

アルテミアの乾燥卵およびノープリウスの細菌相

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Short Paper

Microflora Associated with Eggs and Nauplii of *Artemia salina*

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Although brine shrimp *Artemia salina* is known as an important diet for larvae and juveniles of marine fish and crustacea, there are little studies on microflora of brine shrimp, except for Austin and Allen.¹⁾ It, however, is feasible that pathogenic bacteria invade those animals by feeding the brine shrimp on which the pathogen attached. In this situation, we investigated the microflora of eggs and nauplii of brine shrimp, reared under different conditions.

In this study, canned eggs (Shintoa Koeki) of brine shrimp imported from China were used. Each 1% (w/v) of dried eggs in sterile seawater was sonicated for 120 s at 50 W since a preliminary experiment showed that the viable count of sonicated eggs, for 60–120 s, was 5–11 times higher than that of unsonicated ones. On the other hand, the brine shrimp eggs were incubated at 25°C for 24 h in either the natural seawater or the sterile one. After incubation, hatched nauplii were individually washed in five changes of sterile seawater and then homogenized. Each sample thus prepared was serially diluted and plated onto 1/20 PYBG agar.²⁾ After incubation at 25°C for 7 days, 20 colonies were isolated at random from each plate and identified according to the previous paper.²⁾

Table 1 shows that Gram-positive bacteria such as *Micrococcus*, *Bacillus* and coryneforms were detected from the eggs and the nauplii hatched in sterile seawater, along with the rearing water. This result indicates that

the microflora associated with the nauplii derived from the eggs. However, all bacteria of the nauplii hatched in natural seawater and the rearing water consisted of Gram-negative bacteria including *Vibrio* and *Pseudomonas*. These results strongly suggest that the microflora of brine shrimp nauplii is significantly influenced by that of surrounding waters.

To certify the findings, bacterial flora in the brine shrimp which was hatched in each bacterial suspension (10^0 – 10^6 CFU/ml) of three strains of *Vibrio* spp. and one of *Pseudomonas* sp. isolated earlier were examined. As shown in Table 2, these bacteria associated with the nauplii were present in high percentage in the results similar to that of rearing water when the suspensions of these strains were higher in concentration.

Although we did not examine the pathogenicity of the *Vibrio* spp. and *Pseudomonas* sp. examined, the present results indicate the possibility that pathogenic bacteria could be introduced into the aquatic animals via the brine shrimp nauplii which was hatched in the tank water contaminated with pathogenic bacteria. Therefore, the hatchery should be careful about the sanitation of tank water of the brine shrimp.

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References

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Table 1. Generic composition of bacteria associated with eggs and nauplii reared in the natural and sterile seawater, along with the rearing water

Genus	Eggs* ¹	Natural seawater		Sterile seawater	
		Nauplii* ²	Water* ³	Nauplii* ²	Water* ³
<i>Vibrio</i>	nd* ⁴	2.72	6.77	nd	nd
<i>Pseudomonas</i>	nd	2.39	6.48	nd	nd
<i>Moraxella</i>	nd	1.22	nd	nd	nd
<i>Micrococcus</i>	7.12	nd	nd	2.60	6.44
<i>Staphylococcus</i>	3.71	nd	nd	nd	nd
<i>Streptococcus</i>	nd	nd	nd	0.47	nd
<i>Bacillus</i>	3.94	nd	nd	2.36	6.58
Coryneforms	2.37	nd	nd	2.05	4.19

*¹ Mean viable count (log CFU/g) (n=3).

*² Mean viable count (log CFU/nauplius) (n=3).

*³ Mean viable count (log CFU/ml) (n=3).

*⁴ Not detected.

Table 2. Percentage of Gram-negative rods associated with the nauplii hatched in different concentrations of bacteria, along with the rearing water

Bacteria	Sample	Concentration (CFU/ml)		
		10^0	10^3	10^6
<i>Vibrio</i> sp. MAI 4	Nauplii	60*	55	88
	Water	95	100	95
<i>Vibrio</i> sp. MAI 5	Nauplii	5	98	100
	Water	5	100	100
<i>Vibrio</i> sp. MAI 9	Nauplii	53	68	75
	Water	60	88	95
<i>Pseudomonas</i> sp. MAI 1	Nauplii	60	68	95
	Water	83	88	90

* Mean value (n=2).

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