Isolation and Characterization of a Novel Agar-Degrading Marine Bacterium, *Gayadomonas joobiniege* gen. nov, sp. nov., from the Southern Sea, Korea

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**Introduction**

Marine microorganisms have received a great deal of attention in various efforts to utilize the abundant biological resources in the sea. One of these resources is agar, which is a major component of red algae [6]. Agar is widely used as a dietary ingredient in food and as a gelling agent in solid culture media for microbial growth. Recently, agar oligosaccharides have also been reported to have antioxidant activity, therapeutic activity in inflammatory disease, and antitumor activity, *etc.* [3, 11, 19], which may broaden its application in the food, cosmetic, and pharmaceutical industries as well as biorefinement or biofuel industries.

For an efficient hydrolysis of agar, research seeking a good agarase has been under intense spotlight. A number of microorganisms with agar-hydrolyzing activity, such as *Pseudoalteromonas marinoglutinosa* [30], *Alteromonas atlantica*...
Glaciecola mesophila [31], Catenovulum agarivorans [37], and Thalassomonas agarivorans [18], which are classified as Alteromonas-like Gammaproteobacteria [14, 16], have been isolated from marine environments.

We recently isolated another Alteromonas-like marine bacterium (designated as strain G7T), which can grow on minimal medium supplemented with agar as the sole carbon source at higher temperature (above 40°C). Genomic sequencing of G7T revealed that it had many genes encoding hydrolytic enzymes: 50 sulfatases, 17 glycoside hydrolases, 13 agarases, 8 β-galactosidases, 3 altronate hydrolases, and 1 cellulase, which may act in the complete hydrolysis of sulfated algal polysaccharides [24]. Because of the great potential for the bacterium to be used as a bioresource for bioconversion of algal polysaccharides, we used a polyphasic taxonomic approach for the classification of strain G7T. This report describes the characteristics of the G7T strain as a novel genus of the family Alteromonadaceae.

Materials and Methods

Chemicals

Agar and agarose were purchased from Amresco Inc. (USA) and Takara Shuzo Inc. (Japan), respectively. All other chemicals were purchased from Sigma Chemical Co. (USA).

Isolation of Agarase-Producing Microorganisms

Coastal seawater of Gaya Island, Republic of Korea, was collected to isolate agar-degrading bacteria. The collected sample was serially diluted from 10^{-1} to 10^{-7}, and 200 µl of each dilution was smeared on an artificial seawater (ASW) agar plate [20] containing 1.0% yeast extract (w/v) and 0.3% bacto peptone (w/v) (ASW-YP), and was incubated aerobically at 40°C for 24 h. The ASW contained 6.1 g Tris base, 12.3 g MgSO_{4}, 0.74 g KCl, 0.13 g (NH_{4})_{2}HPO_{4}, 17.5 g NaCl, and 0.14 g CaCl_{2} dissolved in 1 L of distilled water. A total of 1,136 colonies were collected and transferred to fresh ASW-YP plates and incubated at 40°C for 24 h. The plate was stained with Lugol’s iodine solution (0.05 M iodine in 0.12 M KI) to detect agarase activity (Fig. 1A). Colonies with high agarase activity were isolated from the replica plate and transferred to a fresh ASW-YP plate. The bacterial colonies were streaked five times to obtain a single colony for pure culture. One marine bacterium with agar-hydrolyzing activity was selected and designated as strain G7T in this study. After incubation in ASW-YP broth at 40°C for 1 day, the culture was supplemented with 10% glycerol (w/v) and stored at -80°C as the stock culture.

Production of Agarase by Strain G7T

Cell growth and agarase activity of strain G7T were observed by incubating it in ASW-YP liquid medium containing 0.1% agar (w/v) at 40°C for 72 h (Fig. 1B). Then, 1 ml of culture broth was sampled at regular time intervals, and the optical density (OD) was measured at 600 nm (OD_{600}) to plot the growth curve. The sample was centrifuged at 14,000 rpm for 10 min and its supernatant was collected to measure agarase activity. A substrate solution containing 0.2% agarose in 10 mM Tris-HCl (pH 8.0) was used for the agarase reaction. Agarase activity was measured by the previously described method [27] using 3,5-dinitrosalicylic acid (DNS). One unit (U) of agarase was defined as the amount of enzyme that produced 1 µmol galactose per minute at 40°C. Galactose was used as a reference reducing sugar for preparing the standard curve.

16S rRNA Sequencing and Construction of Phylogenetic Tree

The bacterial strain was cultured in ASW-YP liquid medium for 3 days, and genomic DNA was extracted with a genomic DNA extraction kit (Promega Co., USA). The 16S rRNA gene was