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Heat treatment of curdlan enhances enzymatic production of bioactive β-(1,3)-glucan oligosaccharides

Curdlan is a water insoluble linear β-(1,3)-glucan, which is industrially produced from bacterium. This polysaccharide displays irreversible gelation by heating, which has been applied in the food industry as stabilizers and food textures. However, those properties are unfavorable for preparation of valuable β-(1,3)-glucan oligosaccharide (LG) from curdlan as discussed below.

LGs are bioactive carbohydrates, which exhibit the induction of monocytes to produce tumor necrosis factor-alpha, the stimulation of the secretion of interleukin1β, and the enhancement of defense responses in tobacco. It has been revealed that LGs with a degree of polymerization (DP) > 4 (LG>4) possess biological activity and work as bioactive materials. Although many researches have attempted to produce LG>4 from curdlan by enzymatic or chemical approach, the regulation of DP of the hydrolysis products is quite difficult, since the water-insolubility and the heat treatment-induced gelation (> 80°C) resist enzymatic hydrolysis and chemical degradation.

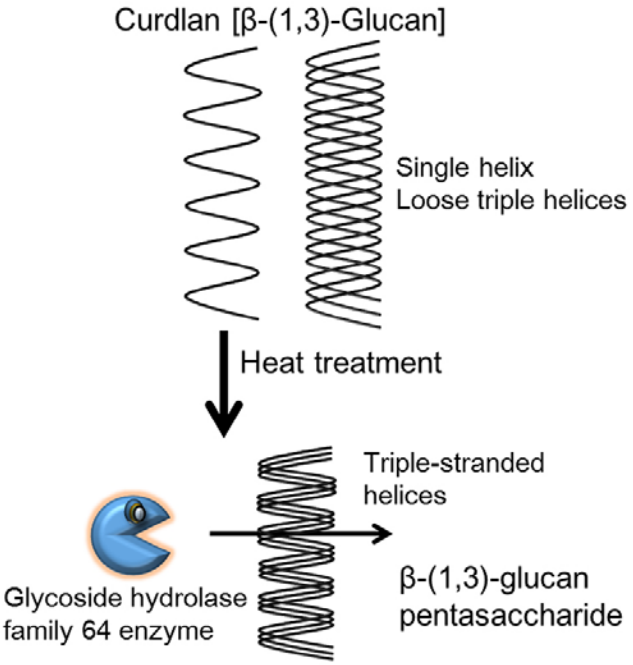

Fig. 1. Schematic of oligosaccharides production from curdlan. The helical lines indicate β-(1,3)-glucan. GH64 enzymes prefer heat treatment curdlan, which formed triple-stranded helices.

For synthesizing LG>4, we utilized a curdlan-degrading enzyme from *Kribbella flavida* NBRC 14399 (KfGH64), which generates LG with DP 5 (LG5) and does not produce small LG with DP < 5. KfGH64 does not degrade intact curdlan, the conformation of which is a mixture of single helix and loose triple helices at room temperature; however, KfGH64 can hydrolyze the heat treatment curdlan, which forms a thermal irreversible gel with triple-stranded helices. The finding indicates

that KfGH64 prefers the triple-stranded helices curdlan. Under optimal reaction conditions, the hydrolysis rate of KfGH64 increases from 3% to 60% when comparing intact curdlan with heat treatment curdlan. By the successive hydrolysis of the curdlan (second or third heat treatment/digestion), the maximum hydrolysis rate in this study, which remains at 75% and does not reach 100%, suggests that the residual curdlan (25%) forms a single helix and loose triple helices that are resistant to attack by KfGH64. It is found that the hydrolysis products do not contain any small size saccharides such as glucose or LGs with DP 2-4. The main product is LG5 (80% of the hydrolysate). The hydrolysis products also include two classes of LG with average DP 13 and DP 130, resulting in the average DP of the whole products is 7.9. The resistance of hydrolysis in saccharides with average DP 13 and DP 130 is caused by their generation of disordered and lamellar structures, respectively, resulting in the inhibition of complete hydrolysis.

An aging society becomes a global problem, and the health care grows increasingly important year by year. Oligosaccharides are well known as functional materials with many beneficial effects on human life such as health maintenance, health promotion, and disease prevention. However, some excellent oligosaccharides are not industrially available due to their difficulty in production; e.g. the synthesis of LG5 from water-insoluble curdlan. From the conformation-altered curdlan with heat treatment, we succeeded in generation of LG5 in good yield by KfGH64-catalyzed degradation. Heat treatment is a simple approach and does not require any further post-process: neutralization with acid or alkaline chemical.

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