

CLOSTAT®

Active Microbial

CLOSTAT® contains a proprietary strain of *Bacillus subtilis* PB6 — a unique, naturally-occurring probiotic that helps maintain the balance of microflora in the gastrointestinal (GI) tract of livestock and poultry. The PB6 in CLOSTAT is proven to help improve performance and health metrics by combating pathogens in the gut in university and field studies.¹

WHY IS INTESTINAL HEALTH IMPORTANT?

Show animals are in fact athletes, and we are asking them to perform outside of their norm. Because of this, we need them to be in the best possible health. Pathogenic bacteria like *Clostridium perfringens* create lesions in the small intestine that compromise the integrity of the intestinal lining (increased permeability or **leaky gut**) — allowing pathogens and toxins to more easily enter the bloodstream. This can result in intestinal **inflammation, disease**, and an environment in which **bacteria** can thrive.

By inhibiting the growth of pathogenic bacteria, the PB6 in CLOSTAT helps maintain a **healthy microbial balance in the digestive tract** and **improve nutrient absorption**.

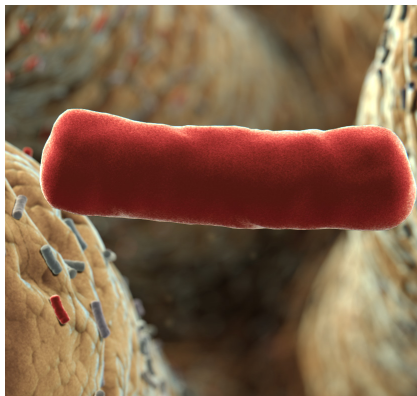


Figure 1: *C. perfringens* in the intestine



Figure 2: Damaged gastrointestinal villi

CLOSTAT® FEATURES AND BENEFITS

- Contains the probiotic PB6, a proprietary strain of *B. subtilis*¹
- Enhances health status, resulting in disease resistance/reduction and reduced shedding of pathogens
- Delivers research-proven efficacy of *B. subtilis* PB6 against a broad range of pathogens *in vitro* and *in vivo*
- Demonstrated safety in livestock and poultry
- Heat stable during pelleting, packaging, and storage
- Compatible with a wide range of feed ingredients including mineral/vitamin premixes and most antibiotics
- Compatible with organic acids and most antibiotics applied through a water medicator

PROVEN PATHOGEN INHIBITION AGAINST *CLOSTRIDIUM*

The efficacy of *B. subtilis* PB6 (or PB6) against *Clostridium perfringens* (or *C. perfringens*) was tested through two common assays to show how it inhibits the growth of the pathogen. In Figure 3, *Clostridium* was streaked vertically across the plate, and two strains of *B. subtilis* was streaked across horizontally. You can see where the *B. subtilis* PB6 breaks the pathogen streak and inhibits growth. *B. subtilis* ATCC 6633 (bottom horizontal streak on plate) did not inhibit *C. perfringens*.

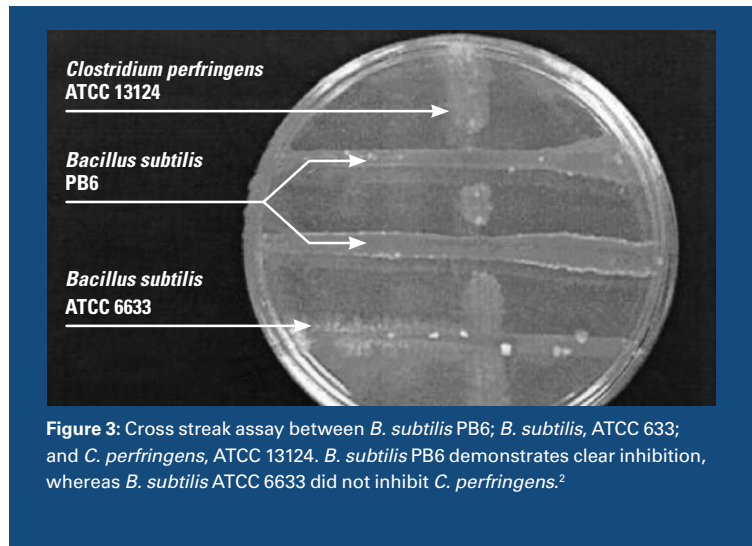


Figure 3: Cross streak assay between *B. subtilis* PB6; *B. subtilis*, ATCC 6633; and *C. perfringens*, ATCC 13124. *B. subtilis* PB6 demonstrates clear inhibition, whereas *B. subtilis* ATCC 6633 did not inhibit *C. perfringens*.²

MODE OF ACTION 1: PATHOGEN INHIBITION

B. subtilis PB6 secretes multiple biocidal proteins that inhibit certain strains of pathogenic bacteria.^{2,4} Its active metabolites have a cyclic structure comprised of seven amino acids, collectively known as surfactins.

Surfactin molecules can form pores on the cell walls of the bacteria, which provides the mode of action.⁵ These proteins disrupt the bacterial membrane, causing the cell contents to leak, ultimately killing the pathogenic bacteria without harming the beneficial gut microflora (Figure 4).

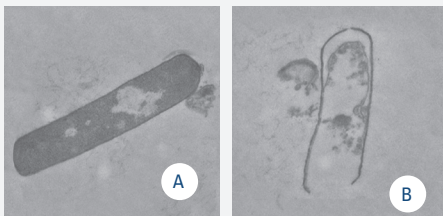


Figure 4: The effect of *B. subtilis* PB6 on *C. perfringens* at 37° C. (a) Disruption of the cell wall and loss of cytoplasmic contents into the exterior after one hour. (b) Rupture and death of cell after 4 hours.³ (Transmission electron micrograph magnification: 29,000X)

MODE OF ACTION 2: REDUCED INTESTINAL INFLAMMATION

Intestinal inflammation can occur if the intestinal lining becomes damaged and allows molecules such as bacteria and pathogens and their toxins to pass between epithelial cells, known as Leaky Gut Syndrome (Figure 5). This triggers an immune response to destroy and remove these invasive organisms, resulting in an inflammatory process that pulls energy from other key performance functions.⁶

B. subtilis PB6 secretes cyclic lipopeptide surfactins through normal metabolism to reduce inflammation.

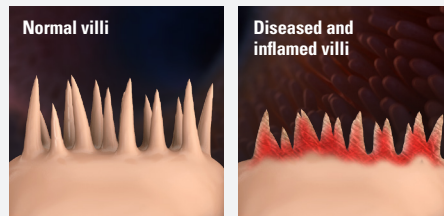


Figure 5: Gut inflammation

MODE OF ACTION 3: QUORUM QUENCHING

Quorum sensing facilitates the initiation of infection, colonization, and disease progression in the intestine where diffusible signaling molecules are produced, secreted, and detected by the bacterial cells in the intestine.⁷⁻¹⁰ Quorum quenching is the mode of action that can disrupt the quorum sensing system through degradation of quorum sensing signaling molecules, the inhibition of signal biosynthesis, and the inhibition of signal detection.¹¹ Quorum quenching also aids in the prevention of toxin production and formation of a biofilm.⁵ *B. subtilis* PB6 can prevent quorum sensing of *C. perfringens*.



Figure 6: *Clostridium perfringens*



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