The non-toxigenic strain Z31 can prevent neonatal diarrhea in piglets caused by *Clostridium difficile*

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INTRODUCTION

Neonatal porcine diarrhea is a current problem on pig farms and is caused by several enteropathogens. Among them, *Clostridium difficile* stands out due to its high prevalence in piglets and its zoonotic potential. Despite the importance of this pathogen, there are no specific measures for the prevention or control of *C. difficile* infection (CDI) in pigs. Thus, the aim of the present study was to characterize genotypically, phenotypically and evaluate the protective potential of a non-toxigenic *C. difficile* strain, named Z31, against CDI in piglets.

MATERIAL AND METHODS

First, it was performed the complete genome sequence of Z31 strain. The next step was the evaluation of the preventive capacity of Z31 strain against CDI, which was performed in hamsters, in an experimental pig model and in a commercial pig farm. Finally, Z31 growth and sporulation was evaluated in five culture media as well as the temporal viability of its spores in two different temperatures (4°C and 25°C).

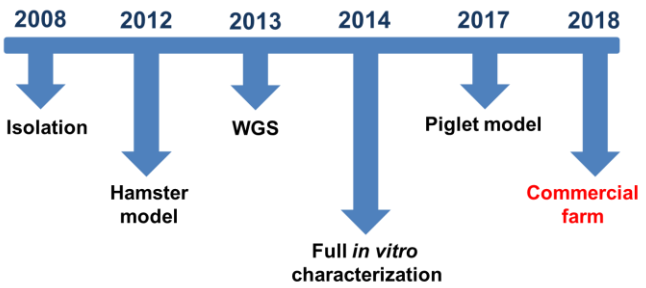

Figure 1: Timeline of the tests with the non-toxigenic Z31 strain. Z31 was isolated from a healthy dog in 2008. Since then, it has been submitted to *in vitro* and *in vivo* tests. Finally, in 2018 it was tested in a naturally infected commercial farm.

RESULTS AND DISCUSSION

Genomic sequencing revealed that Z31 strain has a circular chromosome of 4,298,263 base pairs (bp) containing genes responsible for spore production and stability (*spo0A*), intestinal adhesion and biofilm formation, but absence of toxins encoding genes. In hamsters, the Z31 strain was able protect all animals against death and lesions associated with CDI.

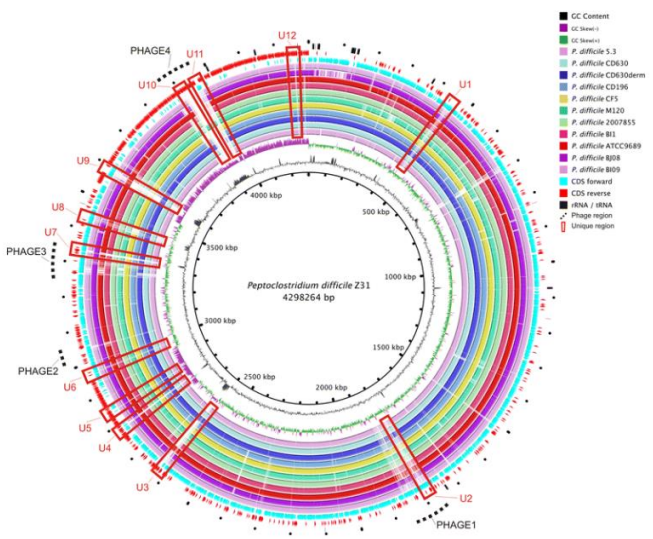

Figure 3: Circular map of Z31 genome. From outside to the center: predicted phage regions by PHAST; RNAs; CDSs on reverse strand; CDSs on forward strand; Blastn hits with BI9, BJ08, ATCC9689/DSM1296, BI1, 2007855, M120, CF5, CD196, CD630DERM, CD630, Cd5.3 strains; GC skew; and, GC content.

Figure 2: Piglets during the evaluation of Z31 in a commercial farm. Piglets marked with a black line received the Z31, while piglets marked with the red line received only placebo.

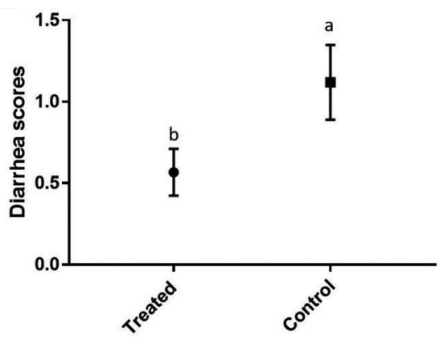

Figure 3: Scores of diarrhea in piglets one day after administration of Z31. Different letters means statistical differences by Mann-Whitney test ($p<0.05$).

In the experimental model in neonatal pigs, the Z31 strain prevented CDI, reduced clinical signs, macro and microscopic lesions and fecal elimination of toxigenic C. *difficile*. In a commercial farm, even in the presence of other enteropathogens, the Z31 administration reduced the occurrence of CDI, the fecal shedding of toxigenic *C. difficile* and the occurrence of neonatal piglet diarrhea. The BHI and RCM media provided the highest in vitro growth rates, reaching concentrations of 6.6×10^6 CFU/ml with spore ratio greater than 98%. After lyophilization, the Z31 strain maintained acceptable viability after 2 years of storage under both temperature conditions.

CONCLUSIONS

The strain Z31 was able to prevent CDI in piglets, reducing toxigenic *C. difficile* spreading and demonstrated desirable characteristics for its potential commercial use.

REFERENCES

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