PS3-037

Unraveling the probiotic potential of two novel *Bacillus* spp.

Krithika V.[1], Jeroen Maertens[2], Antonio Luis Gonzalez Sanchez[2], Karen Vermeulen[2] and Natasja Smeets[2]
[1]Kemin Industries South Asia Pvt. Ltd., #C3, First Street, Ambattur Industrial Estate, Chennai, 600058, India
[2]Kemin Europa NV, Toekomstlaan 42, 2200 Herentals, Belgium

Introduction

Extensive use of antibiotic growth promoters (AGP) in the livestock industry has led to the development of resistant strains of pathogenic microbes. Probiotics are becoming the most relevant alternative to AGP's for supporting intestinal health and performance. The present study aimed to explore the probiotic attributes of two new isolates of *Bacillus subtilis* (FXA, ATCC PTA 127114) and *Bacillus licheniformis* (G3, ATCC PTA 127113) which exhibited *in vitro* antimicrobial efficacy against common pathogens in preliminary studies.

Materials and Methods

In vitro gastrointestinal tolerance:

Table 1. *In vitro* tolerance (pH and Bile)

	Incubation time (min)	Sampling points (min)	Colony count method
pH (pH 3)	60	0, 30, 60	Pour plate
Bile (0.2%)	120	0, 120	

In vitro adhesion capacity:

Auto-aggregation

Bacillus culture suspension, left for gravitational sedimentation and absorbance measured from top at regular intervals at 600nm.

Cell surface hydrophobicity

Bacillus culture suspension, mixed with solvents, the absorbance of aqueous phase measured at 600nm after 30mins

Co-aggregation

Bacillus culture suspension, mixed with pathogen culture suspension (*E.coli* ATCC 25922), (*Salmonella enterica* ATCC 13076),left to sediment, absorbance measured at 600nm from top at regular intervals

In vitro safety assessment:

In vitro susceptibility and compatibility

The susceptibility against antimicrobials of human and veterinary importance and compatibility with commercial coccidiostat were tested by broth microdilution method

Cytotoxicity to Vero cells

Vero cell lines were exposed to the cell-free supernatant of the two strains for 16 h and cytotoxicity was analyzed by Roberts *et al*., 2001 method

Hemolysis

Hemolytic activity was tested using Columbia blood agar plate containing 5% (w/v) sheep blood. A clear zone around the colonies indicates a positive test for β-hemolysis

Genomic analysis: Toxigenicity

The genome sequence of the strains were analyzed for the presence of toxins/virulence factor genes/plasmids by querying against known databases using BLASTp/tBLASTn tool.

Results

In vitro Gastric pH and Bile tolerance:

Table 2. pH stability and bile tolerance

Parameter	FXA	G3
pH stability (pH 3)	+++	+++
Bile tolerance (0.2%)	+++	++

Both the *Bacillus* spp. showed good tolerance to gastric pH 3 and bile at 0.2%, ensuring their survivability in poultry intestinal environment.

In vitro adhesion capacity:

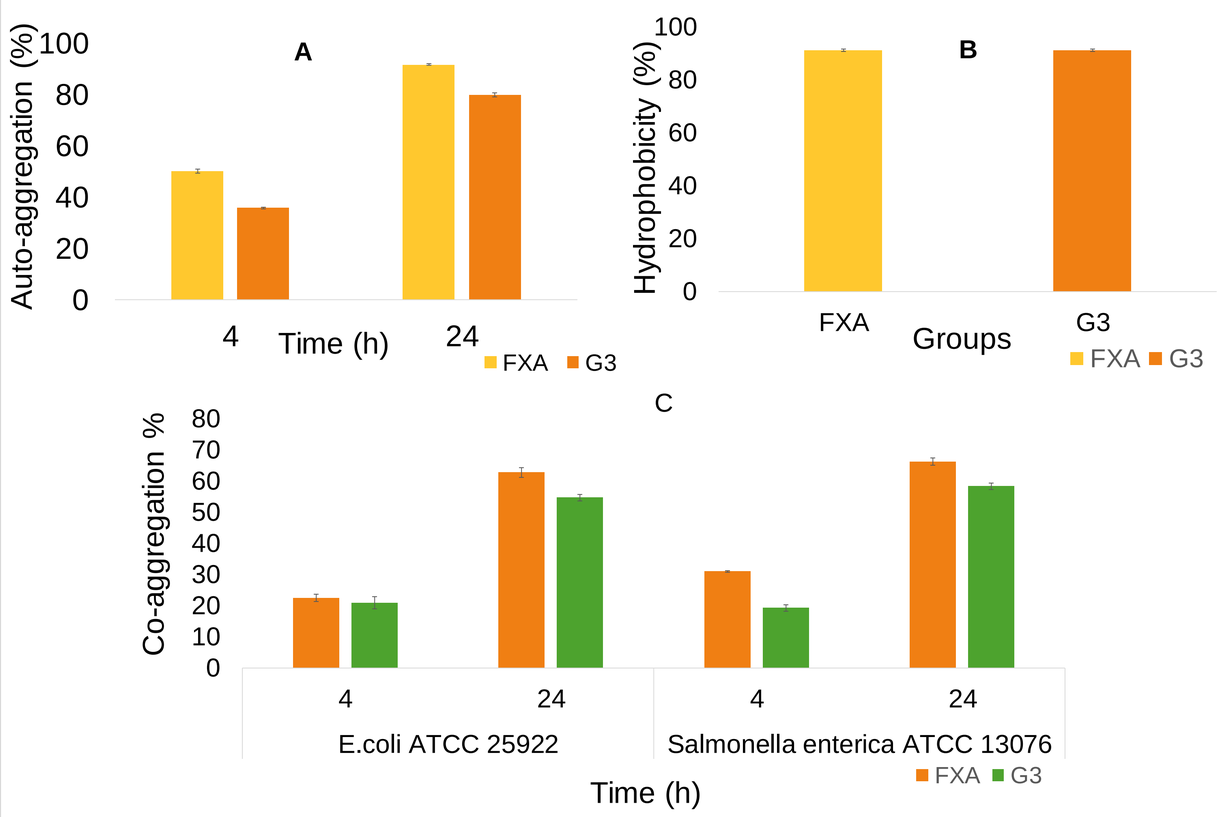

Figure 1. Auto-aggregation % at 4h and 24 h of incubation (A). Bacterial adhesion to hydrocarbons using hexane as a solvent (B). Co-aggregation % with pathogens *E. coli* ATCC 25922 and *Salmonella enterica* ATCC 13076 at 4h and 24h of incubation (C). The bars represent the mean ± SEM values of three replicates.

Both the *Bacillus* spp. showed good auto-aggregation (80-90%), cell surface hydrophobicity (90%), and co-aggregation with pathogens (55 – 60%)

In vitro safety assessment:

Table 3. Safety assessment of *Bacillus* spp. FXA & G3

Antibiotic susceptibility	Coccidiostat compatibility	Cytotoxicity	Hemolysis	Genomic toxigenicity
Susceptible to gentamicin, kanamycin, streptomycin, tetracycline, erythromycin, clindamycin, chloramphenicol, vancomycin.	**Compatible** with amprolium, diclazuril and monensin	**Not toxic** to Vero cells	No zone of clearance – **Non-β-hemolytic**	**No toxin genes** or plasmids

Conclusion

Both *Bacillus subtilis* FXA and *Bacillus licheniformis* G3 exhibited functional probiotic potential at the tested conditions and can be considered safe for commercial application in animals.