

BIOCHEMICAL PROPERTIES OF A NOVEL NEOAGAROTRIOSE-PRODUCING B-AGARASE FROM *GILVIMARINUS AGAROLYTICUS* JEA5

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An agar degrading bacterium was isolated from seawater, collected from the east coast of Jeju Island, republic of Korea and identified as *Gilvimarinus agarolyticus* JEA5. The β -agarase gene from *Gilvimarinus agarolyticus* JEA5 (rGaa16B) was identified from draft genome sequence by BLAST. Gaa16B has 1800 bp of open reading frame encoding 636 amino acids (aa), and include glycosyl hydrolase family 16 (GH16) β -agarase module and two carbohydrate binding module 6 (CBM6). The Gaa16b was cloned and overexpressed as a MBP-fusion recombinant β -agarase (without signal peptide and two CBM6) in *E. coli*. rGaa16B showed highest activity at 60°C and pH 7. After incubation at 45°C for 90 min, rGaa16B showed over than 95% of its initial activity. rGaa16B were enhanced in the presence of MnCl₂, KCl₂, MgCl₂, FeSO₄. rGaa16B showed 2112.1 unit/mg in the presence of 2.5 mM of MnCl₂. rGaa16B produce mainly neoagartetraose (NA4) and neoagarobiose (NA2). Interestingly, we observed neoagartriose (NA3) from hydrolytic products of rGaa16B. LC/Mass analysis was performed to confirm the hydrolytic products containing neoagarotriose. We found three different hydrolytic products which showed 324.28, 468.41, 630.55 Da of molecular weight, respectively.

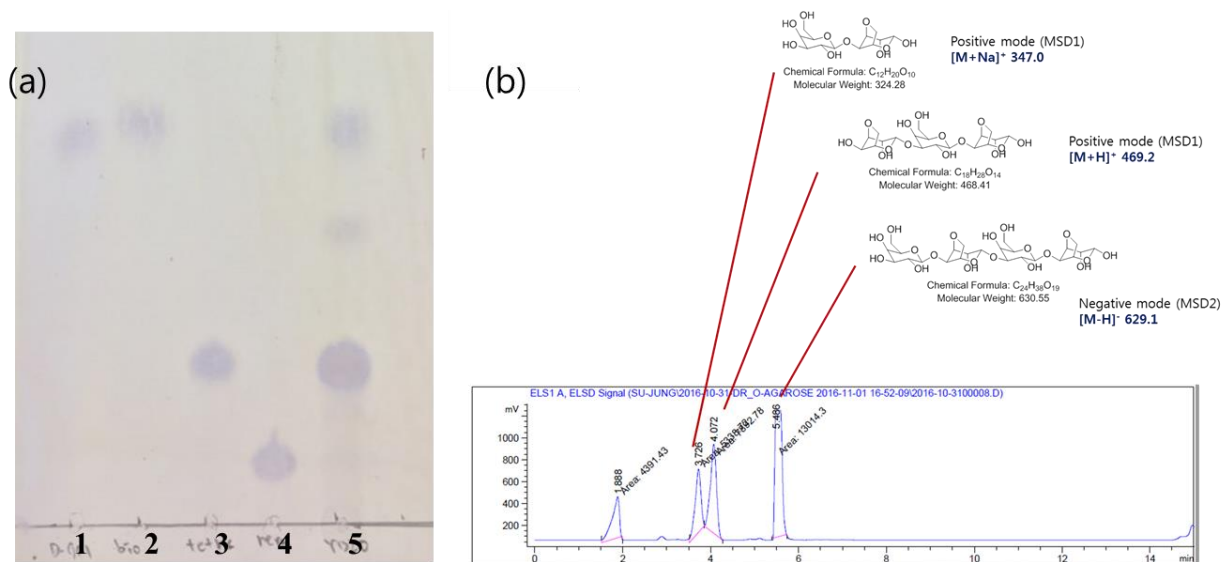


Figure 1. TLC (a) and LC/mass result of hydrolytic products of rGaa16B. 1: D-galactose, 2: neoagarobiose (NA2), 3: neoagartetraose (NA4), 4: neoagarohexaose (NA6), 5: hydrolytic product of rGaa16B.