

Improved stability of live attenuated vaccine *gdhA* derivative *Pasteurella multocida*B:2 by freeze drying method for use as animal vaccine

ABSTRACT

The efficacy of attenuated strain of *gdhA* derivative *Pasteurella multocida*B:2 mutant as a live vaccine to control haemorrhagic septicaemia (HS) disease in cattle and buffaloes has been demonstrated. In order to use *P. multocida* B:2 mutant as a commercial product, it is essential to optimise its formulation for high viability and stability of the live cells. The effectiveness of freeze-drying process using different protective agent formulations for improving cells viability was explored. Sugar and nitrogen compounds were used as protective agents in freeze-drying and the capability of these compounds in maintaining the viability of mutant *P. multocida* B:2 during subsequent storage was investigated. A complete loss in viability of freeze-dried mutant *P. multocida* B:2 was monthly observed until 6–12 months of storage at $-30\text{ }^{\circ}\text{C}$, $4\text{ }^{\circ}\text{C}$ and $27\text{ }^{\circ}\text{C}$ when nitrogen compound or no protective agent was added. Trehalose and sucrose showed significantly high survival rate of 93–95% immediately after freeze-drying and the viability was retained during the subsequent storage at $-30\text{ }^{\circ}\text{C}$ and $4\text{ }^{\circ}\text{C}$. A smooth cell surface without any cell-wall damage was observed for the cells formulated with trehalose under scanning electron micrograph. This study presented a freeze-drying process generating a dried live attenuated vaccine formulation with high stability for commercial applications.

Keyword: *gdhA* derivative *Pasteurella multocida* B:2 mutant; Protective agents; Freeze drying; Cell viability; Storage; Cell morphology