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Bacterially induced stolon settlement in the scyphopolyp of *Aurelia aurita* (Cnidaria, Scyphozoa)

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ABSTRACT: Unsettled stoloniferous scyphopolyps of *Aurelia aurita* Lamarck were offered different substrates for settlement under defined conditions. On addition of different biogenic and abiotic substrates, a pure strain of bacteria, a species of Micrococcaceae, was observed to trigger the settlement of the stolon. The settlement reaction only takes place following direct contact with the bacteria; sterile filtrated culture medium of the same bacterial strain was not able to induce settlement. The bacteria were found to be effective on stolon settlement during the logarithmic growth phase, but not during the stationary phase.

INTRODUCTION

During the metagenetic life cycle of *Aurelia aurita* Lamarck, the pelagic sexually reproducing medusa alternates with the substrate-bound vegetatively reproducing polyp during the metagenetic developmental cycle. While the polyps of the upside-down jellyfish *Cassiopea andromeda* (Rhizostomeae), for example, cannot leave the site they have attached to during metamorphosis, polyps of *Aurelia aurita* are capable of changing their site using special structures called pedal stolons (Halisch, 1933; Gilchrist, 1937). Besides the scyphopolyps of *Aurelia aurita*, other species of the Semaeostomeae also show stolon formation and locomotion (Chuin, 1930; Truitt, 1935).

The pedal stolons of *Aurelia* polyps are multifunctional; they serve not only for locomotion and additional attachment sites, but also establish the first contact between the buds and their substrate. Neither the stolons of the buds nor those of the parent displayed noticeable morphological or specific behavioural differences.

Free stolons were seen only rarely in the stock culture, but almost every scyphistoma was additionally attached to the substrate by means of one or several pedal stolons. The settling stolons seemed to favour substrates (glass, rocks) that were overgrown by various species of algae and that were covered by detritus. However, any clear-cut preference for a special substrate could not be derived from these observations.

The following investigation was designed to determine whether the settlement reaction of the stolon tip can be influenced by special properties of the substrate.

MATERIALS AND METHODS

The animals for the experiments were from a clone of planula larvae from a medusa of the Mutsu Bay in the North of the Hondo Island (Japan). The culture was kindly provided by Mr. van den Sande, Zoological Garden of Antwerp (Belgium).

The scyphistomae of *Aurelia aurita* were kept in 4-l glass tanks at 19.5 ± 0.5 °C on a 16 : 8 h light: dark cycle in seawater (30 ‰ salinity). The animals were fed nauplii of *Artemia salina* twice weekly. The seawater was replaced 3–5 h after feeding. For some of the experiments, seawater containing antibiotics was used (100 mg Penicillin G – potassium salt [Serva], 129 mg Streptomycin – sulfate [Serva]/1000 ml pasteurised seawater).

For settlement experiments, non-budding scyphistomae without stolons were chosen. The basal fifth of the animals was dissected a day before the beginning of the experiment with an ophthalmological scalpel.

These polyps were then cultivated in 150-ml Boveri dishes filled with seawater containing antibiotics. Normally each animal developed 1 stolon within 24 h after the operation, so that animals with stolons of the same stage were available for the experiments.

Settlement of the stolon tips was considered to have taken place when the stolon tip could not be removed from the substrate without tearing the shaft of the stolon when probed with a sterilized needle.

Stock culture of bacteria. Agar set-up: 40 g tryptic soy agar (Difco), 5 g agar (Merck), 25 g NaCl/1000 ml distilled water.

Liquid culture of bacteria. Liquid set-up: 60 mg Bacto-Pepton (Difco), 300 mg D (+)-Glucose/300 ml seawater. The preparations were autoclaved for 30 mins at 0.5 bars.

For the biological tests a sample was taken from each suspension culture and was adjusted to an extinction of $E_{460} = 0.250$ in order to have a constant quantity of bacteria for reference. In early growth phases this was achieved by means of concentrating the bacteria by dilutions with sterilized seawater. The extinction was determined after thorough whirling of the suspension using a Mixomat. Before the onset of the experiment, the bacteria were washed again 3 times in sterilized seawater to remove the culture medium completely.

RESULTS

Development of a pedal stolon: Pedal stolons normally arise in the basal third of the polyp's trunk, more rarely in the immediate neighbourhood of the foot. They never occur below the tentacles.

In the earliest recognized stage the stolon appears like a cone-shaped protuberance containing both ecto- and endoderm. This protuberance elongates to form a tendril-shaped mobile ciliated process. During elongation the tip of the stolon thickens to form a knob-like structure. This thickening is caused by specialized ectodermal cells which secrete an adhesive substance, as soon as the tip of the stolon attaches to a suitable substrate. The ability to attach to the substrate does not appear to be correlated with the length of the stolon. After attachment, the tip of the stolon broadens and flattens, while

the stolon itself appears as a taut process connecting the site of attachment with the trunk of the polyp.

After attachment the ectoderm of the stolon contracts. The endoderm is first compressed, then the endoderm of the stolon is reintegrated into the endoderm of the polyp. The endodermal strand can be torn in places and at the base of the stolon it often appears spirally curled. Eventually, the stolon appears as a hollow ectodermal process stretched out between the polyp and the substrate. Endodermal cell aggregations can be seen occasionally in its lumen.

On attachment, the stolon can contract so strongly that the polyp slowly detaches from its original site and is drawn towards the attached stolon. After moving from one site to another, the tip of the stolon can become the new foot of the polyp, or else can be withdrawn into the wall of the stalk, while the polyp again settles with its original foot.

The initiation of the settlement reaction under various experimental conditions was examined. Different experiments were constructed to determine the conditions optimal for stolon settlement. Samples of the culture medium were taken from every Boveri dish in order to record the potential presence of any microorganisms that could be significant to stolon attachment.

After 24 h, only a few stolons had settled in the experimental dishes containing abiotic substrates, while the number of settled stolons dramatically increased on addition of biological materials, especially when non-sterile seawater and thalli of *Caulerpa* were added.

Table 1 shows that there was a significantly higher percentage of fixed stolons after addition of fragments from *Caulerpa* thalli. Most stolons were in direct contact with the fragments, but sometimes they were also attached to the glass surface of the Boveri dish.

After 48 h, all polyps in experiments containing a biological substrate had settled by means of their stolons. In the experiments containing the abiotic substrate, the settlement of stolon was observed in some, but not all, of the polyps (Table 1).

These observations show that a preference of the stolons for a certain substrate exists, but settlement was possible under each of the experimental conditions. However, it was interesting to note that the time taken for the settlement of all stolons differed from one condition to another. For instance, after only 24 h the greatest number of fixed stolons was observed in dishes containing *Caulerpa*. This means that thalli of *Caulerpa* must have had some quality especially favourable for the settlement of stolons. This quality promotes a relatively rapid settlement of the stolons as compared with the other substrates. All agar dishes containing culture medium of varying composition, whether with or without added substrate, contained bacteria. Agar dishes containing seawater and treated with antibiotics showed none or only few bacterial colonies. Dishes that had been treated with culture medium from the most effective substrates contained the most dense growth of microorganisms in comparison with the other dishes. In seawater containing antibiotics, stolon attachment was not observed. This leads to the assumption that microorganisms promote the settlement of the stolons. The microorganisms did not seem to be exclusively restricted to a special substrate.

Four species of bacteria were isolated from swabs taken from *Caulerpa*. Out of these, one species of the Micrococcaceae was found to be effective in inducing the initiation of the settlement reaction of stolons.

For further experiments, a pure strain of this bacteria was cultured. Since the

Table 1. *Aurelia aurita.* Number of stolons attached after addition of various substrates

Culture medium	Time (h)	Sterile seawater with substrate			Control 1 Sterile seawater without substrate			Control 2 Sterile seawater containing antibiotics		
		No. of stolons			No. of stolons			No. of stolons		
Substrate		n	attached	free	n	attached	free	n	attached	free
Sand	24	20	0	20	15	2	13	15	0	15
	48		9	11		4	11		0	15
Sandstone	24	15	1	14	15	2	13	15	0	15
	48		10	5		12	3		0	15
Dirty seawater	24	32*	22	10	25*	4	21	25*	0	25
	48		32	0		8	17		0	25
Egg shells of *Artemia*	24	20	6	14	15	0	15	15	0	15
	48	20	0			1	14		0	15
Biogenic coating	24	20	7	13	15	3	12	15	0	15
	48		20	0		9	6		0	15
Caulerpa thallus	24	45**	38	7	45**	10	35	45**	0	45
	48		45	0		29	16		0	45

* Values from 2 independent repetitions
** Values from 3 independent repetitions
The abiotic substrates were sterilized prior to the beginning of the experiment (3 h at 160 °C)

relationship between the titre and the extinction during growth of this bacteria was found to be constant in 7 repetitions, extinction values were chosen for reference in the following experiments.

The rise of the titre of bacteria during growth is shown in Figure 1, along with the extinction values during growth.

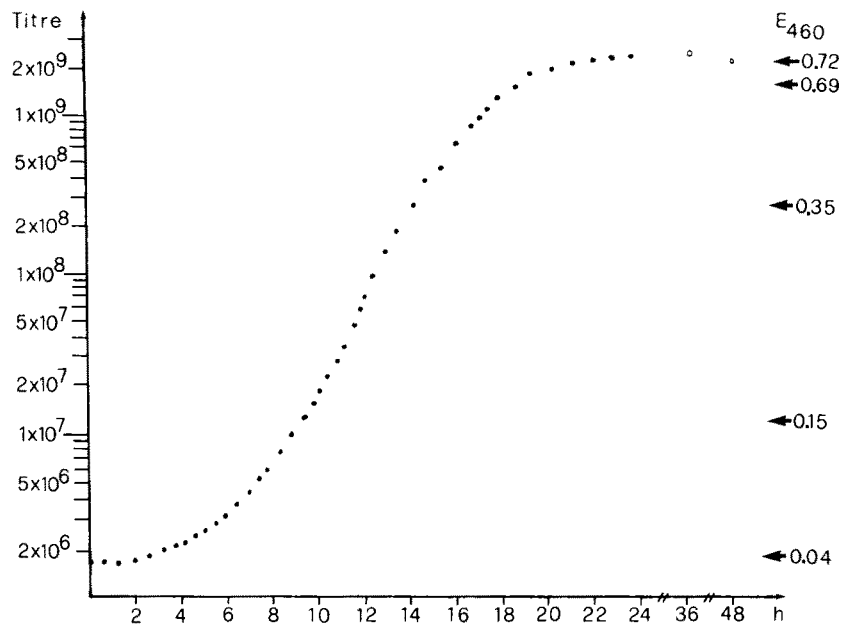

Fig. 1. Graph of bacterial growth: mean values from 7 independent suspension cultures of the pure strain belonging to the Micrococcaceae that was used throughout the series of experiments; in photometry, seawater was used as a reference. E_{460} : 0.04 = starting titre; samples were taken for biological tests at E_{460} : 0.150, 0.350, 0.690; stationary phase at E_{460} : 0.720

The efficiency of inducing attachment of the bacteria in relation to phase of growth and dose was also studied. In order to answer the question as to whether bacteria are responsible for initiating stolon attachment only in certain phases of growth (in relation to the start of the test), suspension cultures were inoculated with bacteria in 150 ml seawater in Boveri dishes and monitored for stolon attachment after different lengths of bacteria incubation.

Bacteria taken from the earliest phase of growth (E_{460} : 0.05) initiated an enhanced settlement of stolons in comparison to control only after 48 h (Table 2). The end plates of the stolons had not broadened and even after careful contact with a sterile needle they could be detached from the substrate. Moreover, the stolons were not taut between their attachment sites and the trunk of the polyp. This is an indication that settlement had taken place only a few hours before the second control was taken.

Bacteria that had been harvested a short time after inoculation (E_{460} : 0.1) were found to be slightly more effective in inducing the attachment reaction of the stolons. However, in this case too, only little resistance was encountered when attempting to detach stolons mechanically.

Table 2. *Aurelia aurita.* Number of attached stolons in relation to the phase of growth of the bacteria* added

Time (h)	O.D. Suspension culture (E_{460})	No. of stolons attached	free
24	0.05	5	35
48		24	16
24	0.1	21	19
48		30	10
24	0.2	35	5
48		37	3
24	0.3	38	2
48		39	1
24	0.4	38	2
48		38	2
24	0.5	37	3
48		38	2
24	0.6	38	2
48		39	1
24	0.69	39	1
48		40	0
24	0.72	22	18
48		26	14
24	ABS‡ without	0	40
48	bacteria	0	40

* Bacteria from 40 ml suspension culture; adapted to E_{460}: 0.250
** Values from 2 tests with 20 polyps each
‡ ABS: Seawater containing antibiotics

On the other hand, bacteria harvested after having entered the logarithmic phase (transition from lag to log. phase, E_{460}: 0.150), those that were in the logarithmic phase (E_{460}: 0.150 −0.690) and those that were on the verge of the stationary phase (transition from log. to stationary phase E_{460}: 0.690), on the other hand, all showed a high incidence of stolon settlement after only 24 h. In settled polyps the end plates of the stolons were invariably broadened. The stolons were stretched between their point of attachment and the base of the polyp. Mechanical detachment was not possible without tearing the stolons.

Bacteria which came from the stationary phase (E_{460}: 0.720) at the beginning of the test showed decreased effectiveness in comparison to experiments containing bacteria from the logarithmic growth phase. Bacteria from the phase of decay only led to sporadic settlement of stolons, the polyps showed signs of damage (shortening of tentacles, permanent contraction of the trunk of the polyp).

In order to investigate whether the reaction of stolon settlement depends on the quantity of bacteria, series of tests were made in which different concentrations of

Table 3. *Aurelia aurita*. Number of attached stolons in correlation with the added quantity of bacteria

Bacterial sediment from (ml) suspension culture	Bacteria early log. growth phase (E_{460}: 0.150)*				Intermediate log. growth phase (E_{460}: 0.350)*				Late log. growth phase (E_{460}: 0.690)*			
	No. of stolons**											
	24 h		48 h		24 h		48 h		24 h		48 h	
	attached	free	attached	free	attached	free	attached	free	attached	free	attached	free
0.01	6	74	17	63	9	71	18	62	11	69	20	60
0.025	16	64	50	30	18	62	52	28	17	63	49	31
0.05	21	59	58	22	19	61	57	23	20	60	68	12
0.1	23	57	60	20	24	56	63	17	19	61	65	15
0.25	31	49	66	14	29	51	70	10	27	53	71	9
0.5	36	44	65	15	39	41	72	8	34	46	73	7
1.00	58	22	71	9	67	13	74	6	71	9	76	4
1.75	72	8	73	7	66	14	74	6	74	6	78	2
3.5	70	10	74	6	75	5	79	1	73	7	78	2
7.	74	6	78	2	71	9	76	4	75	5	80	0
20.	71	9	77	3	73	7	78	2	75	5	77	3
50.	68	12	71	9	78	2	79	1	75	5	77	3
70.	72	8	77	3	69	11	75	5	74	6	75	5
100.	57	23	63	17†	47	33	51	29†	53	27	59	21†
150.	14	66	27	53†	17	63	18	62†	21	59	23	57†
200.	5	75	7	73††	11	69	11	69††	6	74	8	72††

* Titre of suspension culture adapted to E_{460}: 0.250
** Values from 4 experiments on 20 polyps each
† Shortened tentacles, contracted
†† Contracted, histolysis

bacteria were used. For this purpose, however, only bacteria from the most effective growth phase were used: transition from lag to log. E_{460} : 0.150; log. E_{460} : 0.350; transition from log. to stationary phase: E_{460} : 0.690. Sterilized seawater served as culture medium.

The number of settled polyps increased independently of the growth phases of the bacteria, but directly proportionally to the quantity of bacteria (Table 3). Only when bacterial pellets of more than 70 ml were added, did the percentage of settled stolons decline. At the highest concentrations attachment was observed occasionally, but the majority of the polyps were deformed (shortening of the tentacles, permanent contraction of the trunk). Sometimes the stolons had also shortened, with a thickening in the proximal portion. Histolysis was observed as early as 48 h in all experiments containing the highest quantities of bacteria.

The settlement of more than 50 % of the animals had taken place within 24 h in a considerable range of concentrations (sediment from 1–100 ml). There was no clear difference regarding the effectiveness of the bacteria from the 3 stages of logarithmic growth, from which samples were taken. The high percentage of settlement was not correlated with the quantity of bacteria, but it was observed in pellets harvested from 1 ml to 100 ml of suspension cultures. A turbidness of the culture medium on addition of larger pellets (from 20 ml to 50 ml from suspension cultures) did not necessarily lead to damage of the polyps.

Generally, the number of stolons attached to the substrate increased in experiments between 0–24 h. The rate of stolon attachment decreased between 24–48 h.

DISCUSSION

The settlement of pedal stolons of *Aurelia aurita* under laboratory conditions was initiated by a species of bacteria belonging to the family of Micrococcaceae. Under experimental conditions the attachment of stolons could only be evoked when live bacteria were added.

There were no apparent differences in ability to induce stolon attachment during the logarithmic phase of bacterial growth, whereas samples from bacteria immediately after the onset of the incubation or from the stationary phase had no effect on stolon attachment. The bacterial sediment from 0.1 ml suspension (adapted to E_{460} : 0.250) in 150 ml of culture medium was the minimal dose that initiated stolon attachment in more than 50 % of the animals after 24 h. The scyphistomae responded similarly to concentrations 50–70 times up to the minimal concentration, without any noticeable adverse effect. A correlation between an optimal settlement and a certain titre of bacteria could not be determined under the chosen conditions.

The observation that bacteria induce settlement of stolons of *Aurelia aurita* is similar to results concerning the behaviour of free-swimming planulae of marine coelenterates. Their attachment to the substrate is also bacteria-dependent. In these cases, however, the bacteria induce attachment to the substrate and then metamorphosis, whereas in *Aurelia aurita* the bacteria induced settlement and attachment to substrate of adult animals. The first example for the induction of settlement by exogenous bacterial factors was described in planulae of *Hydractinia echinata* (Müller, 1969, 1973a, b; Müller & Buchal, 1973). The metamorphosis of planulae and buds of *Cassiopea andromeda*

(Neumann, 1976; Müller et al., 1976; Hofmann et al., 1978; Neumann, 1979) is also induced by bacteria.

Bacterial induction of settlement in different species of coelenterates occurs at different times during the bacterial growth phase. *Vibrio* sp. induces settlement of planulae and buds of *Cassiopea andromeda* during the early log-phase (Hofmann et al., 1978; Neumann, 1979; Neumann et al., 1980). *Alteromonas* sp., which induces settlement and metamorphosis of planulae of *Hydractinia echinata*, exhibits its maximal effect in the stationary phase (Müller, 1969, 1973b).

In the cases described, the presence of specific bacteria could indicate that the substrate conditions are favourable for survival. The larvae then settle on the substrate and undergo metamorphosis in response to this information. The high specificity of reaction to certain bacteria can be explained by the fact that neither scyphistomae of *Cassiopea andromeda* nor hydroid polyps of *Hydractinia echinata* are capable of reattachment after the initial attachment. Hence, the choice of habitat is final.

The polyps of *Aurelia aurita*, however, are not inseparably bound to their habitat. They can still change their sites of settlement by means of the stolons during the scyphistoma stage. The stolons can settle at a distance from the polyp and can move the scyphistoma by contractions towards the site of attachment which is distinguished by the presence of certain bacteria.

This method of changing habitats by the polyps of *Aurelia aurita* appears to be very restrictive, since the length of the stolons is limited, whereas the highly mobile buds and planulae have a greater chance of finding a suitable substrate. However, the scyphistomae of *Aurelia aurita* can increase their opportunities for contact by a repeated change of site. In this way, the determination of an attachment site that depends on the presence of bacteria may be an advantage in polyps of *Aurelia aurita*, since the polyp may move repeatedly to new sites.

Details regarding the character of the substance to which the bacterial effect on stolon settlement in *Aurelia aurita* is due, will be presented in another communication.

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