# Prebiotic Activity Assessment of Chlorella spp.

Major active ingredients in Chlorella are extracellular polysaccharides. These extracted polysaccharides show numerous health benefits and can be used as a prebiotic. (Wang et al., 2018)

Criteria used to classify a compound as a prebiotic:
(i) it should be resistant to acidic pH of stomach, cannot be hydrolyzed by mammalian enzymes, and should not be absorbed in the gastrointestinal tract
(ii) it can be fermented by intestinal microbiota
(iii) the growth and/or activity of the intestinal bacteria can be selectively stimulated by this compound and this process improves the host's health.

## Experimental Design

Experiments designed to assess prebiotic potential of the below products are as follows:

(i) **In Vitro GI Digestion**. Place product samples in simulated GI fluid to "digest". After digestion use Barfoed's test to check if residue contained monosaccharides/free sugars. Undigested residue would show no red precipitate thereby satisfying the criteria that the prebiotic material is resistant to acidic pH of the stomach and cannot be hydrolyzed by mammalian enzymes.
(ii) **In Vitro Fermentation**. Use pure culture method to test if potential prebiotic can be fermented by bacteria strains typically present in intestine. Use pure bacterial strains (Lactic Acid Bacteria and E.coil) for this method.
(iii) **Prebiotic Activity Score**. The ability to utilize each potential prebiotic as a carbon source is calculated using the following formula:

$$\text{Prebiotic Activity Score} = \frac{\{(\log \text{CFU/mL of WF-1 with prebiotic at 24h} - \log \text{CFU/mL of WF-1 with prebiotic at 0h})}{(\log \text{CFU/mL of WF-1 with glucose at 24h} - \log \text{CFU/mL of WF-1 with glucose at 0h})\}} \text{ subtracted from } \frac{\{(\log \text{CFU/mL of E.coli with prebiotic at 24h} - \log \text{CFU/mL of E.coli with prebiotic at 0h})}{(\log \text{CFU/mL of E.coli with glucose at 24h} - \log \text{CFU/mL of E.coli with prebiotic at 0h})\}}$$

Each broth (MRS and TSB) is made without glucose (modified broth). 1g of prebiotic sample is added to 50mL of modified broth. FOS was used as a positive control. The higher the score the greater potential the product has to be used as a potential prebiotic.

## Assessment Results for Phytoplankton: Chlorella spp.:

**(i) In Vitro GI Digestion**

- No red precipitate was formed. Therefore, satisfying the criteria that the prebiotic material is resistant to acidic pH of the stomach and cannot be hydrolyzed by mammalian enzymes.

**(ii) In Vitro Fermentation**

- **pH measurement**. Starting pH was 7.33, pH after 24 hours was 6.28.
- **Enumeration of Wolf Strain Mix in prebiotic**. Starting count was $1.17 \times 10^5$ CFU/mL, after 24 hours the count was $4.8 \times 10^8$ CFU/mL. The wolf strain mix was able to utilize Chlorella spp. as a carbon source for fermentation.

**(iii) Prebiotic Activity Score**

Score was lower than the positive control but still has the potential to be a prebiotic that can support bacterial fermentation. (See graph below). Note production of a gas by product was detected in the test tube after 24 hours.