMP of *P. multocida* P210 with protein H of *P. multocida*, P2 of *H. influenzae* and OmpC of *E. coli*

The amino acid composition of porin H of *P. multocida*, P2 of *H. influenzae* and OmpC of *E. coli* has been described by Chevalier *et al.* (1993), Hansen *et al.* (1989) and Mizuno *et al.* (1983), respectively. ND, Not determined.

Amino acid	Composition (mol %)			
	<i>P. multocida</i> MOMP	<i>P. multocida</i> H	<i>H. influenzae</i> P2	<i>E. coli</i> OmpC
Asx	18.83	12.57	12.32	18.20
Glx	8.34	7.63	11.14	9.25
Ser	5.44	5.60	4.99	4.62
Gly	12.54	12.18	11.73	13.87
His	1.48	1.30	2.05	0.29
Arg	8.82	4.04	4.69	3.76
Thr	5.43	4.69	7.04	6.94
Ala	7.63	8.21	7.04	6.94
Pro	0.82	1.51	0.88	0.87
Tyr	4.42	4.55	6.74	8.38
Cys	ND	0.00	0.00	0.00
Val	5.80	9.23	7.04	6.07
Met	0.42	0.67	0.29	0.87
Ile	1.33	3.09	4.40	2.89
Leu	5.47	7.90	7.04	6.36
Phe	4.52	5.01	3.81	5.49
Lys	12.54	8.95	8.80	4.33
Try	ND	2.82	0.00	0.87

Fig. 4. SDS-PAGE and Western blot analysis of whole-cell preparations (12 µg protein per lane) from *P. multocida* serotype D 5152 (lane 1), 3881 (lane 2), 4065 (lane 3), 3018 (lane 4) and 2153 (lane 5) and from serotype B 5120 (lane 6), 656 (lane 7) and 4992 (lane 8) isolated from chicken, cattle, rabbit, swine, turkey, cattle, bison and swine, respectively. Proteins were stained with Coomassie brilliant blue (a) or transferred onto nitrocellulose membranes and incubated with murine hyperimmune serum (b) and hybridoma culture supernatant of mAb MT1 (c). Molecular mass markers are indicated on the left.

32 kDa MOMP (Fig. 4a, lane 5). The murine hyperimmune serum against purified 32 kDa MOMP recognized both serotype B and D strains bearing either a 32 or a 37 kDa MOMP (Fig. 4b, lanes 1–8), whereas mAb MT1 recognized a major band of 32 kDa only in serotype D strains (Fig. 4c, lanes 1–4). However the reactivity of the major band with murine hyperimmune serum from serotype D strains bearing the 32 kDa MOMP (Fig. 4b, lanes 1–4) was stronger than that with serotype B strains bearing the 32 kDa MOMP (Fig. 4b, lanes 6–8) or a serotype D strain bearing the 37 kDa MOMP (Fig. 4b, lane 5). The present data indicate that the 32 kDa MOMP of *P. multocida* are antigenically heterogeneous and possess both specific and cross-reacting epitopes. Ramdani & Adler (1994) produced a type-specific mAb directed against the 33 kDa MOMP of *P. multocida* strains associated with haemorrhagic septicaemia. It is suggested that detection of type-specific epitopes on the MOMP of *P. multocida* strains using mAbs may have potential implications regarding the feasibility of developing a serotyping system.

Besides the 37 kDa MOMP described earlier as protein H by Chevalier *et al.* (1993), 32 kDa MOMP as shown in Fig. 4(a) may also be considered as protein H as suggested by Lugtenberg *et al.* (1984). Based on the discrepancies in the antigenic properties, amino acid composition and N-terminal amino acid sequences between MOMP of 37 and 32 kDa (Table 1; Figs 3 and 4), it is evident that *P. multocida* strains possess two different major porin proteins. Perusal of literature shows that a 32 kDa porin protein has not been previously identified as a protein distinct from the 37 kDa porin protein of *P. multocida*. Further analysis of the genes encoding the 32 and 37 kDa MOMP is needed to elucidate the genetic difference between the two MOMP.

Surface localization

Immunogold electron microscopy (Fig. 5a, b) was used to assess the surface exposure of the 32 kDa MOMP. The

number of gold particles that bound to capsulate strain 4275 (Fig. 5a) with murine hyperimmune serum was five times less than that of non-capsulate strain 4275 B (Fig. 5b). Both capsulate and non-capsulate strains probed with normal mouse serum did not bind any gold particles (data not shown). It is speculated that the 32 kDa MOMP on the surface of the capsulate *P. multocida* strain may not be adequately accessible to the antibody because of the masking of outer-membrane components by the capsule produced by this organism. More recently Jacques *et al.* (1994) reported that capsule production by *P. multocida* strains decreased under conditions of iron limitation *in vitro*, and bacterial cells grown *in vivo*, whereas expression of several OMPs increased under the same conditions. In addition, mice and rabbits immunized with non-capsulate *P. multocida* were protected against challenge with homologous capsulate *P. multocida* organisms (Rebers & Heddleston, 1974). These results suggest the involvement of outer-membrane components in protection against pasteurellosis. Experimental animals immunized with purified porins of *Salmonella typhi* (Isibasi *et al.*, 1992), *Pseudomonas aeruginosa* (Gilleland *et al.*, 1984) and *H. influenzae* (Murphy & Bartos, 1988; Munson *et al.*, 1983) were protected against challenge infection with corresponding organisms. Furthermore, a mAb against a 37.5 kDa MOMP of *P. multocida* inoculated intranasally protected rabbits and mice against an homologous challenge (Lu *et al.*, 1991b). Conversely, Abdullahi *et al.* (1990) reported that the MOMP of *P. multocida* were not protective antigens in a mouse model of pasteurellosis. However, the role of the 32 kDa MOMP in the protection against *P. multocida* infection remains to be studied.

A colony blotting assay was used to screen the surface localization of 32 kDa MOMP on *P. multocida* strains by using mAb MT1. All of the serotype D strains bearing the 32 kDa MOMP reacted with mAb MT1, although differences were observed in their intensity. In contrast, mAb MT1 failed to react with serotype D strain 2153 which lacked the 32 kDa MOMP and all three serotype B strains

Fig. 5. Immunogold labelling of whole cells of *P. multocida* capsulate strain 4275 (a) and its non-capsulate variant 4275B (b) with murine hyperimmune serum. Bar, 200 nm.

which possessed the 32 kDa MOMP (Fig. 6). The capsulate *P. multocida* strain 4275 and its non-capsulate variant 4275 B revealed differences in their reactivities with the mAb MT1 by the colony blotting assay as compared by immunogold electron microscopy. The colony blotting procedure may be considered a simple, sensitive and rapid screening assay for demonstrating the surface-localized epitopes of porin proteins. These results are in agreement with those obtained by Mutharia & Hancock (1985) regarding the surface accessibility of porin F of *P. aeruginosa*, as assessed by colony blotting assay.

In this study, we purified a 32 kDa MOMP of *P. multocida* by affinity chromatography using mAb MT1 and demonstrated conclusively that it is related to the porins of Gram-negative bacteria, notably to the protein H of *P. multocida*. Also, this protein was shown to be antigenically heterogeneous. Based on the data obtained, it is suggested that detection of type-specific epitopes on the 32 kDa MOMP using an mAb may have potential implications

Fig. 6. Colony immunoblot showing the interaction of *P. multocida* serotype D strain P210 MOMP specific mAb (mAb MT1) with *P. multocida* serotype B 5120 (1), 656 (2) and 4992 (3) and serotype D 2153 (4), 5152 (5), 3881 (6), 4065 (7), 3018 (8), 4275 (9) and 4275 B (10) strains.

regarding the feasibility of developing a serotyping system for *P. multocida*.

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