ETEC Piglets Endolysin Simulated intestinal fluid

Are endolysins promising agents in controlling *E. coli* associated post-weaning diarrhea in piglets?

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Enterotoxigenic *Escherichia coli* (ETEC) associated infections are the major cause of piglets' mortality in weaning and post-weaning period, resulting in significant economic losses to the swine industry. Furthermore, the increase of multidrug resistant ETECs have been recognized a public health danger due to the potential transfer of resistance into the food chain. Bacterio(phages) endolysins are enzymes produced in the end of phage lytic cycle that are responsible for cell lysis. So far, no resistance has been reported, which make endolysins an attractive alternative to antibiotics. In the present work, endolysins were exploited to tackle ETECs in piglets. Two enzymes previously cloned were tested against ETEC SP23 strain exponential cells. First, Lys68 (used herein as proof of concept) was tested alone or in combination with 5 different organics acids (already implemented in the piglets' diet) – citric, malic, formic, lactic and sorbic acids – in 20 mM HEPES, 149 mM PBS and 140.33 mM simulated intestinal fluid (SIF). Later, PlyF307 endolysin was also tested with malic and citric acids in SIF. Results demonstrated that both enzymes reduced ETEC concentration in more than 4 orders of magnitude in HEPES. Lys68 together with malic acid displayed the best antibacterial activity in PBS, being able to reduce approximately 1 order of magnitude. However, enzymes efficacy was drastically reduced when tested in buffers that mimicked physiological conditions. In SIF, Lys68 and PlyF307 did not display antibacterial activity. In summary, phage endolysins revealed to be ineffective to treat ETEC bacterial load in more complex environments.