

HUMAN GUT MICROBIOTA DEGRADE THE RED SEAWEED-DERIVED DIETARY FIBER CARRAGEENAN

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Objectives: A fluctuating fiber-rich diet drives the human gut microbiota to develop strategies to metabolize novel dietary polysaccharides. One such recent addition is a highly sulfated marine red algal-derived polysaccharide called carrageenan. Due to its gelling properties, carrageenan is widely used as a food additive in dairy products and confectionary. Although considered as a safe dietary fiber, recent studies have shown that exposure to low molecular weight carrageenan called poligeenan causes intestinal ulcerative colitis in animal models. We explore the role of a human gut microbe isolated from North American population, *Bacteroides thetaiotaomicron* in carrageenan degradation to poligeenan.

Methods: Our study applies metagenomics and proteomics to identify carrageenan-utilizing cluster of genes in human gut bacterial isolates. We use recombinant protein expression, carbohydrate gel electrophoresis and ion chromatography techniques to reveal the catabolic action of gut bacterial encoded enzymes on carrageenan. Lastly, we could trace the fate of carrageenan in human gut isolate *B. thetaiotaomicron* using super-resolution fluorescence microscopy.

Results: We present one of the first evidence of an upregulated, carrageenan-utilizing gene locus in human gut bacterium *B. thetaiotaomicron* that is laterally transferred from marine microbes to gut *Bacteroides*. The key protein players encoded within this locus includes a range of transporters, glycoside hydrolases (GH), sulfatases and regulators. We show the activity of highly efficient endo-acting Type 1 sulfatases and carrageenases GH82 and GH16 that could be the first to encounter carrageenan in the gut. These enzymes desulfate and cleave the complex carrageenan substrate into low molecular weight poligeenan-like fragments, which are taken up in the bacterial periplasmic space.

Conclusion: Our findings strongly suggest that carrageenan gene locus is a recent adaptation of gut microbiota in North American people due to the increased use of carrageenan in the diet. Therefore, the industrial application of carrageenan as a dietary fiber should be carefully revisited since our investigations provide the *in vitro* evidence

of gut bacterial enzymatic digestion of carrageenan into potentially harmful poligeenan. These studies on biochemical and microbial mechanism of carrageenan degradation contribute to the general understanding of complex polysaccharide utilization in gut and its effects on human health.