MOLECULAR CLONING AND BIOCHEMICAL PROPERTIES OF GH-16 B-AGARASE FROM *GILVIMARINUS AGAROLYTICUS* JEA5

Youngdeuk Lee, Korea Institute of Ocean Science & Technology
lyd1981@kiost.ac.kr
Eunyoung Jo, Korea Institute of Ocean Science & Technology
Do-Hyung Kang, Korea Institute of Ocean Science & Technology
Chulhong Oh, Korea Institute of Ocean Science & Technology

Key Words: Gilvimarinus agarolyticus, agarase, neoagarooligosaccharide, agar.

Agar is complex polysaccharide founds in the cell walls of some red algae and up to 70 % of the algal cell wall can be agar polymers. Agar was formed by a mixture of two polysaccharides named agarose and agaropectin. Agarose can be hydrolyzed by α-agarase (E.C. 3.2.1.158) and by β-agarase (E.C. 3.2.1.81); the former cleaves the α-1, 3 linkage of agarose to generate agaro-oligosaccharides, and the latter cleaves the β-1,4 linkage to generate neoagaro-oligosaccharides. Agarases have been isolated from many sources, including seawater, marine sediments, marine algae, marine mollusks, fresh water and soil. Recently, *Givimarinus chinensis*, *G. polysacchalyticus*, *G. agarilyticus* were identified and their agarolytic activity also reported. However, there are no report published that molecular and functional characterization of agarase from *Givimarinus* genus. In this study, we first report molecular characterization and biochemical properties of agarase from *Gilvimarinus* genus.

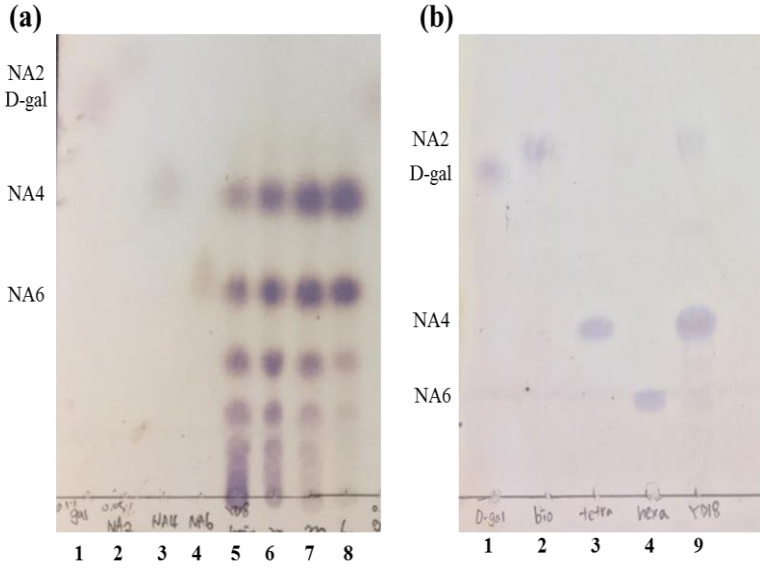

Figure 1. TLC hydrolysis products of the purified rGaa16A. 1: D-galactose, 2: neoagarbiose (NA2), 3: neoagarotetraose (NA4), 4: neoagarohexaose (NA6), 5-8: agarose with rGaa16A after 10, 20, 30 and 60 min incubation. 9: NA6 with rGaa16a after 2 hrs incubation

A gene (Gaa16a) encoding a β-agarase from *Gilvimarinus agarolyticus* JEA5 was cloned and expressed in *E. coli*. The recombinant protein was purified as a fusion protein and biochemically characterized. The purified recombinant agarase (rGaa16A) showed maximum activity at 55°C and pH 7. Interestingly, the thermostability of rGaa16A was improved in the presence of $CaCl_2$ rGaa16A showed specific activity toward agarose with 103.5 unit/mg. rGaa16A highly maintained its activity in the presence of $CaCl_2$.Products of the enzyme reaction of rGaa16A were determined by TLC. TLC results showed that agarose was rapidly degraded to neoagarotetraose (NA4), Neoagarohexaose (NA6), and neoagarooligosaccharides larger than NA8 during early stage of the reaction (Fig 4a). After 60 min incubation, amount of NA4 and NA6 lager than NA8. However, after 2 hours incubation, only neoagartetraose and a trace amount of neoagarbiose were detected.