



Expanding Probiotic Possibilities

Deerland Enzymes has genome sequenced and clinically tested a highly effective strain of *Bacillus subtilis*, a very stable probiotic spore that works as a complement to many of the non-spore strains on the market today.

PROBIOTICS FOR DIGESTIVE AND IMMUNE HEALTH

The human body carries nearly 100 trillion bacteria in the gut...that's more than 10 times the total number of human cells in the entire body. Probiotics are those "good" bacteria that help keep the intestines healthy and assist in digestion and nutrient absorption. Researchers are also finding evidence that certain bacteria in the gut influence the development of aspects of the immune system^{1,2}. In fact, the gut accounts for 25% of the immune cells in the body which provides 50% of the body's immune response.



Probiotics work to help maintain balance in the intestinal microbiota. By enhancing the intestinal flora, these microorganisms may have a larger effect in terms of keeping people in good health. Understanding the type and quantity of microorganisms in the gut has become a critical goal in the pursuit of overall wellness. Recent research on the microbiome has shown that its influence extends far beyond the gut, playing a crucial role in both our digestive and immune systems. In fact, studies are showing the microbiome's role in such areas as brain health, memory and even mental health.

Consumers today have the ability to influence their gut microbiota like never before—from supplements to food, people are seeking sources of good bacteria.

PROBIOTIC STRAINS: SPORE AND NON-SPORE FORMERS

The majority of probiotics currently available are bacteria which are non-spore formers, such as *Lactobacilli* and *Bifidobacteria*. These probiotic strains have been widely studied for their health benefits and are a popular choice for use in dietary supplements or cold-processed foods, such as yogurt.

Spore forming bacteria are a diverse group of very

hardy bacteria, characterized by their ability to form endospores to protect themselves in varying conditions such as high temperatures and the acidic environment of the gut. The *Bacillus subtilis* species of microorganism has been known for almost 100 years, having first been isolated and described in 1915. It is considered to be a normal inhabitant of the gut in animals and humans³.

Bacillus subtilis has the ability to form spores that protect the microbes from harsh conditions until they enter an environment ripe for germination, such as the GI tract. Because of this spore-forming ability, *Bacillus subtilis* offers additional benefits as a probiotic:

- Remains viable under a wide temperature range, doesn't require refrigeration⁴
- Survives passage through the acidic environment of the GI tract⁵
- Can persist in the GI tract, increase its numbers and then re-sporulate⁶
- Supports the normal immune reaction of intestinal cells^{7,8}
- Crowds out bacterial pathogens and maintains healthy gut flora^{9,10}
- Communicates with intestinal cells to maintain gut barrier function¹¹

Each type and strain of probiotic, spore and non-spore forming, performs a different role with particular benefits in terms of digestive and immune systems' health, as well as where in the GI tract they act. Multi-strain probiotic supplements provide a broad spectrum of benefits.

BACILLUS SUBTILIS - DE111

Deerland Enzymes has genome sequenced and tested a highly effective strain of *Bacillus subtilis*, a probiotic spore that works as a complement to many of the non-spore strains on the market today. DE111 has been fully sequenced and uploaded to GenBank, the National Institutes of Health genetic sequence database.



Clinical Results

A randomized, double-blind, placebo controlled human clinical study has shown the ability of DE111 to support a healthy gut microflora by controlling microbial populations as well as support digestion and maintain general health.

CONTROLS MICROBIAL POPULATIONS

DE111 is a classic *Bacillus subtilis* strain that supports the normal proliferation of beneficial bacteria and crowds out other bacteria.

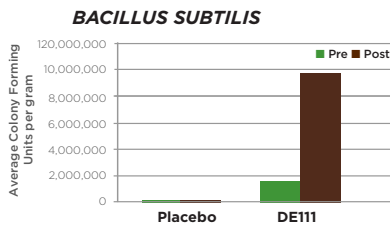


Figure 1. Subjects who were administered the placebo demonstrated a slight decrease in intestinal levels of the probiotic *Bacillus subtilis*, while those who were administered DE111 experienced a significant increase.

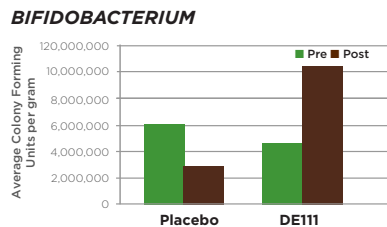


Figure 2. Subjects who were administered the placebo demonstrated a decrease in intestinal levels of the probiotic *Bifidobacterium*, while those who were administered DE111 experienced a significant increase.

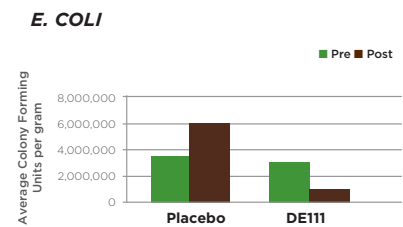


Figure 3. Subjects who were administered the placebo demonstrated an increase in levels of the pathogenic bacteria *E. coli*, while those who were administered DE111 experienced a slight decrease.

SUPPORTS DIGESTION AND MAINTAINS GENERAL HEALTH

DE111 supports the normal breakdown of complex carbohydrates and fats, promoting proper digestion and nutrient absorption.

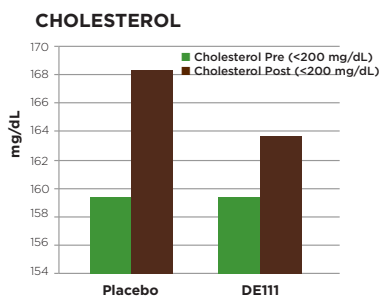


Figure 4. Subjects taking the placebo experienced a larger increase in cholesterol levels than those taking DE111.

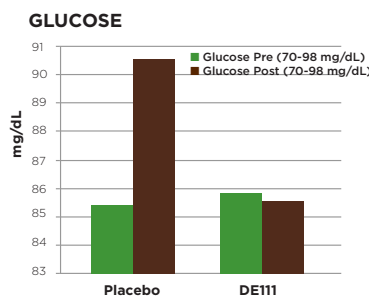


Figure 5. Subjects taking the placebo experienced an increase in glucose levels, while the levels of subjects taking DE111 were maintained.

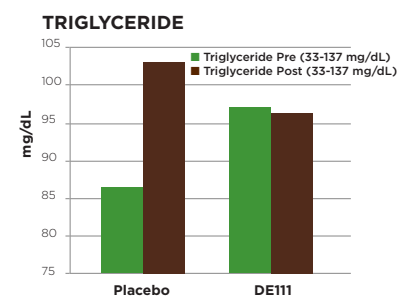


Figure 6. Subjects taking the placebo experienced an increase in triglyceride levels, while the levels of subjects taking DE111 were maintained.





Applications - Food

Because DE111 remains viable under a wide temperature range, the probiotic is ideal for use in supplements, foods and beverages.

FOODS AND BEVERAGES: PROCESSING STABILITY

Food Product	Processing Simulation Method	Average % CFU Survival
Green Tea (Hot)	A green tea bag containing DE111 was placed in boiling water and steeped for 5 minutes. A cell count was performed on the hot tea.	94%
Chocolate Bar (70% Cacao)	A bar of chocolate was melted to 48°C, DE111 was added. The mixture was cooled to 27°C and heated back to 32°C before the chocolate was poured into molds to set.	98%
Instant drink powder (chocolate)	DE111 was added to the drink powder and combined with cold milk. A cell count was performed on the prepared drink.	89%
Dairy Processing	DE111 was added to 100 mL of skim milk. The milk was heated to 80.6°C for 22 seconds, then immediately chilled and a cell count was performed.	100%

SUPPLEMENTS: SHELF STABILITY

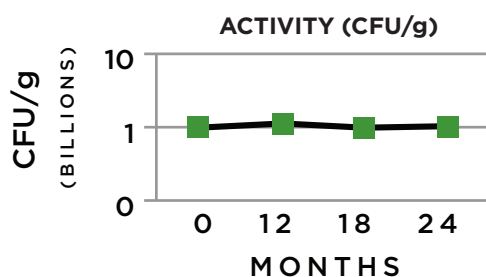


Figure 8. In stability testing, DE111 experienced virtually no loss of colony forming units (CFU) over 24 months, when stored at room temperature. (25°C)



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