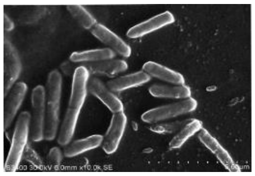
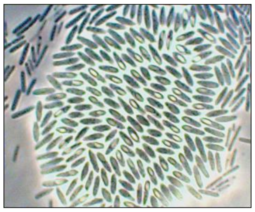
Chemistry+Biology

State-of-the-art research and advanced process development deliver performance-adding value to everyday products

What are Clostridia?



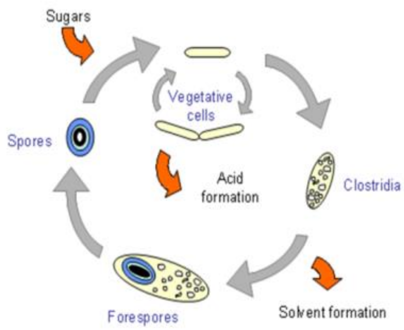


Clostridia are a large class of microorganisms that were traditionally grouped together based on the characteristic "look" of the family of cells. The word *Clostridium* is derived from the Greek word *Kloster* meaning spindle. When examined under a microscope, these bacterial cells are rod (or 'spindle') shaped. Microbes of the *Clostridia* family are anaerobic, which means they will only grow in the absence of oxygen, or where oxygen levels are very low. Clostridia can be found in soil, water, sewage, or even in human and animal intestines as part of the normal gut microbes, and are able to change shape and hibernate when conditions for growth are not optimal. Clostridia are hardy, versatile, and robust microbes, and while there are many similarities across the *Clostridia* family, individual members (or strains) of the class *Clostridia* have a wide range of functions and usefulness to industrial processes.

Several *Clostridia* strains have known beneficial roles in the environment and industry including bioremediation of contaminated soil and production of commercially valuable chemicals. Some *Clostridia* strains are even being explored as potential cancer therapies. Like many bacteria, there are some strains that can be pathogenic, or cause disease in humans, for example *Clostridium tetani*. At Green Biologics, our focus is on *Clostridia* strains that have been shown to naturally produce chemicals of importance to our everyday lives.

The *Clostridia* strains in the Green Biologics library are non-pathogenic, highly productive strains that produce a high concentration of bio-based n-butanol and acetone in Green Biologics' proprietary advanced fermentation process (or AFP™) in a process called "ABE", for **A**cetone, **B**utanol and **E**thanol. In fact, during World War I, the ABE process, using *Clostridia* microbes as biocatalysts, was the only commercial route to production of acetone for cordite used in manufacturing the smokeless munitions used in the Great War. The process was commercialized in 1915 by Chaim Weizmann, a Russian emigrate and professor at the University of Manchester in England. This invention helped turn the tide of war in Europe, and Professor Weizmann was honoured by the UK parliament for his efforts. The ABE process was the primary route to n-butanol and acetone until 1938, with the development of the modern petrochemical route to n-butanol and acetone.

Clostridia fermentations can be defined as the transformation of sugars derived from crops or biomass, into solvents, such as n-butanol and acetone, in the absence of oxygen. The main *Clostridia* strains that have been studied and optimized for commercial use are *Clostridium acetobutylicum*, *Clostridium beijerinckii* and *Clostridium saccharobutylicum*. Each is able to produce n-butanol, acetone and ethanol in varying ratios although n-butanol is always the major product. The choice of strain used is largely dependent on the substrate to be fermented. *C. acetobutylicum*, for example, is able to ferment starchy feedstocks such as corn mash, which *C. beijerinckii* and *C. saccharobutylicum* are unable to use efficiently. While the process was initially commercialized to produce acetone, the invention of butyl acetate and its use for automobile lacquers in 1927 by DuPont led to a dramatic increase in demand for butanol, and a shift in ABE fermentations toward n-butanol as the main product. Eventually, ABE could no longer compete with petrochemical methods of butanol production. The last US facilities were shuttered in 1954; but Russia and South Africa continued to produce using ABE fermentations until the mid-1980's.





Strains related to *C. acetobutylicum, C. beijerinckii,* and *C. saccharobutylicum* make up a significant proportion of Green Biologics' *Clostridia* strain collection, or library. With advances in technology, GBL can take advantage of the robust nature of the *Clostridia* bug to marry individual microbes to feedstocks (like corn cobs or stover, or forestry residuals) to greatly enhance the efficiency of the ABE fermentation process. Although GBL's advanced technology now allows detailed manipulation of strains, our current strain improvement methodologies utilise strain adaptation and lab-based selection methods very similar to those used by Weizmann and the plant operators 100 years ago.

These microbial advances, along with advanced fermentation and product recovery and purification process improvements developed by GBL, have allowed us to optimize our strains with a wide range of feedstocks that enable the *Clostridia* ABE process to become a cost effective, low cost, and much more sustainable alternative to petrochemical based processes.