Identification of immunodominant proteins of *Histophilus somni* in commercial vaccine antigen production

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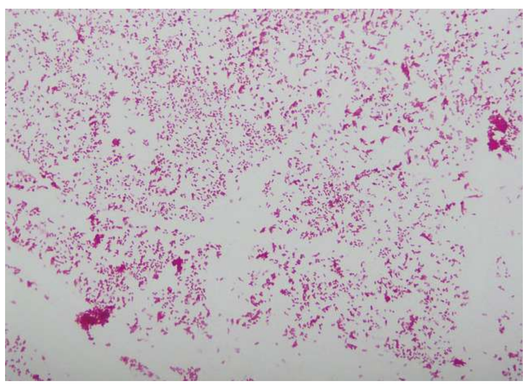
INTRODUCTION

Histophilus somni, a member of the *Pasteurelaceae* family (Yatsentyuk et al., 2023), is commonly known for its association with bovine respiratory diseases (BRD) and thrombotic meningoencephalitis (TME) (Angen et al., 1998), but can also cause other diseases, such as pleuritis; polysynovitis; arthritis; bronchopneumonia; septicaemia; myocarditis; infertility; abortion; and mastitis in the infected host (Headley et al., 2018).

Of these, BRD also known as shipping fever is the most common (Dassanayake et al., 2017) and most costly disease caused by *H. somni* in the cattle livestock industry (Magstadt et al., 2018). BRD is difficult to diagnose with infections resulting in a high rate of morbidity and mortality (Petruzzi et al., 2020), due to antimicrobial drug resistance, which consequently has a negative financial impact on the cattle livestock industry (Magstadt et al., 2018).

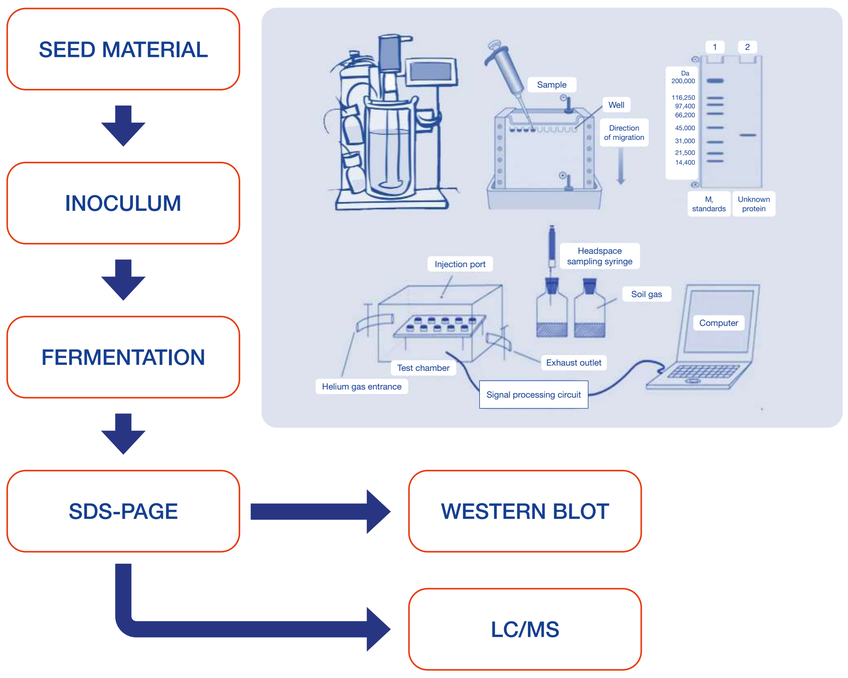


Therefore, alternative prevention and treatment approaches, such as vaccination with inactivated whole cell bacterins, have been used to alleviate the impact of *H. somni* in the cattle livestock industry (O'Toole and Sondgeroth, 2016). Existing vaccines for *H. somni* include either killed cells or bacteria-free outer membrane proteins which have proven to be moderately successful.

To improve efficacy, vaccines with relevant and efficacious antigens are paramount. In this study, immunoproteomic approaches are used to identify immunodominant outer membrane proteins and virulent antigens in commercial *H. somni*-containing vaccines.

MATERIALS AND METHODS

- *H. somni* culture was inoculated on blood tryptone agar plates (BTA) then incubated facultatively anaerobically at 37°C for ± 72 hours.
- The growth on the pates was aseptically scrapped from the plates and inoculated into a sterile seeding flask with broth before incubating statically at 37°C overnight.
- The bioreactor was inoculated with the seeding flask before the fermentation was initiated.
- The bioreactor fermentation was run for ±16 hours. After the fermentation cycle, samples were taken for analysis.
- Sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) was performed on the sample following the relevant protocol. The SDS-PAGE was run on two gels (one for Western blot and one for band excision for liquid chromatography/mass spectrometry (LC/MS)).
- Western blot was done on the SDS-PAGE gel using sera obtained from cattle vaccinated with *H. somni* containing vaccines from Design Biologix.
- The other SDS-PAGE gel was used to exercise protein bands of interest for protein mapping by LC/MS.



RESULTS

Figure 1 displays the SDS-PAGE gel of various proteins expressed over 24 hours. Samples collected at 8 hours, 14 hours, and 24 hours were loaded in duplicate for experiments 1 and 2. However, due to well limitations, only an 8-hour sample was loaded for experiment C. Across all experiments, the expressed proteins exhibited remarkable similarity. The arrows highlight bands that were excised and subsequently identified using LC/MS. Specifically:

The band indicated by the **blue arrow** corresponds to Ornithine decarboxylase. The **orange arrow** points to the Outer membrane protein assembly factor BamA (Beta-barrel Assembly Machinery A). The **green arrow** designates 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase.

Figure 1: SDS-PAGE gel showing the expression of proteins at different times during fermentation.

Figure 2: Western blot image of expressed proteins identified by LC/MS.

Figure 2 depicts the western blot image of immunogenic proteins expressed during the growth of *H. somni* in the SUB. The western blot was probed using sera from animals vaccinated with Multisomni (Reg. No. G4357, Act 36/1947).

CONCLUSION

The generated data provides a comprehensive immunoproteomic characterization of the inactivated whole-cell *H. somni* vaccine component, offering evidence of its quality and optimal antigen manufacturing process.

Additionally, our results highlight immunogenic proteins of interest as potential subunit vaccine candidates. Specifically, the proteins expressed (as shown in **Figure 1**) were identified as follows:

- **Ornithine Decarboxylase (ODC):**

  ODC's impact on cell transformation and its involvement in polyamine synthesis make it a relevant protein for understanding *H. somni* pathogenesis and developing vaccines.

- **Outer Membrane Protein Assembly Factor BamA:**

  BamA is a critical player in the outer membrane assembly of *H. somni*, impacting virulence, host interactions, and immune responses.

- **2,3-bisphosphoglycerate-dependent Phosphoglycerate Mutase:**

  This intriguing protein's involvement in metabolic pathways, biofilm formation, and potential immunogenicity warrants further study for vaccine development against bovine respiratory diseases.

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