博 士（理 学） ナフィース アハメド

学 位 論 文 題 名

Study on the Growth Process of Barnacle on Soft Substrates

（ソフト基板上でのフジツボの成長過程に関する研究）

学位論文内容の要旨

The fouling problems caused by marine sessile organisms (barnacles, shells, algae, diatom, etc.) on the marine submerged solid surfaces, such as ship's hull, fishing nets, power plants, have created great economic threat. Barnacles are found to be unwilling to adhere on natural soft substrates such as sea weed, sea squirt, dolphin, etc. because of the softness of these surfaces. This antifouling property of living surfaces has drawn the attention of the marine scientists. In this connection, scientists have been emphasizing on synthetic soft materials (silicone elastomers) without having any toxicity or reactivity since 1980's for coating purpose. After the banning of tributyltin (TBT) since 2008 for its high endocrine disruption, the scientist casted the gaze on the wide range of soft materials other than silicone elastomer. Consequently, since last one decade hydrogels have been considered to be another type of soft and wet materials which can be treated as the antifouling agents against barnacles.

This dissertation is based on the investigation of the growth process of barnacle, *Balanus amphitrite*, on different soft substrates (silicone rubber and hydrogel) revealing the concept of antifouling. As the low settlement and smaller basal size of barnacles on soft substrates were previously observed in the field test, it was a great demand to investigate the long-term real-time growth of barnacles on the soft substrates in the laboratory environment to acquire detail information avoiding some interfering factors (tidal waves or friction, competition of other organisms). In this case PDMS (poly-dimethyl siloxane) and DN (double network) gel were chosen because of their soft property, different wettability and durability inside the seawater for long time. As barnacle settlement is inhibited by the soft materials, the growth of barnacles can also be impeded by the soft substrates. Regarding the growth of barnacles several new concepts of antifouling have been developed.

In Chapter 2, the detail of the culture of barnacles was described. The culture protocol of barnacles has two processes, e.g., the maintenance of adult barnacle and the culture of nauplius larvae to obtain the cyprid larvae. Finally, we succeeded to establish the culture of barnacles in the laboratory environment.

In Chapter 3 the comparative effectualness of the three systems (24-well polystyrene plate (PSP), 09-cell nylon plankton net cage (NPNC) and polystyrene petri dish (PSPD) systems) and the substrates for the settlement and growth of barnacles were discussed. Very low settlement of barnacle was observed on either physically or chemically crosslinked PVA (polyvinyl alcohol) gel. On the other hand, reasonable number of barnacle was found to adhere on PAMPS/PAAm (poly-(2-Acrylamide-2-methylpropanesulfonic acid)/poly-acrylamide) DN (double network) gel. PDMS (poly-dimethyle siloxane) exhibited as mediocre fouling favoured material against barnacle. The investigation of the settlement and growth of barnacle on soft substrates were carried out implementing all three systems except 24-well polystyrene plate (PSP) system, because of its inconvenience to the growth experiment.
In the growth observing experiment it was observed that the basal diameter and the growth rate of barnacle had been reasonably higher on PDMS (E = 0.37 MPa) compared with other two soft substrates, PAMPS/PAAm DN gel (E = 0.30 MPa) and PC PVA-2 (E = 0.05 MPa). These results inspired us to search for the influence of the elastic modulus on the growth of barnacle. Though 09-cell NPNC system was good to conduct the experiment of the growth of barnacle, very low settlement of cyprids and the problem in handling the sample during the growth observation compelled us to develop polystyrene petri dish (PSPD) system.

In PSPD system we used three soft substrates (PVA, DN gel and PDMS) with different elasticity and no influence of elastic modulus of the same substrate was found on the basal diameter of barnacle except DN gel. DN gel exhibited slight declining pattern of basal diameter with elasticity. However, in this case it was surprisingly observed that growth rate slightly varied with the type of the substrate rather than on the elastic modulus.

After considering the result it was unanimously considered that the newly developed PSPD system can be susceptible of investigating settlement and growth of barnacle. These results motivated us to search the settlement and growth of barnacle on soft substrates with different elastic modulus and wettability. In this case, it was not only noticed the susceptibility of the system but also justified the suitable soft materials to carry out the experiment on the settlement and long-term growth of barnacles in the laboratory environment. So, it can be culminated that the soft substrates having considerable fouling property against barnacle along with the durability to survive in the seawater for long time should be chosen. The chapter 4 will be focused on the detail information concerning these aspects.

In Chapter 4 it was explained that PDMS (hydrophobic) and PAMPS/PAAm DN gel (hydrophilic) exhibited the increment of the settlement of pre-metamorphosed barnacle (cyprid larvae) with their corresponding increase in elastic moduli (E = 0.01 – 0.47 MPa) but the growth of barnacles on both of these substrates was found to be independent on the elastic modulus of the substrate. Furthermore, "self-release" tendency of barnacles from the hydrophobic PDMS surface at post-metamorphosed state proved the high antifouling activity of PDMS against barnacle. On the other hand, PDMS as well as PAMPS/PAAm DN gel exhibited growth inhibitory activity which was evidenced by comparing the growth rate of barnacles on hard substrate (PS) (about 4 times higher) with that on soft PDMS and DN gels (both of them were nearly the same) after more than two months observation of the growth kinetics.

In Chapter 5 the dominating role played by the elasticity of the substrates within wide range to influence the basal morphology and morphometry of barnacles growing on PS, PDMS and DN gel over that within narrow range (E = 0.01 – 0.47 MPa) was discussed. Furthermore, the authors were able to find out the connection between different basal morphology and 3D-shape of barnacles influenced by different substrates. Substrates influenced the shape of barnacle which finally regulated the basis morphology. These findings were supported by the higher value of OBR (orifice to base ratio), volume and SSA (shell to substratum angle) of barnacles on PDMS and DN gel at latter growth stage compared with those on PS. Regarding this relation the model for the 3D-shape of barnacle was explored based on the barnacle muscle modulus hypothesis. These observations also revealed another concept of antifouling property of soft substrates upon regulating the shape and basal morphology. As in the chapter 4 three types of antifouling properties (settlement inhibition, growth inhibition and self-release) of soft substrates were discussed, another type of antifouling property of soft substrata was explored in this chapter. Basal morphology and shapes of the barnacles on soft substrates (PDMSs and DN gels) were found to be deviated from the typical outlook. This has created a new concept of antifouling by the soft substrates.

Eventually, this study revealed the new concept of antifouling property of soft materials against barnacles. Till now the meaning of antifouling activity of the soft materials has been stacked up onto the settlement inhibition criterion. However, through the long-term real time investigation of the growth of barnacles on soft substrates with different elasticity and wettability it has been discovered that antifouling activity is not only comprised of the lower settlement of cyprid larvae of barnacle but also the 'self-release' tendency (for the barnacles on PDMS) and the growth inhibitory activity (for the barnacles on DN gel and PDMS) of the soft substrates during the growth period after being metamorphosed to juvenile barnacle. On the other hand, the change in the shape and morphology of base and shell of barnacles on aforementioned substrates during growing process were also discovered.

学位論文審査の要旨

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Therefore, we acknowledge that the author is qualified to be granted the doctorate of Science from Hokkaido University.