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Symbiotic relations between bacteria and cephalopods

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INTRODUCTION

Chronic heterotrophic symbiosis is known from many invertebrate taxa, including cephalopods. The symbionts can be intracellular or extracellular, and entertain with the host parasitic, commensal or mutualistic relationships. The transmission from one generation to the other is either hereditary (vertical), or non-hereditary (horizontal), the infection occurring after birth.

In cephalopods, two types of symbiosis are now well documented: the sepiolid *Euprymna*/luminous bacterium *Vibrio fischeri* association, and the accessory nidamental glands/microbial community symbiosis.

(1) Many cephalopods are bioluminescent, and in some species of sepiolids and loliginids the light is emitted by extracellular symbiotic bacteria, which live in the crypt of a light organ located on the ink sac. In this case, the host provides an adequate environment for the heterotrophic bacterium, and the host derives luminescence from the bacteria. At birth, the sepiolid is aposymbiotic and acquires free-living *Vibrio fischeri* from the surrounding sea water within hours (WEI & YOUNG, 1989; MCFALL-NGAI & RUBY, 1991, 1998; MONTGOMERY & MCFALL-NGAI, 1994). Only some tissues of the light organ are influenced by the presence of *Vibrio*, which induce light organ morphogenesis (DOINO & MCFALL-NGAI, 1995).

(2) As far as the accessory nidamental glands (ANG) microbial community symbiosis is concerned, we know that these organs harbour a dense bacterial community, colourless in immature animals, which becomes red-orange in mature females (BLOODGOOD, 1977). Sequencing of bacterial 16S DNA has allowed to show that various bacteria species are present in the ANG of *Loligo pealei* (BARBIERI *et al.* 1996). ANG symbiotic bacteria are present in sepioids and in myopsid squids, but their role is not yet totally understood. The ANG are closely associated with nidamental glands and oviduct, and their role might be to coat the eggs with bacteria in charge of protective antimicrobial activity (BARBIERI *et al.*, 1997). In agreement with this suggestion, similarities in the microbial community of both ANG and egg cases were shown. As far as the transmission is concerned, bacterial colonization of ANG is thought to occur after hatching (LUM-KONG & HASTINGS, 1992; KAUFMAN *et al.*, 1998).

In this paper, we investigate the presence of symbi-

otic bacteria in different organs of the sepioid *Sepia officinalis*, the nautiloid *Nautilus macromphalus* and of the octopod *Eledone sp.* the sepioid *Sepia officinalis* is known to harbour symbiotic bacteria in the ANG, and Schipp *et al.* (1985) suggest the presence of bacteria in the pericardial appendages of *Nautilus*. In *Eledone* no symbiotic association is known so far.

MATERIAL AND METHODS

***Sepia officinalis*:** 8 adult specimens were collected in the English Channel (Luc-sur-mer). Various organs were obtained by aseptic dissection: the accessory nidamental gland (ANG); the nidamental gland, the anterior and posterior renal sac, the heart, the branchial heart, the gills, the digestive gland, the blood, the pericardial gland, the spermatophore.

***Nautilus macromphalus*:** 9 adult specimens were collected in New Caledonia. Various organs were analysed: the gills, the heart, the pericardial appendages, the digestive gland, the renal appendages.

***Eledone sp.*:** 3 adult specimens were collected in the Mediterranean (Banyuls-sur-mer). The organs analysed were the renal sac, the coelomic liquid, the branchial heart, the gills.

Samples for **PCR analysis** were stored in TE buffer pH8 (10 mM tris/HCl pH7.2, 1mM EDTA) at -20°C until use. Total DNA extraction was performed by classic phenol purification and ethanol precipitation after 10mg/ml Lysozyme and 10mg/ml Proteinase K digestion. DNA was resuspended in 50µl of TE and stored at -20°C.

The amplification primers are listed in table 1. We used the universal bacterium-specific 27F-1385R or UNIL-UNIR, which produced almost the entire 16S rRNA gene fragment and the eubacteria specific EUB-UNIL pair. Two other amplification primer pairs were used: the AMOR-AMOF pair specific for ammonifying bacteria (amplification of the gene coding for the ammonia-monooxygenase), and the NARGF-NARGR pair specific for denitrifying bacteria (amplification of the gene coding for the nitrate reductase).

PRIMER	SEQUENCE
27F	5'GAGTTTGATCCTGGCTCAG3'
1385R	5'GCCACACATGTTCCGGG3'
UNIR	5'ATGGTACCGTGTGACGGGCGGTGTA3'
UNIL	5'ATTCTAGAGTTTGATCATGGCTCA3'
EUB	5'GCTGCCTCCCGTAGGAGT3'
AMOR	5'AGACTCCGATCCGGACTACG3'
AMOF	5'TGGGGCATAACGCATCGAAAG3'
NARGF	5'TTACTTCAAACAGAAGGGTGAAACCTTT3'
NARGR	5'TTTCGCTTTATCGGCGTCTTCAATGAT3'

Tab. 1 - Amplification primers sequences.

PCR was conducted with dNTP, *Taq* polymerase and buffer (Eurogentec S.A.) in a Thermojet Thermocycler (EquiBio, Belgium) with a denaturing step at 94°C for 5 min, 32 cycles of 94°C (30 sec), 55°C (30sec) and 72°C (1 min) and a final elongation step of 72°C for 7 min. For amplification with NARGF-NARGR primers, Kloos *et al.* (1995) protocol was followed. Length of PCR products was controlled by electrophoresis in 1.5% agarose gel.

RESULTS AND DISCUSSION

Several organs were tested for bacterial symbiosis based on PCR amplification, in three species of cephalopods, *Sepia officinalis*, *Nautilus macromphalus*, *Eledone sp.*, using 4 different primer pairs. The results obtained are presented in table 2.

Table 2. Results of PCR amplifications of different bacterial strains from various organs of three cephalopods. *: positive results; **: highly positive results; -: negative results; nd: not done. ANG: accessory nidamental gland; Ant.RS: anterior renal sac; BH: branchial heart; Coel.Liq.: coelomic liquid; DG: digestive gland; NG nidamental gland; P.App: pericardial appendages; P.Gl: pericardial gland (branchial heart appendages); Post.RS: posterior renal sac; Renal App: renal appendages; Sperm: spermatophore.

(A) *Sepia officinalis*

Primers\ organs	Gills	BH	Heart	Blood	NG	ANG	Sperm	P.Gl	DG	Ant. RS	Post. RS
Universal bact.	*	-	*	-	-	**	-	-	-	*	**
EUB	*	-	*	-	-	**	-	-	-	**	**
bAMO	-	-	-	-	nd	-	nd	-	nd	-	*
NAR-G	nd	nd	nd	nd	nd	*	nd	-	nd	-	-

(B) *Nautilus macromphalus*

Primers\ organs	Gills	BH	Heart	P.App	DG	Renal App
Universal bact.	**	-	-	**	-	**
EUB	nd	-	-	**	-	**
bAMO	-	-	-	-	nd	*
NAR-G	nd	nd	nd	nd	nd	-

(C) *Eledone sp.*

Primers\ organs	Gills	BH	Coel.Liq	Renal Sac
Universal bact.	*	-	-	-
EUB	**	-	-	-
bAMO	*	-	nd	-
NAR-G	nd	nd	nd	nd

As expected the accessory nidamental glands of *Sepia officinalis* give highly positive results (universal bacteria, eubacteria, NAR-G). But also some other organs of this species appear to harbour dense bacteria populations, in particular the renal sacs (universal bacteria, eubacteria, bAMO).

The presence of symbiotic bacteria in the pericardial appendages of *Nautilus* was suggested by Schipp *et al.* (1985), based on electron micropscopy observations. This is confirmed here: the results obtained with universal bacteria and eubacteria specific primers are highly positive. In addition, this species also appears to have, like *Sepia*, bacteria associated to the renal appendages. In both species, the excretory organs harbour among eubacteria, ammonifying bacteria.

As far as *Eledone* is concerned, the only positive result concerns the gills and might be fortuitous. Preliminary results with in situ hybridization do not reveal the presence of bacteria in the gill tissues of *Eledone* (unpubl. res.). Accordingly, the positive result obtained with PCR is considered as doubtful. The origin of the bacteria present in the gills of the three taxa might be environmental, since these organs are in contact with external sea water.

Thus, bacterial symbiotic associations appear to concern several organs of *Sepia* and *Nautilus*, whereas no significant association could be identified, so far, in *Eledone*. This might be related to a different buoyant system. *Eledone* is a benthic species with no need for buoyancy. *Sepia* and *Nautilus* live in the sea water column and both have a chambered shell, either internal (*Sepia*) or external (*Nautilus*), playing an important part in the regulation of buoyancy by accumulating gas, mainly N₂, in the chambers. Due to these shelled species low excretion rates compared to non shelled ones, it was suggested that the origin of N₂ accumulation might be the result of protein catabolism end-product (NH₄⁺) recycling by symbiotic bacteria (BOUCHER-RODONI & MANGOLD, 1994). The results obtained here are in agreement with this hypothesis, since only the two shelled species *Sepia* and *Nautilus* appear to harbour symbiotic bacteria, including ammonifying or denitrifying strains. In parallel to in situ hybridization aimed to localize the bacteria within the tissues, the specific identification by 16S rRNA sequencing of the bacteria present in the various organs of *Sepia* and *Nautilus* is currently underway and should confirm if some of them could effectively be involved in the Nitrogen cycle.

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