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O122 Intrinsic properties of 3 *Bacillus* spp. strains from animal origin constituent of a Direct Fed Microbials/protease blend having growth performance in pigs fed high fiber diet

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Keywords: Probiotic; Screening; Enzymatic activity; Pig; Fibers

Introduction

Although dietary fibers reduce nutrient digestibility, fiber-rich feeds have been widely used in pig diets because of lower feed costs. Thus, to secure and maintain animal performances, feed additives that can improve or support fiber fermentation gained more attention. Probiotics (also named Direct Fed Microbials) for animals such as spore-forming *Bacillus*, can survive high temperatures and low pH as spores. When these *Bacillus* spores germinate in the intestine of the pig, they may produce a wide variety of fiber-degrading enzymes. Danisco has developed a feed additive consisting of three *Bacillus* strains and a protease that has successfully demonstrated *in vitro* greatest structural breakdown of fiber ingredients and protein solubilization (Payling et al., 2017a) and growth performance in pigs fed high fibre diet (Payling et al., 2017b). Here is reported the scientific approach (*in vitro* and *in silico*) and high-throughput model applied to screen and select the top three *Bacillus* candidates considering their enzyme production profile.

Material and Methods

The enzymatic activity of 1300 bacteria isolates from animal and environmental sources was tested by growing the cultures in 0.5ml TSB at 32°C for 24hrs in an orbital shaking incubator. High-throughput screening of these test strains was performed by replicate spot plating of 2 µl liquid culture onto 15.0 ml media supplemented with agar and varying substrates in 100 x100 x15mm grid plates. Assay plates were left to dry for 30 minutes following culture application, and then incubated at 32°C for 24hrs. The following substrates were tested: polysaccharides (corn starch, Carboxymethyl Cellulose -CMC, xylan, mannan, pectin), proteins (soy and zein – insoluble corn protein), and lipids (tributyrin and tween 80). Assays were run in triplicate and radius of the zone of clearance around colonies indicating substrate utilization, were measured and categorized as follow: from 0 to 29 mm "no activity"; 30 to 49 mm "small activity"; 50 to 69 mm "good activity" and above 70 mm "very good activity". *Bacillus* strains showing largest clearance zone for xylan, mannan and CMC based-media, were selected for subsequent experiments. Ten candidates were selected for whole genome sequencing using next-generation sequencing technologies. Genomes were assembled using SPAdes and Unicycler and annotated using RASTtk in PATRIC. Their genomes were mined for the presence of genes encoding extracellular enzymes responsible for the breakdown of other degradative enzymes and compared to publicly available genomes.

Results and Discussion

The high-throughput substrate agar assays provided fast visuals results of enzymatic activity on different substrates (Figure 1), such as no xylanase activity (radius = 0 mm, strain BL21) or strong xylanase activity (radius = 74 mm and 73 mm, respectively. for strains 3BP5 and BS918). From the 1300 strains tested, ten candidates were selected for further analyses based on their superior zone of clearance radius on

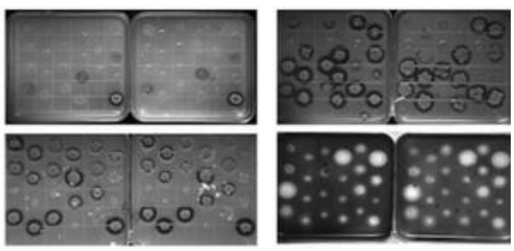

Figure 1. Bacterial colonies and zones of clearance around culture colonies indicative of enzymatic activity. Top left-bottom right: esterase, soy protease, zein protease and xylanase assay.

xylan, mannan and CMC substrates suggesting superior xylanase, β-mannanase and cellulase enzymatic activities. They were selected for whole-genome sequencing and *in silico* analysis. *In silico* analysis of the whole genome confirmed the presence of genes encoding for the enzymatic activity identified *in vitro* and was used to extend search of other degradative enzymes. Three strains, one B. subtilis subsp. *subtilis* 3BP5, and 2 *B. velezensis* (formerly *B. amyloliquefaciens* subsp. *plantarum*) BS918 and BS1013, were selected for their highest phenotypic enzymatic activities as well as for their broader and unique enzymatic potential as detected based on genomic analysis. Thus, as a blend, those 3 strains contained genes encoding for the production of identified enzymes (all tested substrates) and more as licheninase, CMCase, xylanase, and β-xylosidase as well as the phy gene (phytase) and corresponding phytase regulatory genes PhoR and PhoP. The amyE gene (saccharifying amylase) was identified in all 3 strains, while 3BP5 contained amyX pullulanase) and BS1013 and BS918 contained amyA (liquefying amylase). Extracellular proteases and esterases were also identified in the genomes.

Conclusion and Implications

These results illustrate the value of the *in vitro* high-throughput substrate plate model, to screen and identify appropriate probiotic strains from non-ruminant origin producing a variable set of enzymes that can effectively improve nutrient utilization of poorly digestible ingredients fed in pig diets as reflected by *in vitro* and *in vivo* data. Such products make it possible to increase the flexibility of diet formulation by allowing use of low-quality ingredients while maintaining the growth performance of pigs and hence reducing the environmental impact associated with high-fiber diets.

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O123 The use of dietary bacteriocins as alternatives to antibiotics in growing piglets

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Keywords: Bacteriocins; Feed additives; Microbiota

Introduction

The use of antibiotics and the development of antibiotic resistance have become a global concern. Considering the contribution of animal production to antibiotic resistance, alternatives are currently the focus of much research. Such an alternative could be provided by bacteriocins, which are ribosomally-synthesized and post-translationally modified anti-microbial peptides produced by gut microbiota (Cotter et al., 2013; Soltani et al., 2020). They possess a narrower spectrum of activity than antibiotics, thus reducing both the chance of resistance and collateral damage to the host's microbiota (Umu et al., 2016; Kang et al., 2021).

Material and Methods

Two hundred and eighty-eight weaned male piglets (21 days of age and 5.9 ± 0.83 kg of weight) were blocked by initial weight and distributed into 48 pens of 6 animals. Four dietary treatments (12 pens/treatment) were applied from day 1 through day 21 post-weaning, i.e., 1) No antibiotics (NAB; negative control); 2) Antibiotics (AB; positive control; 660 ppm chlortetracycline hydrochloride; 3) Microcin McJ25 (MIC); and 4) Nisin (NIS). Animal performance was monitored through day 42 of the nursery phase. Feed and water intakes were recorded weekly and body weight measured on day 0, 7, 14, 21, 28, and 42. Fecal samples were collected on day 0, 14, 21 and 42, and analyzed for volatile fatty acids (VFA) and for microbiota composition using 16S-RNA gene sequencing. Data were analyzed under a mixed-effects model with repeated measures including the random effect of pen, and the fixed effects of treatment, time and their interaction.

Results and Discussion

Total fecal VFA concentrations as well as the molar proportion of acetate, propionate and butyrate were not affected by treatment, but increased over time ($P < 0.001$). Feed intake was 65 g/day lower in NAB compared with MIC, NIS and AB treatments from days 7 to 14 ($P < 0.05$). Water intake increased over time ($P < 0.001$) and was not affected by treatment. There was a treatment × time interaction for average daily weight gain (ADWG), which was lowest in NAB relative to the other groups on weeks 1, 2 and 5 ($P < 0.001$). Interestingly, AB presented the lowest ADWG on week 3, but was not different from other groups at any other time point. Live weight was 710 g higher in MIC compared to NAB on days 14, 21, 28, and 42 ($P < 0.001$), whereas no differences were detected between MIC and the NIS and AB groups, except on day 42, where piglets from the MIC group were 790 g heavier compared with NIS ($P < 0.01$). Firmicutes (71.0 ± 9.9%) and Bacteroidota (27 ± 8.9%) were the predominant phyla in fecal samples, followed by proteobacteria (1.2 ± 3%). Firmicutes were not