学位論文内容の要旨

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学位論文題目

Genome analysis of the alginate degrading marine bacterium *Vibrio halioticoli*

（*Vibrio halioticoli* のゲノム解析）

In maintaining a sustainable society in global warming era, the development of key technologies for renewable energy sources has become an important challenge. As the next-generation feedstock, marine macroalgae have emerged as a viable option. It can be used to produce biofuel or precursors, but there are still many difficulties to overcome. It contains a variety of unique carbohydrates, and alginate is a major component of brown macroalgae. The oxidized carbohydrate is resistant to biofuel conversion, but attempts have been successful in some terrestrial bacteria such as *E. coli* and *Sphingomonas* sp. However, up to now we have not had much success using marine microbes.

V. halioticoli IAM 14596^{T} was isolated from the gut of the abalone *Haliotis discus hannai*. The bacterium has a high native ability to ferment mannitol and alginate. The wild type cannot convert alginate to ethanol directly, but recently created metabolically engineered cells with installed “Production of Ethanol” operon can produce ethanol. Symbiotic association to the host abalone has been considered. Therefore *V. halioticoli* is a biotechnologically and ecologically important marine bacterium, but the complete genome sequence has not yet been achieved. To improve the biocatalysts in producing bioethanol more efficiently and to know how symbiotic association has been established between the host abalone and bacteria, the detailed metabolic pathway of alginate and the gene expression controls of such genes in the bacterium should be elucidated. The aim of this study is to obtain complete genome sequencing of *V. halioticoli* IAM 14596^{T} and genome wide mining of genes responsible for alginate degradation and its expression.

Using a sequencer combination including PacBio, the genome sequence of *V. halioticoli* IAM 14596^{T} was completed. On the basis of this work, two chromosomes and one plasmid were obtained. It consisted of 2,785,698 bp of larger chromosome (Chr. 1), 1,098,310 bp of smaller chromosomes (Chr. 2) and 244,363 bp of plasmid with G+C contents of 43.23%, 42.39%, and 41.18%, respectively. This is the first time to obtain the complete genome sequence of *V. halioticoli*, and is only the second complete genome in Halioticoli clade species followed by *V. breoganii* FF50.

In total 3,602 CDSs, 33 rRNAs and 30 tRNAs were predicted and covered more than 85% of the genome. The annotation rate is approximately 75%. I can identify, the replication origins of each

chromosome by confirming the presence of typical genes and unique sequences. A Rep-3 replication initiation protein gene with Dna box is found in the 3rd reconstructed genome, I decided that it could function as a plasmid. On the plasmid, there is a set of genes for conjugation, which means the plasmid also could function to be a conjugative plasmid. A 21,277 bp identical region is also found in both Chr. 2 (position 516756-538033) and the plasmid (position 5028-26305), which may show the plasmid could be integrated in the *V. halioticoli* genome.

Among 3,602 CDSs on the *V. halioticoli* genome, 2100 CDSs were annotated in BlastKOALA corresponding to 58.3%. Most of the CDSs encode products are involved in Metabolism, Genetic Information, Environmental Information, Cellular Processes, Human Diseases, and Organismal Systems. In metabolic pathways, *V. halioticoli* possesses genes responsible for EM, PP, ED pathway in carbohydrate metabolism. *V. halioticoli* possesses CDSs classified into 32 GH, 26 GT, 23 PL, 7 CE and 2 AA in total. Some of them possess CBMs. A total of 140 types of transporter genes being capable of transport 35 substrates, and 6 two-component systems, were found to respond to environmental changes and stimuli. *V. halioticoli* possessed a set of genes for utilization of mannitol, alginate, and the other components of marine biomass. Cloning and expression of these genes may help us to understand the alginate metabolism in the bacterium.

The gene mining and the expression studies reveal a total of 15 alginate lyase gene candidates in the whole genome data of *V. halioticoli*. Further motif/domain and localization analysis of these gene shows that at least 11 genes were more likely to function as alginate lyase. At least six types of alginate lyases were contained in the genome of *V. halioticoli*; 1) extracellular secreting (or periplasmic) 35-40 kDa PL7 types (S2G1, S2G3, S2G4, S4G1 and S4G8), 2) a cytoplasmic 80 kDa PL17 type (S2G2), 3) a cytoplasmic 70 kDa dual domain PL7 (S4G2), 4) an extracellular 57 kDa dual domain alginate lyase (S4G3), 5) localization unknown 81 kDa dual domain PL17 type (S4G4 and S4G5), and 6) cytoplasmic 79 kDa PL15 type alginate lyases (S4G10). S2G1, S4G1, and S4G8 showed polyG specific activities.

In addition, a new species in the Halioticoli clade species was found off Ishigaki Island in coral reef water and has been named as *Vibrio ishigakensis* sp. nov. Optimum growth temperature is likely to be shifted upwards from that of *V. halioticoli*.

In conclusion, the genome sequence of *V. halioticoli* was completely sequenced. The bacterium produces at least active 7 extracellular secreting alginate lyases and active oligoalginate lyases S2G2 and S4G10. The genome provides a new starting point to help us more clearly understand the organism's phenotype and genotype characteristics in the bacterium and the related spices including *V. ishigakensis*, and push forward the biotechnology research.