A Screening Method for Test Substances on Attachment Using Larval Barnacle, *Balanus amphitrite*, in the Laboratory.*

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Methods for the screening of substances that induce or inhibit the attachment of barnacle cyprids have been reported by RITTSCHOF *et al.* (1984) and MAKI *et al.* (1988). However, these methods were not suitable for the evaluation of selectivity for substrata by the larvae simultaneously. In their methods, cyprids were placed into polystyrene petri dishes coated with or without test materials or microorganisms, so that the cyprids in the a dish could have no chance to select substrata. Therefore, another methods is required to compare the effect for attachment in the same experimental vessel.

We developed a screening method that takes advantage of the selectivity for substrata by cyprids at the same time, and examined its practicality. *Balanus amphitrite* DARWIN was used as test organism. Nauplii of this species were reared until stage VI to cyprid at 24°C with diatom *Chaetoceros calcitrans* as food (KADO, 1991). The experimental equipment consists of a plastic water tank, a cage and a water pump (Fig. 1). The cage, made by converting a plankton net of 96 µm meshes into a bag form (20×15×10 cm), was used to prevent cyprids from settling on the wall of tank, and suspended into the tank containing 8 liter of seawater. The sea-

Fig. 1. Screening equipment using larval barnacle.

water was continuously circulated and poured from the tank into cage by the water pump, creating a water current. Fifteen each of glass plates (25×75×2 mm, as control) and the same size test plates were fixed onto stainless steel wires and hung down into the cage at random. When about one-third of the reared nauplii metamorphosed into cyprid (about six days after hatching), approximately 1000 of nauplii and cyprid were placed into the cage. The larvae were kept at 24°C for 72 hrs under a dark condition and then the number of those underwent metamorphosis after attachment to the test plates and control plates were counted.

Using this methods, we performed two experiments on the reaction of barnacle larvae; 1) to a slime and 2) to an antifouling paint. The slime was developed on glass plates immersed for one week into the sea (1 m in depth) in Aburatsubo Bay, Kanagawa. Attached

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Table 1. Results of screening tests for a slime film and an antifouling silicon type paint by present method

Test material		No. of cyprids examined	The survival rate of larvae (%)	Total no. of attached larvae (%)	No. of attached larvae on plate	
					Control (%)	Sample (%)
Slime	A	1885	93	145 (7)	50 (35)	95 (65)
	B	1349	89	113 (5)	32 (28)	81 (72)
	C	2259	97	243 (18)	95 (39)	148 (61)
AF Silicon paint	A	568	69	16 (2)	16 (100)	0
	B	813	90	23 (4)	23 (100)	0

bacteria was counted at about 10^4 cells/mm² on it. A silicon type paint, which is one of the antifoulants and known to be highly preventive of biofouling, was coated on glass plates. Untreated glass plates were used as control in the two experiments.

More than 80% of the larvae used in each screening test were cyprids at the end of the experiments. Results for the two experiments are shown in Table 1. Three trials were performed on the selectivity for substrata between the slime and control plate. The survival rate of larvae was 89~97%. Percentage of attachment was 5~18%; and 61~72% of the attached cyprids chose the slime plates. On the other hand, in the two trials using silicon paint plates, the survival rate was 69% or 90%. Percentage of attachment was as low as 2% or 4%. Nevertheless, all of the attached cyprids chose the control plates.

These results confirm that this method is practical for the assay of test substances in the laboratory. In addition, this method is also useful for the evaluation of toxicity of various substances by following the survival rate of larvae. Evaluation of various substances for their inducing or inhibiting properties on larval attachment are now in progress using this methods.

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摘 要

フジツボの付着期幼生を用いた付着関連物質のスクリーニング法とその検討

フジツボのキブリス幼生の付着率を判定基準として、付着促進または防止物質のスクリーニング法を考案しその有効性を検討した。試験は 8 l の海水を入れた水槽に目合い 96 μ m の篩絹籠を垂下し、試験板と対照板とキブリス幼生を収容後、海水を循環させ 24°C 暗条件下で 72 時間放置後の試験板と対照板への幼生の付着率を求める。本法により海域に浸漬して形成させたスライムとシリコン系防汚塗料について試験した結果、前者には付着促進、後者には付着防止効果が認められた。

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