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ORIGINAL ARTICLE

Structural Features of a Highly Conserved Omp16 Protein of *Pasteurella multocida* Strains and Comparison with Related Peptidoglycan-associated Lipoproteins (PAL)

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Abstract Bacterial lipoproteins of varying size and functional role in physiology/pathogenesis are widely prevalent among Gram-negative bacterial species. Among virulent bacteria, *Pasteurella multocida* is known to be associated with diverse infectious diseases of livestock worldwide and possess several outer membrane proteins (OMPs) as virulence factors. In the present study, we cloned and sequenced *omp16* gene of different *P. multocida* strains (n = 9) of Indian origin and compared with representative related bacterial species from *Pasteurellaceae* (n = 28) as well as others (n = 11). Phylogenetic and multiple sequence alignment revealed high degree conservation of Omp16 among *P. multocida* strains. However, it significantly differed from similar peptidoglycans-associated lipoproteins of other Gram-negative bacterial species. Notably, a conversed lipobox at N-terminus of proteins was noticed among all bacterial species. Further, three dimensional homology model of Omp16 was predicted and its structural features were analyzed. The study indicated the potential possibilities to use either native or recombinant Omp16 protein in developing species-specific diagnostic assay or broadly active subunit vaccine along with suitable adjuvant for pasteurellosis in livestock.

Keywords Omp16 outer membrane lipoprotein · *Pasteurella multocida* · Pasteurellosis · *Pasteurellaceae* · Sequence analysis · Structural features

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Introduction

Pasteurella multocida, a Gram-negative bacterial pathogen, is associated with a variety of infectious diseases in wide range of animals/birds causing haemorrhagic septicaemia (HS) in cattle and buffaloes [1], fowl cholera in poultry [2], atrophic rhinitis in pigs, snuffles in rabbits, pneumonic and septicaemic pasteurellosis in sheep, goats, wild animals [3] and occasional infections in humans through dog/cat bites, which are collectively termed as 'pasteurellosis' [4]. Members of the species are subgrouped on the basis of their capsular (A, B, D, E and F) and somatic (1–16) antigens, which are classified on the basis of indirect haemagglutination assay (IHA) and agar gel precipitation tests (AGPT). Considerable variation has been observed among different serogroups with respect to host predilection, virulent factors, mechanism of pathogenesis and immunity. Although, prophylactic strategies involving several vaccine formulations are being implemented [4], none of them were found to be absolutely efficacious for control of pasteurellosis in livestock [5]. Since, there is a need to develop an improved broadly cross-protective novel vaccine as well as differentiating diagnostic assays for infections caused by the pathogenic members of *Pasteurellaceae*, it has been envisaged that identification of widely distributed and immunogenic

bacterial surface proteins is of prime importance in future development of recombinant outer membrane proteins (OMPs) based subunit vaccines or diagnostic assays [6–11].

Lipoproteins are one of the most abundant proteins in the outer membrane of Gram-negative bacteria which are considered as potential target antigens for vaccine development against many infectious diseases including pasteurellosis [12–14]. Among them, especially in the recent past, the role of peptidoglycan-associated lipoproteins (PAL) belonging to several Gram-negative bacterial species in pathogenesis and potential applications in development of diagnostics as well as subunit vaccines have been extensively studied [15, 16]. Initially, a 16 kDa protein isolated from *P. multocida* strain showed high similarity to P6 protein of *Haemophius influenzae* which has been shown to elicit a protective immune response. Further, bioinformatic analysis revealed that Omp16 belongs to the OmpA-like PAL superfamily [17]. Subsequently, immuno-reactivity was noticed against recombinant P6-like protein in all 16 *P. multocida* serotypes. A recent bioinformatics analysis using *P. multocida* genome sequence data predicted 129 proteins as secreted and located in the outer membrane, or lipoproteins with a total of 79 proteins as outer membrane localized [18]. Among them, most commonly prevalent 16 kDa OMP (Omp16) is considered as immuno-dominant. However, the role of the Omp16 protein in virulence, if any, is unknown. Since antibodies to P6, a homolog of Omp16, are potentially protective as observed previously, and there exists a potential possibility to incorporate Omp16 into vaccine formulations, it is important to establish the degree of antigenic homogeneity or heterogeneity of Omp16 among various *P. multocida* strains. Moreover, till now, no attempts were made to analyze the structural features of Omp16 and its sequences among members of *Pasteurellaceae* comparatively as well as its potential implications.

In the present study, we describe the comparative *omp16* gene sequence analysis of *P. multocida* strains and in members of *Pasteurellaceae* as well as its related peptidoglycan-associated OMP P6 lipoprotein sequences from Gram-negative bacterial species. A structural feature of Omp16 as predicted by homology model has also been discussed.

Materials and Methods

Bacteria, Vector and Primers

Pasteurella multocida strains (n = 9) maintained in the 'Clinical Bacteriology Laboratory', Indian Veterinary Research Institute (IVRI), Mukteswar, Uttarakhand (UK), India, were used. All these strains as mentioned in Supplementary Table 1 (Sl # 1 to 9) were previously isolated from clinical samples collected during the natural disease outbreaks at varied time period in the respective host species belonging to different geographical regions of India. For construction of clones, pJET1.2 vector (MBI-Fermentas, USA) and *Escherichia coli* TOP10 cells were used. One set of primers as described below were synthesized (IDT-DNA, USA) and procured for *omp16* gene amplification.

omp16 Gene Amplification and Sequencing

The DNA of all the strains was extracted using genomic DNA miniprep kit (MDI, India). PCR amplification of *omp16* gene from respective strains was carried out using a primer set and standard reaction conditions as described previously. A set of primers used were;

O16SF: 5′-ATGAAAAAACTAACT-3′ and O16SR: 5′-TTAGTATGCTAACACAGCAC-3′.

All the amplified *omp16* gene products were purified from the agarose gel using a gel extraction kit (Qiagen, USA) and ligated to pJET1.2 vector using CloneJET PCR Cloning Kit. Following transformation to *E. coli* TOP10 cells and selection of recombinant clones, the extracted plasmids from respective clones using plasmid mini kit (Qiagen, USA) were sequenced using an automated DNA sequencer (ABI PRISM 3100; Perkin Elmer, Applied Biosystems, USA).

Phylogenetic Analysis and Multiple Sequence Alignment of Omp16 Sequences

The *omp16* gene encoding for Omp16 belonging to Indian *P. multocida* strains (n = 12) as well as from foreign countries (n = 17) isolated from different host species and disease conditions as described in Supplementary Table 1 were used along with other Gram-negative bacterial species (n = 11) as mentioned in Supplementary Table 2 for comparative analysis. Phylogenetic tree construction and multiple sequence alignments were carried out using pairwise ClustalW method of MegAlign (DNASTAR). The percentage of identities and divergence at nucleotide as well as deduced amino acid level in these isolates/strains were also compared using the DNASTAR program.

Prediction of Omp16 Protein Characteristics and Homology Modeling

The *omp16* gene (GenBank Acc # AJ271673) from *P. multocida* serogroup B:2 strain P52 [19], an Indian HS vaccine strain, was used for prediction of matured Omp16 protein characteristics using the PROTEAN program (DNASTAR), PSIPRED as well as proteomics tools from

the ExPASy website. LipoP v1.0 server was used for prediction of lipobox in Omp16/18/P6 lipoproteins among representative members of Gram-negative bacterial species.

An automated homology model of Omp16 of *P. multocida* B:2 strain P52 was done by SWISS-MODEL [20] based on a recently described solution NMR structure of the periplasmic domain of PAL of *Hamophilus influenzae* (PDB ID-2AIZ-P) as template [15].

Results and Discussion

Although, it is well known that several bacterial species belonging to the family *Pasteurellaceae* are involved in causing highly infectious and contagious diseases with high morbidity/mortality rate in livestock with their varied ecological preferences for specific surfaces and hosts, very little is known about the factors that govern the host specificity. To date, there are 73 species included under 18 genera of family *Pasteurellaceae,* which contribute for diversity in antigenicity as well as pathogenesis/immunity. Antigenic heterogeneity in many of the surface antigens in *P. multocida* strains suggests that a highly conserved, immunogenic molecule is required for formulation of an effective vaccine. In view of establishment of molecular conservation of Omp16 which probably has potential implications in development of specific diagnostic and/or a cross-protective vaccine to prevent pasteurellosis, the present study was undertaken.

In this study, following PCR targeting full length *omp16* gene, all the *P. multocida* strains resulted in similar amplicon of 453 bp in size (Fig. 1, inset). The complete edited nucleotide sequences of *omp16* gene of nine *P. multocida* strains were submitted to GenBank and their accession numbers are presented in Supplementary Table 1. No change in the length of *omp16* gene/protein (453 bp and 150 aa) among various *P. multocida* strains was noticed. Previous results of PCR amplification and the Southern blot analysis also reflected the presence of *omp16* gene among *P. multocida* serotypes. However, variable lengths (gene: 453–627 bp; protein: 150–208 aa) in related PAL proteins in other Gram-negative bacterial species were also noticed.

Phylogenetic tree constructed based on Omp16/PAL protein sequences is depicted in Fig. 1. All the strains belonging to *P. multocida* were clustered into single group with an absolute homogeneity. However, members of family *Pasteurellaceae* were branched out independently making their separate Cluster-A than that of Cluster-B, which comprised of Gram-negative bacterial species. Similar tree was also noted based on their nucleotide sequences (data not shown). Multiple sequence alignment revealed that all Indian strains of *P. multocida* had absolute homogeneity (100 %) at amino acid level, however, at nucleotide level, the percentage of identity and divergence varied from 99.3 to 100 and 0 to 0.7 % respectively. A synonymous nucleotide changes were noticed at 228, 264, and 318 positions in *omp16* sequences of *P. multocida* strains. The percentage of identity/diversity among PAL proteins of representative members belonging to family *Pasteurellaceae* and *Enterobacteriaceae* is mentioned in Supplementary Fig. 1.

We observed high degree conservation of Omp16 protein among Indian strains of *P. multocida.* A similar sequence homology was also observed in an earlier report [19] indicating a wide distribution of *omp16* gene among different serotypes of *P. multocida* (n = 9) and found to localize in a 6.0 kb *Hind*III fragment of complete *P. multocida* genome. Comparison of the *omp16* gene sequences from various epidemiologically and serologically unrelated strains demonstrated 100 % homology at the amino acid level and 97–99 % homology at the nucleotide level. Omp16 of *P. multocida* was distinctly divergent from that of members belonging to *Pasteurellaceae* as well as *Enterobacteriaceae*. Despite the diversity of strains, the high level of conservation of Omp16 sequence likely reflects strong selective pressure on *P. multocida* to maintain the protein.

Predicted characteristics of Omp16/PAL proteins revealed identification of lipobox motifs in different members of Gram-negative bacterial species as mentioned in Fig. 2. The location of lipobox was found to be fairly constant among the members at 33–38 aa position of N-terminus. However, in genus *Pasteurella*, a conserved location was noticed at 17–20 aa position [L–S–G–C] with a cleavage site between $_{19}$G and C$_{20}$ aa. Lysine (L) and Cysteine (C) residues were almost largely conserved among all lipobox regions. The variable residues at 1 (V/I/A/L), 2 (V/A) and 3 (G/S/A) position of lipobox was noticed. The cleavage site was found to be between 3 (G/A) and 4 (C) residues position.

Following the initiating Methionine (M), there exist signal peptide region composed of 20 strongly hydrophobic amino acids, which is an essential feature at the N-terminal end of proteins that are membrane bound or secreted through membranes. It was noted that majority ($\sim$88 %) of well characterized prokaryotic signal peptides end with an Alanine or Glycine and minority ($\sim$10 %) end with a Serine, Cysteine, or Threonine [21]. We also observed consistent prevalence of signal peptide ($_{17}$LAAC$_{20}$), which was described for lipoproteins found to be conserved as potential leader sequence among all Omp16 sequences of *P. multocida* strains. The putative Omp16 signal peptide ends with the deduced amino acid sequence Ala–Cys–Gly. In addition, similarity of signal sequence carboxyl terminus

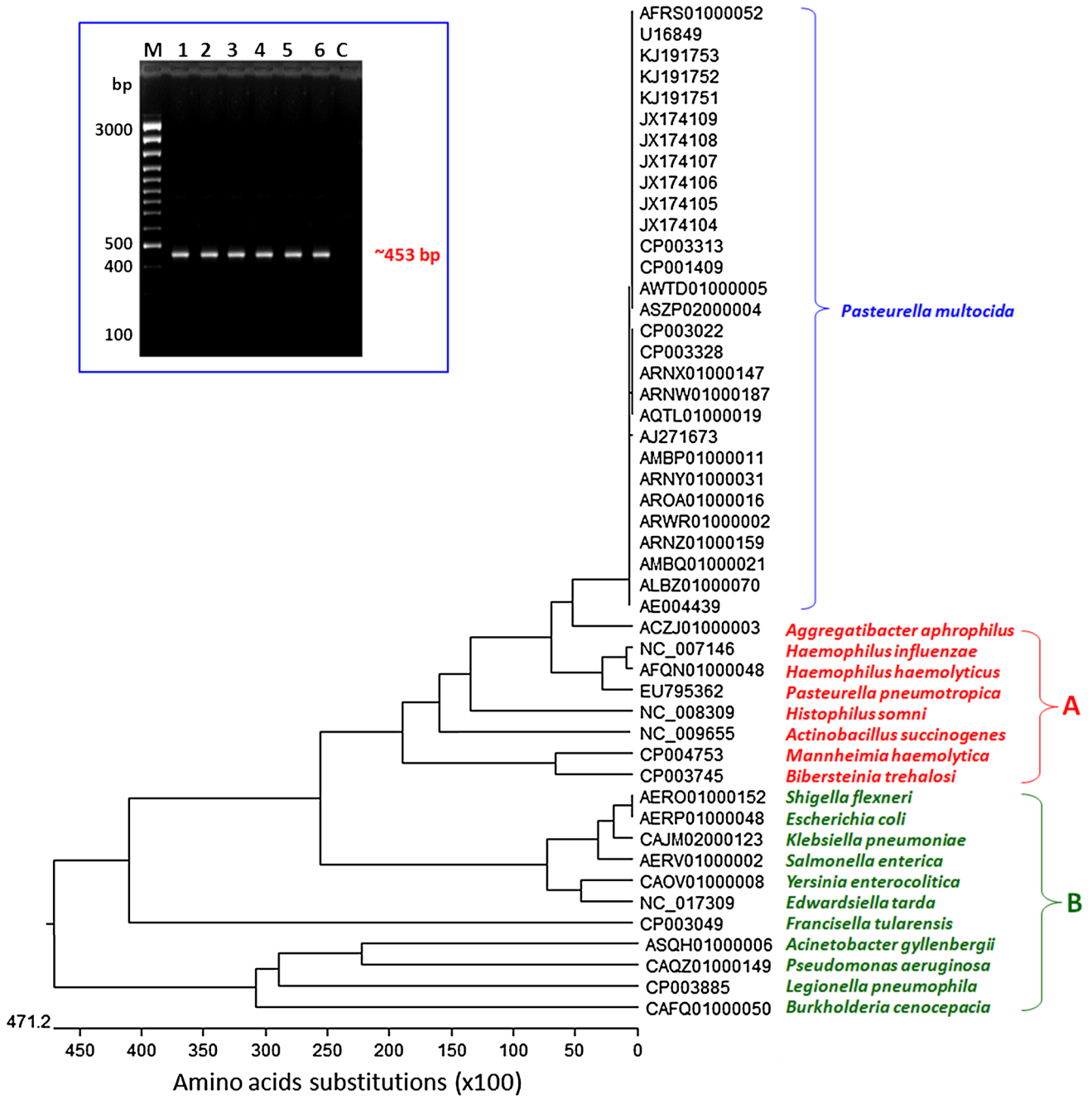

Fig. 1 Phylogentic tree based on Omp16/18 kDa/P6 proteins of different bacterial species. Omp16/18 kDa/P6 protein sequences of strains belonging to family *Pasteurellaceae* as well as representative members of family *Enterobacteriaceae* available in GenBank as listed in Supplementary Tables 1 and 2, were used in phylogenetic tree construction using the ClustalW method (DNASTAR). *Inset* PCR amplified product (453 bp) of *omp16* gene. *Lane M*: DNA marker; *Lanes* 1–6: Representative *omp16* gene products from *P. multocida* strains viz., IndPm113, IndPm115, IndPm116 IndPm167, IndPm219 and IndPm224 sequentially. *Lane C*: Negative control

with a common sequence found in bacterial lipoproteins strongly suggested that Omp16 is a lipoprotein. It was noted that the consensus sequence of amino acids in signal peptide region is a common structural feature thought to play a role in the modification and cleavage of bacterial prolipoproteins. The unmodified precursor of Omp16 contains a total of 150 amino acids with a calculated molecular size of 16,212 Daltons having isoelectric point 8.082. Post-translational proteolytic cleavage by signal peptidase II would likely to result in a protein composed of 131 amino acids (~14.32 kDa) with isoelectric point 6.115. The difference of ~2 kDa may partly be accounted

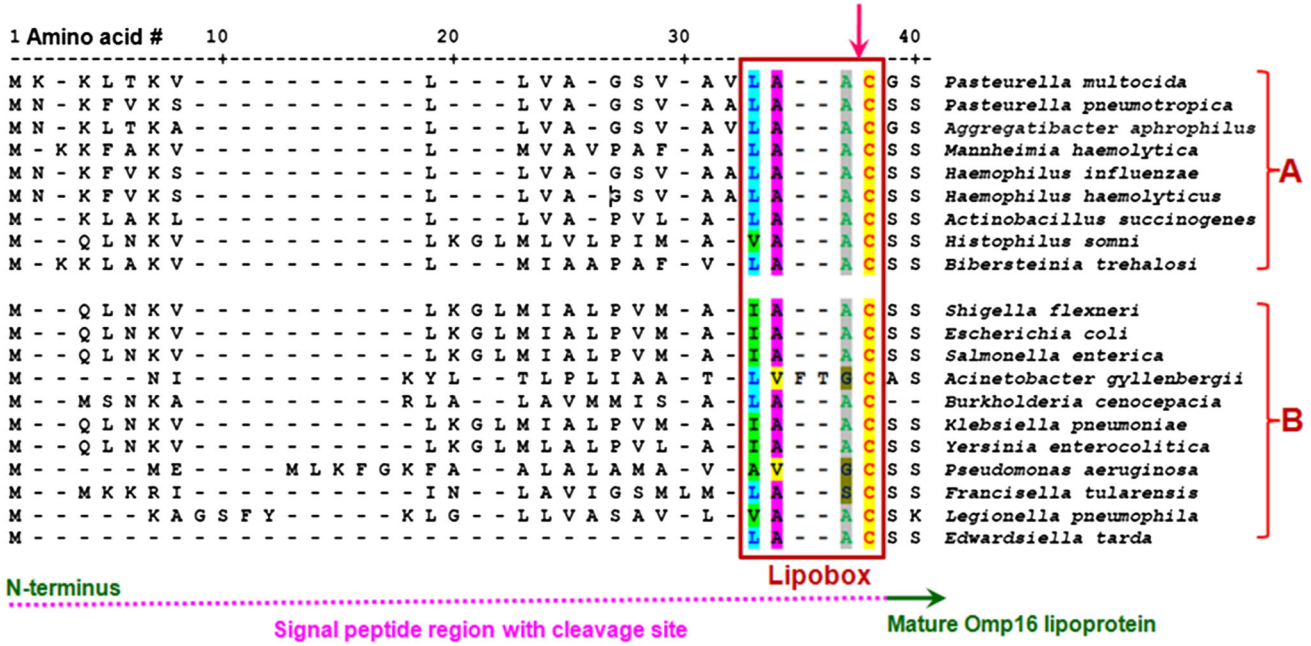

Fig. 2 A predicted lipobox region in PAL (Omp16/18 kDa/P6) proteins of different bacterial species. A total stretch of 40 amino acids at the starting of amino terminus of Omp16-like proteins of different bacterial species are indicated. The lipobox residues as predicted by LipoP v1.0 are indicated by a *red colored box*. A *blue colored square box* denotes variable lipobox region among *Pasteurellaceae*. A *pink colored downward arrow* denotes cleavage site within the lipobox. A *green colored horizontal arrow* denotes starting of mature Omp16/18/P6 lipoprotein. Panel **A** Representative members of family *Pasteurellaceae;* **B** Representative members of family *Enterobacteriaceae*. (Color figure online)

for a series of post-translational modifications, accompanying proteolytic removal of the signal peptide, in the formation of a mature lipoprotein. These modifications might include the transfer of a glyceryl moiety to the sulfhydryl group of the Cysteine, acylation of the glycerylcysteine, and attachment of a fatty acid to the alpha-NH_2 group of the Cysteine. A variable mobility of Omp16 proteins of *P. multocida* strains during SDS-PAGE analysis [22] were noticed, contrary to the fact that P6, an homologue of Omp16 in *Haemophilus influenzae* strains, showed absence of variations in mobility despite recently demonstrated variable P6 sequences among 10 % of non-typeable *H. influenzae* strains [23]. It warrants further studies on varied mobility patterns of PAL proteins among different bacterial strains.

On contrary to in silico proteome [24], in vitro OM proteome analysis of eight different *P. multocida* isolates recovered from different host species resulted in identification of 22 OMPs under iron-rich or iron-limited growth conditions [22]. Among them, Omp16 was commonly present across different *P. multocida* isolates irrespective of growth conditions but displayed molecular weight (~16–18 kDa) heterogeneity [22]. It was also noted that the expression of Omp16 was not affected by iron limitations. The conservation of Omp16 among different isolates reflects the broader host range of *P. multocida.* Since Omp16 is highly conserved, it is likely that it performs an important function for the bacterial cell. However, its role in physiology/pathogenesis has not yet been determined directly. Nevertheless, several indirect evidences indicate that it performs a structural function for the cell.

Three dimensional homology model of Omp16 of *P. multocida* based on similar PAL protein (82.8 % sequence identity) of *Haemophilus influenzae* is depicted in Fig. 3, Panel A. Structurally, mature Omp16 protein was found to posses N-terminus structureless region (18 residues) followed by four helix (α1–α4) and β-strand (β1–β4) regions which were linked by a varied flexible loop regions (4–9 residues). Omp16 formed a monomeric α/β sandwich with the secondary elements arranged in the order; α1–β1–α2–β2–α3–β3–α4–β4. The length of each helices ranged from four (α1) to maximum of twenty two (α3) residues, whereas β-strands were in the range of five (β1) to eight (β3) residues. Cysteine (C) residue, a point of lipidation, was found at N-terminus of the predicted mature protein.

The PAL family of proteins make up a relatively small proportion of the total protein content of the outer membrane is widespread in Gram-negative bacteria with sequence homologous in more than 100 organisms [15]. Bioinformatics analysis indicated that Omp16 belongs to the OmpA-like PAL superfamily. The N-terminal region of Omp16 is likely to form a flexible tail, which binds the

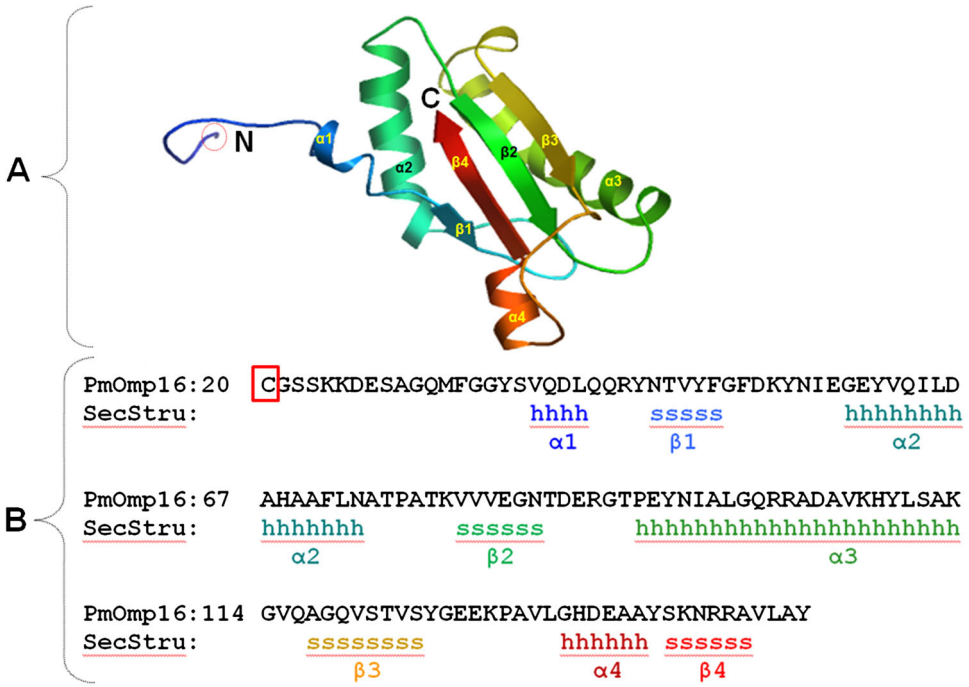

Fig. 3 Predicted structural features of Omp16 of *Pasteurella multocida*. Panel **A** Three dimensional model of Omp16 from *P. multocida* B:2 strain P52. It was generated using automated homology prediction by SWISS-MODEL server. A total stretch of 131 amino acids of mature Omp16 protein starting from N-terminus amino acid $_{20}$C and ending on C-terminal amino acid Y_{150} was used in developing a model using a PDB ID-2AIZ-P as template [15]. A dotted red circle at N-terminus denote site of lipidation. Panel **B** Predicted secondary structures of Omp16 along with its sequence. The abbreviations are as follows: *N* amino terminus; *C* carboxyl terminus; *h/α* helix; and *s/β* strand; *PmOmp16* outer membrane protein 16 of *P. multocida*; SecStru-secondary structure. *Numerical* along with α/β denotes their sequential helix/strand numbers starting from N-terminus. *Red colored square box* with Cysteine residue at N-terminus indicate region of lipidation. (Color figure online)

protein to the inner leaflet of the OM via lipid moieties linked to the Cysteine at the 20 position. The majority of Gram-negative bacterial outer membrane lipoproteins are tethered to the membrane via an attached lipid moiety and oriented facing in toward the periplasmic space; however, a few lipoproteins have been shown to be surface exposed [16]. Omp16/PAL protein is known to stabilize the outer membrane by providing a non-covalent link to the peptidoglycan (PG) layer through a periplasmic domain [15]. A specific region of PAL proteins including Omp16 was known to provide a pocket for the m-Dap residue of PG-P, a component of the cell wall present in the peptidoglycan layer of all Gram-negative bacteria, leading to formation of a cross-bridge between the OM and peptidoglycan [15]. Since, the solution NMR structure of the 134-residue periplasmic domain of *H. influenzae* PAL is similar to the crystal structure of a truncated 108-residue homologue from *E. coli* [15]; our homology predicted structure of mature Omp16 protein of *P. multocida* would likely to resemble similar architectural features. Recently, on the basis of flow cytometry analysis [25], it was demonstrated that P6 protein of non-typeable *H. influenzae* has a dual orientation, existing infrequently as surface exposed and predominantly as internally oriented toward the periplasmic space in a manner similar to its homologue PAL in *E. coli*. Since, Omp16 shares homology with the PAL protein of *E. coli* as well as P6 protein of *H. influenzae,* it may also be similarly involved in anchoring the outer membrane to peptidoglycan. Further, surface exposure of Omp16 in *P. multocida* strains could be ascertained by immune-electron microscopy of intact cells and identification of specific surface epitopes by monoclonal antibodies.

Ever since the OMPs of *P. multocida* have been identified as major immunogens, a number of reports have focused on the role of individual OMPs in eliciting an immune response [6, 7]. Omp16/PAL protein is known to constitute ~1–5 % of the protein content of the bacterial outer membrane. Unlike the variability in virulence genes such as *ptfA* [26, 27] and *plpE* genes, our study demonstrating a very high conservation of Omp16 among *P. multocida* strains irrespective of geography, serogroup and host species, is of considerable value since it could act as potential candidate antigen in multi-component vaccine for pasterurellosis. Recently, homogeneity and immunogenicity potential of highly conserved VacJ outer membrane lipoprotein of *P. multocida* has been reported [10, 14]. Another practical implication may be to use *omp16* gene

sequencing as a tool for identification of *P. multocida* strains alternatively to existing methods of PCR assays [28]. Further, understanding of Omp16–Tol complexes might also facilitate the design of new antibacterial drugs.

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References

1. Biswas A, Shivachandra SB, Saxena MK, Kumar AA, Singh VP, Srivastava SK (2004) Molecular variability among strains of *Pasteurella multocida* isolated from an outbreak of haemorrhagic septicaemia in India. Vet Res Commun 28:287–298. doi:10.1023/B:VERC.0000026656.77847.5b
2. Shivachandra SB, Kumar AA, Gautam R, Saxena MK, Chaudhuri P, Srivastava SK (2005) Detection of multiple strains of *Pasteurella multocida* in fowl cholera outbreaks by PCR based typing. Avian Pathol 34:456–462. doi:10.1080/03079450500367963
3. Kumar AA, Shivachandra SB, Biswas A, Singh VP, Singh VP, Srivastava SK (2004) Prevalent serotypes of *Pasteurella multocida* isolated from different animal and avian species in India. Vet Res Commun 28:657–667. doi:10.1023/B:VERC.0000045959.36513.e9
4. Shivachandra SB, Viswas KN, Kumar AA (2011) A review of haemorrhagic septicaemia in cattle and buffalo. Animal Health Res Rev 12:67–82. doi:10.1017/S146625231100003X
5. Ahmad TA, Rammah SS, Sheweita SA, Haroun M, El-Sayed LH (2014) Development of immunization trials against *Pasteurella multocida*. Vaccine 32:909–917. doi:10.1016/j.vaccine.2013.11.068
6. Hatfaludi T, Al-Hasani K, Boyce JD, Adler B (2010) Outer membrane proteins of *Pasteurella multocida*. Vet Microbiol 144:1–17. doi:10.1016/j.vetmic.2010.01.027
7. Hatfaludi T, Al-Hasani K, Gong L, Boyce JD, Ford M, Wilkie IW, Quinsey N, Dunstone MA, Hoke DE, Adler B (2012) Screening of 71 *Pasteurella multocida* proteins for protective efficacy in a fowl cholera infection model and characterization of the protective antigen PlpE. PLoS One 7:e39973. doi:10.1371/journal.pone.0039973
8. Shivachandra SB, Kumar AA, Amaranath J, Joseph S, Srivastava SK, Chaudhuri P (2005) Cloning and characterization of *tbpA* gene encoding transferrin binding protein (TbpA) from *Pasteurella multocida* B:2 (strain P52). Vet Res Commun 29:537–542. doi:10.1007/s11259-005-2495-6
9. Shivachandra SB, Yogisharadhya R, Ahuja A, Bhanuprakash V (2012) Expression and purification of recombinant type IV fimbrial subunit protein of *Pasteurella multocida* serogroup B:2 in *Escherichia coli*. Res Vet Sci 93:1128–1131. doi:10.1016/j.rvsc.2012.02.010
10. Shivachandra SB, Kumar A, Yogisharadhya R, Viswas KN (2014) Immunogenicity of highly conserved recombinant VacJ outer membrane lipoprotein of *Pasteurella multocida*. Vaccine 32:290–296. doi:10.1016/j.vaccine.2013.10.075
11. Kumar A, Yogisharadhya R, Ramakrishnan MA, Viswas KN, Shivachandra SB (2013) Structural analysis and cross-protective efficacy of recombinant 87 kDa outer membrane protein (Omp87) of *Pasteurella multocida* serogroup B:2. Microb Pathog 65:48–56. doi:10.1016/j.micpath.2013.09.007
12. Kovacs-Simon A, Titball RW, Michell SL (2011) Lipoproteins of bacterial pathogens. Infect Immun 79:548–561. doi:10.1128/IAI.00682-10
13. Wu JR, Shien JH, Shieh HK, Chen CF, Chang PC (2007) Protective immunity conferred by recombinant *Pasteurella multocida* lipoprotein E (PlpE). Vaccine 25:4140–4148. doi:10.1016/j.vaccine.2007.03.005
14. Shivachandra SB, Kumar A, Mohanty NN, Yogisharadhya R, Chacko N, Viswas KN, Ramakrishnan MA (2014) Homogeneity of VacJ outer membrane lipoproteins among *Pasteurella multocida* strains and heterogeneity among members of *Pasteurellaceae*. Res Vet Sci 96:415–421. doi:10.1016/j.rvsc.2014.03.016
15. Parsons LM, Lin F, Orban J (2006) Peptidoglycan recognition by PAL, an outer membrane lipoprotein. Biochem 45:2122–2128. doi:10.1093/jb/mvp061
16. Godlewska R, Wisniewska K, Pietras Z, Jagusztyn-Krynicka EK (2009) Peptidoglycan-associated lipoprotein (PAL) of Gram-negative bacteria: function, structure, role in pathogenesis and potential application in immunoprophylaxis. FEMS Microbiol Lett 298:1–11. doi:10.1111/j.1574-6968.2009.01659.x
17. Babu MM, Priya ML, Selvan AT, Madera M, Gough J, Aravind L, Sankaran K (2006) A database of bacterial lipoproteins (DOLOP) with functional assignments to predicted lipoproteins. J Bacteriol 188:2761–2773. doi:10.1128/JB.188.8.2761-2773.2006
18. Al-Hasani K, Boyce J, McCarl VP, Bottomley S, Wilkie I, Adler B (2007) Identification of novel immunogens in *Pasteurella multocida*. Microb Cell Fact 6:3. doi:10.1186/1475-2859-6-3
19. Goswami PP, Chaudhuri P, Tiwari V, Parihar NS, Harbola PC (2004) Cloning and sequencing of a 16 kDa outer membrane protein gene of *Pasteurella multocida* P52. Vet Res Commun 28:17–25. doi:10.1023/B:VERC.0000009529.26278.21
20. Schwede T, Kopp J, Guex N, Peitsch MC (2003) SWISS-MODEL: an automated protein homology-modeling server. Nucl Acids Res 31:3381–3385. doi:10.1093/nar/gkg520
21. Watson MEE (1984) Compilation of published signal sequences. Nucl Acids Res 12:5145–5164. doi:10.1093/nar/12.13.5145
22. Wheeler R (2009) Outer membrane proteomics of *Pasteurella multocida* isolates to identify putative host-specificity determinants. Biosci Horizons 2:1–12. doi:10.1093/biohorizons/hzp002
23. Chang A, Kaur R, Michel LV, Casey JR, Pichichero M (2011) *Haemophilus influenzae* vaccine candidate outer membrane protein P6 is not conserved in all strains. Human Vaccine 7:102–105. doi:10.4161/hv.7.1.13351
24. E-Komon T, Burchmore R, Herzyk P, Davies R (2012) Predicting the outer membrane proteome of *Pasteurella multocida* based on consensus prediction enhanced by results integration and manual confirmation. BMC Bioinform 27:13–63. doi:10.1186/1471-2105-13-63
25. Michel LV, Snyder J, Schmidt R, Milillo J, Grimaldi K, Kalmeta B, Khan MN, Sharma S, Wright LK, Pichichero ME (2013) Dual orientation of the outer membrane lipoprotein P6 of nontypeable *haemophilus influenzae*. J Bacteriol 195:3252–3259. doi:10.1128/JB.00185-13
26. Siju J, Kumar AA, Shivachandra SB, Chaudhuri P, Srivastava SK, Singh VP (2007) Cloning and characterization of type 4 fimbrial gene *(ptfA)* of *Pasteurella multocida* serogroup B:2 (strain P52). Vet Res Commun 31:397–404. doi:10.1007/s11259-006-3372-7
27. Shivachandra SB, Kumar A, Yogisharadhya R, Ramakrishnan MA, Viswas KN (2013) Carboxyl terminus heterogeneity of type IV fimbrial subunit protein of *Pasteurella multocida* isolates. Vet Res Commun 37:269–275. doi:10.1007/s11259-013-9569-7
28. Gautam R, Kumar AA, Singh VP, Singh VP, Dutta TK, Shivachandra SB (2004) Specific identification of *Pasteurella multocida* serogroup-A isolates by PCR assay. Res Vet Sci 76:179–185. doi:10.1016/j.rvsc.2003.10.005