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www.ahperformance.com

Technical Bulletin – *Bacillus velezensis* 747

Introduction

Bacillus velezensis are Gram-positive, spore forming bacteria recognized as QPS (qualified presumption of safety) under the European Food Safety Authority. Through full genome analysis and comparison, *B*. *velezensis* 747 has been positively identified as a member of the *Bacillus velezensis* group.

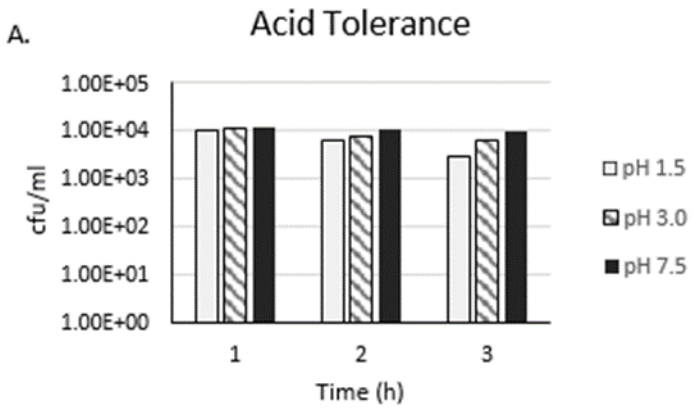
B. velezensis 747 was originally selected for its ability to control the populations of pathogenic bacteria. However, recent research has demonstrated an additional role for this strain to regulate digestive health.

Gastrointestinal Performance

Probiotics are defined as live microorganisms that confer a health benefit to the host when administered in adequate numbers (WHO/FAO). Consistent with this, *Bacillus* are naturally found in the gastrointestinal tract of humans and animals, and many species have a long safe history of use in food preservation and production with multiple defined health benefits.

Survival in the gastrointestinal tract requires a variety of attributes, including acid tolerance and the ability to withstand bile salts. *In vitro* trials have demonstrated *B. velezensis* 747 is resistant to low pH conditions (pH 1.5 and 3.0) and survives in the presence of physiological concentrations of bile salts (Figure 1) for extended periods of time.

Figure 1.



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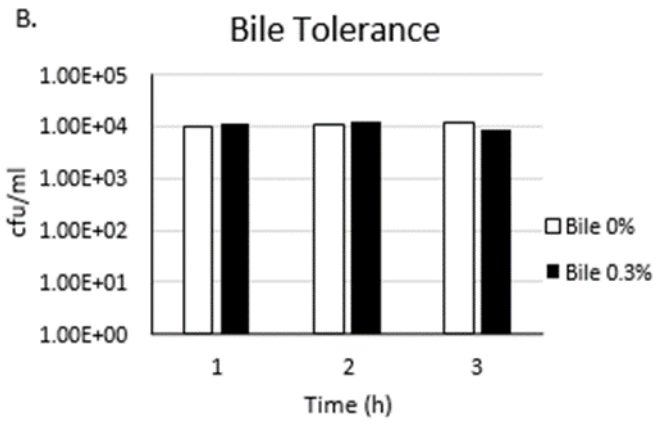

Figure 1. Acid and Bile tolerance of *B. velezensis* 747. A) Survival of *B. velezensis 747* at varying pH levels (1.5, 3.0, and 7.5) demonstrate strong cell viability over a 3-hour period. B) Survival of *B. velezensis* 747 in the presence of physiological concentrations of bile over a 3 hour period.

Inhibition of Pathogens.

The ability of probiotic organisms to target and protect against pathogens is important for gastrointestinal, as well as whole organism health.

Many bacteria produce compounds that can inhibit other bacteria, commonly known as bacteriocins. The function of bacteriocins is to allow the producer cells to compete with other microbes in their natural environment. They generally increase membrane permeability by forming pores in membranes of target cells or inhibit cell wall synthesis thereby preventing growth of susceptible microbes. Other beneficial attributes of bacteriocins are resistance to low pH and heat and little, if any, negative effects on host cells. These bacterially produced antimicrobial peptides are very similar to those produced by the host organism itself.

In vitro assays using bacterial supernatant are typically used to investigate the effects of probiotic strains on pathogen survival. *Bacillus* strains were selected as candidates for use as probiotics based on functional inhibitory assays against pathogenic *E. coli, C. perfringens, C. difficile, and Salmonella. Bacillus* strains were grown overnight, cells removed by centrifugation and cell-free supernatants were prepared by filter sterilization. Antimicrobial assays contained representative strains from diverse genetic clusters inoculated in growth medium and mixed with *Bacillus* supernatants. Assays were incubated overnight and the growth compared to assays of the same indicator strain without the *Bacillus* supernatants. *B. velezensis* 747 displayed the strongest inhibitory profile against the broadest collection of *E. coli*, *Clostridia* and *Salmonella* indicator strains. These results were recapitulated in *in vivo* models.

% Pathogen Inhibition	E. coli	>97
	C. perfringens	97
	C. difficile	>97
	Salmonella	96

Intestinal Barrier Maintenance.

An intact intestinal barrier is the first line of defense in the immune system. Maintenance of the barrier becomes of critical importance as changes in intestinal permeability can allow solutes and pathogens to cross into the underlying tissue resulting in inflammation.

In vivo animal trials (Gadde et al., 2017) have demonstrated the ability of *B. velezensis* 747 to regulate tight junction proteins ZO-1 and Occludin, suggesting a modulation of the intestinal tight junctions. Follow up *in vitro* assays demonstrated an increase in barrier tightening as demonstrated by Transepithelial Electrical Resistance (TEER). Briefly, human intestinal epithelial Caco-2 cells were grown on permeable inserts. Cells were then stimulated with the inflammatory cytokine TNF-α for 24 hours, which increases intestinal permeability. *B. velezensis* 747 reversed the TNF-α induced loss of barrier function resulting in a barrier consistent with the untreated control epithelium.

Figure 2.

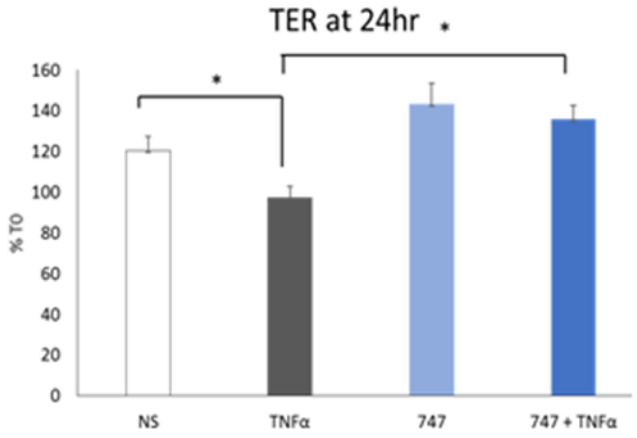

Figure 2. *B. velezensis* 747 induced barrier function. TNFα increased epithelial permeability compared to NS, (no stimulation). *B. velezensis* 747 reversed the loss in barrier function induced by TNFα. * P < 0.05.

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Genetically Characterized

Bacillus velezensis 747 has been characterized and classified by genetic interrogation methods such as complete 16S rRNA gene sequencing, and full genome sequencing confirming its identity as a novel strain and safety. The complete genome has been studied and compared against antibiotic resistance data bases including the Comprehensive Antibiotic Resistance Database (CARD) and Antibiotic Resistance Genes Database (ARDB) to assure there are no transmissible antibiotic genes present.

References

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sanders ME. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014 Aug;11(8):506-14

Gadde UD, Oh S, Lee Y, Davis E, Zimmerman N, Rehberger T, Lillehoj HS. Dietary Bacillus subtilis-based direct-fed microbials alleviate LPS-induced intestinal immunological stress and improve intestinal barrier gene expression in commercial broiler chickens. Res Vet Sci. 2017 Oct;114:236-243.

Gadde U, Oh ST, Lee YS, Davis E, Zimmerman N, Rehberger T, Lillehoj HS. The Effects of Direct-fed Microbial Supplementation, as an Alternative to Antibiotics, on Growth Performance, Intestinal Immune Status, and Epithelial Barrier Gene Expression in Broiler Chickens. Probiotics Antimicrob Proteins. 2017 Dec;9(4):397-405.