

## Integrated Defensive Strategy against Microbial Infection in Honeybee Colonies

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The atmosphere inside a honeybee colony is usually warm and humid with abundant food (honey and pollen) that provides ideal conditions for microorganisms. However, the actual intracolony environment is kept clean. We hypothesized that the inside of a honeybee colony, which is often called a 'super organism', is analogous to inside the body of an ordinary insect. Honeybees seem to have evolved integrated defensive systems that exploit exogenous materials such as propolis, and endogenous substances such as antibacterial peptides and vaporous compounds of various origins.

### Strategy 1: Individual Level

Antibacterial activities were induced in the hemolymph of individual honeybees injected with viable *E. coli* (Casteels *et al.* 1989). We speculated that larvae and pupae have poorer inductive antibacterial activity than adults because they are nursed at the centre of the colony under aseptic conditions. However, antibacterial activities were induced in the hemolymph not only of adults but also of larvae and pupae (Fig. 1). No difference was found in the inductive ability among aging workers or

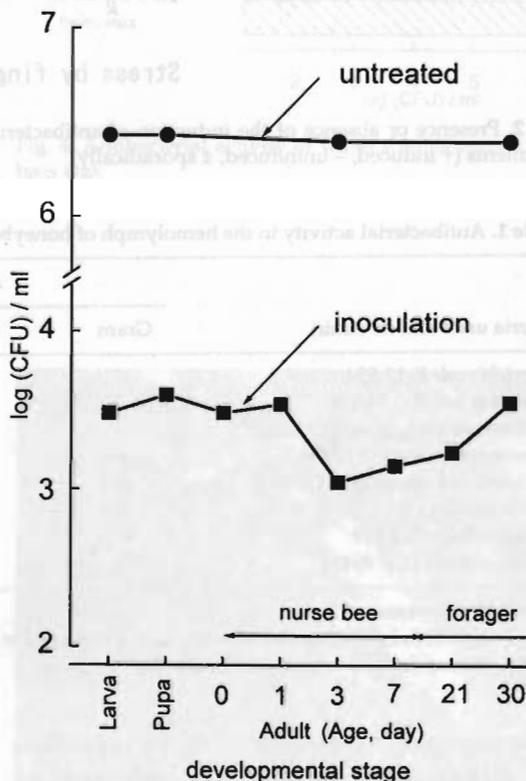


Fig. 1. Changes in antibacterial activity with the developmental stages and adult aging [inoculation: hemolymph (100  $\mu$ l/20 bees) was diluted to 25%; untreated: not diluted]

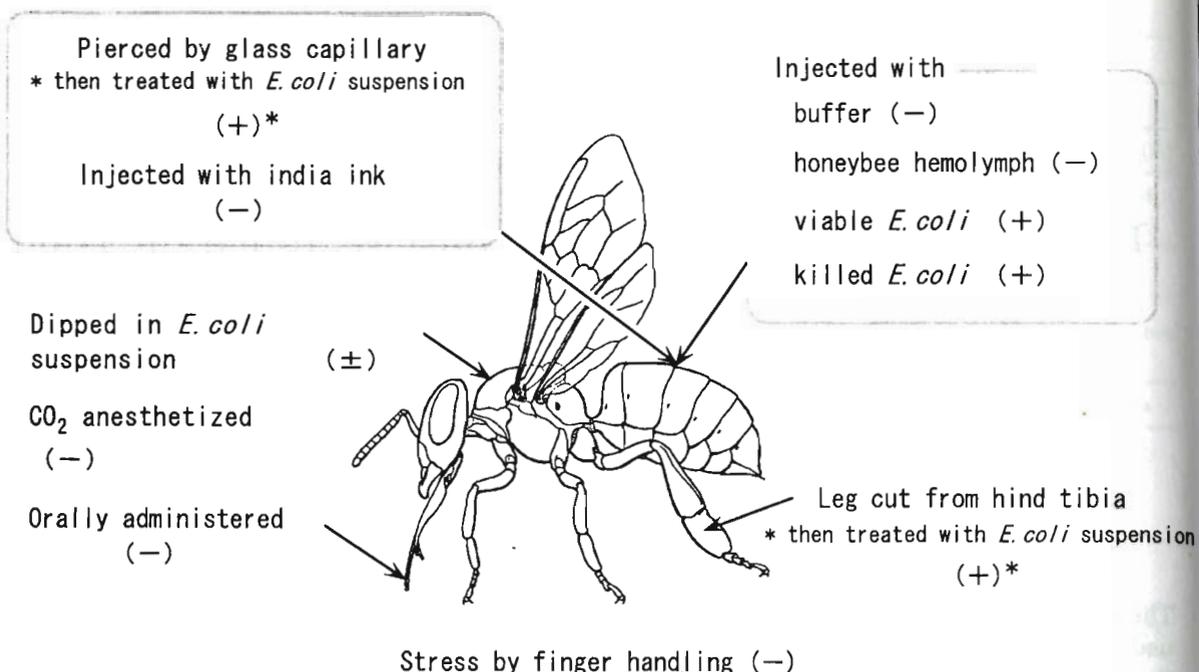


Fig. 2. Presence or absence of the induction of antibacterial activity in the hemolymph of honeybees received various treatments (+ induced, - uninduced, ± sporadically)

Table 1. Antibacterial activity in the hemolymph of honeybees injected with viable *E. coli* K-12

Bacteria used and its strain	<i>A. mellifera</i>			<i>A. cerana japonica</i>	
	Gram	untreated	inoculation	untreated	inoculation
<i>Escherichia coli</i> K-12 594	-	0.0	17.7	0.0	17.7
<i>Escherichia coli</i> IFO 12734	-	-	14.4	-	17.8
<i>Pseudomonas aeruginosa</i> IFO 12689*	-	-	8.0	-	8.0
<i>Micrococcus luteus</i> IFO 12708	+	-	17.2	-	15.9
<i>Staphylococcus aureus</i> IFO 12732	+	-	17.6	-	14.0
<i>Bacillus subtilis</i>	+	0.0	9.8	0.0	-
<i>Bacillus subtilis</i> PCI 219	+	-	0.0	-	8.0
<i>Bacillus larvae</i> ATCC 9545T*	+	-	0.0	-	0.0

\* Honeybee diseases.

Hemolymph (5.5 µl / bee) of 100 µl was put in a cup (inside diameter 6 mm) and cultured at 30 °C for 24 h. Inhibitory zone (mm) was measured with slide calipers.

those engaged in different work. To reproduce the situation of honeybees infected by microorganisms in natural conditions, no activity was induced by oral administration of *E. coli* but

was occasionally induced by external application (Fig. 2). Antibacterial activity is not shown against *Bacillus larvae*, which is a pathogen of American Foul Brood (Table 1).

### Strategy 2: Antimicrobial Measures

To make inside the colony aseptic, honeybees seemed to use antibacterial principles in beeswax. We speculated that antibacterial activity was not due to propolis because activity was found even in *Apis mellifera* colonies confined in a cage separate from their beeswax, and in *A. cerana* colonies that never use propolis (Fig. 3). However, antibacterial activity was found in water-soluble extract of virgin wax scales (Fig. 3) that had just been secreted from the abdomen and not yet mixed with saliva (Fig. 4). Development of stronger antibacterial activity with processing (Fig. 5) suggests that the active substance originates from saliva or exogenous propolis. Antibacterial activity of water-soluble extract from beeswax showed up against *Bacillus larvae*, which is a pathogen of American Foul Brood (Table 2). Also, water-soluble extract of cell

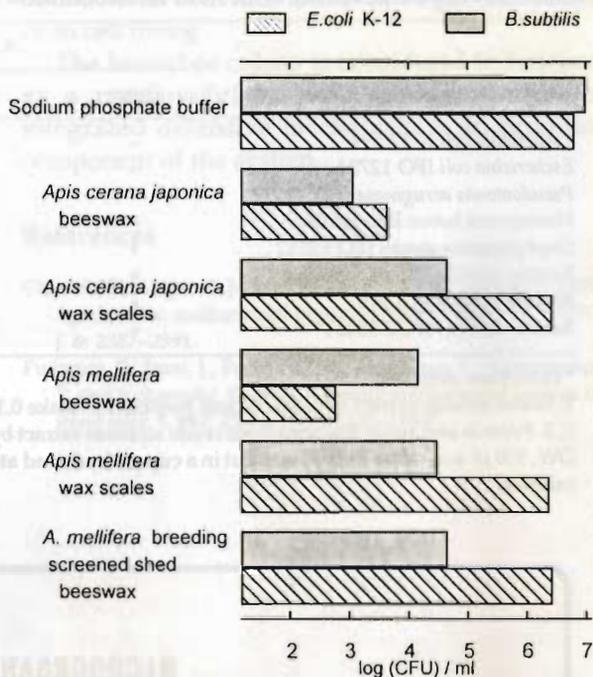


Fig. 4. Antibacterial activity of water soluble extract from bees wax



Fig. 3. Wax scales



Fig. 5. Beewax processed by honeybees



lining also had antibacterial activity that might protect the pupae from exogenous infection.

### **Strategy 3. Integrated Defense to Microorganisms**

Anti-microorganismic measures in honeybee colonies are summarised in Fig. 6. As already mentioned, the larvae and pupae have similar host defense mechanisms to that of the adult. The external secretion of 'royalycin' (Fujiwara *et al.* 1990) could be analogous to internal phenomenon if the colony was regarded as an individual organism. The same can be said for

antibacterial activities found in virgin wax scales or in cell lining.

The honeybee colony is considered to function as a purposeful 'superorganism', and the integrated defensive mechanism is an essential component of the system.

### **References**

- Casteels, P., Ampe, C., Jacobs, F., Vaeck, M. and Tempst, P. 1989. Apidaecins: antibacterial peptides from honeybees. *EMBO J.* **8**: 2387-2391.
- Fujiwara, S., Imai, J., Fujiwara, M., Yaeshima, T., Kawashima, T. and Kobayashi, K. 1990. A potent antibacterial protein in royal jelly. *J. Biol. Chem.* **265**: 11333-11337.