# Septicemia Occurrence in Cocoons as Related to Silkworm Rearing Conditions

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The control of silkworm diseases at the larval stages has been emphasized so far, whereas the disease occurrence in cocoons has rather been neglected. However, silkworms which died of diseases and putrefied in cocoons contaminate the inside of the cocoons, causing deterioration of cocoon filament quality. As the occurrence of such contaminated cocoons has increased widely, the problem of silkworms died in cocoons has suddenly come to be regarded very important. Silkworms infected with diseases at the larval stage are almost unable to produce cocoons. Therefore, dead silkworms in cocoons seem to occur at a limited condition: infection when the cocooning is finished and then disease occurrence.

Septicemia is regarded a disease which causes infection, death, and putrefaction in cocoons within an extremely short time. A series of experiments were carried out to clarify the problem, i.e., by what route the pathogenic bacteria of septicemia can enter into the cocoons and cause the disease[1,2,3]?

## Bacteria causing septicemia in cocoons

It is known that septic bacteria (which cause septicemia of silkworm) isolated from flachrie silk-

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**Table 1. Septic bacteria isolated from dead silkworms in cocoons**

| | Rearing season | | |
|---|---|---|---|
| | Spring | Summer | Autumn |
| *Serratia marcescence* | 25.0% | 72.2% | 80.8% |
| *Pseudomonas* sp. | 50.0 | 6.9 | 5.2 |
| *Proteus* sp. | 11.4 | 11.1 | 3.5 |
| *Aeromonas* sp. | 0 | 0 | 0 |
| *Streptococcus faecalis* | 11.4 | 9.7 | 10.5 |
| *Bacillus* sp. | 2.3 | 0 | 0 |
| Numbers of species | 44 | 72 | 114 |

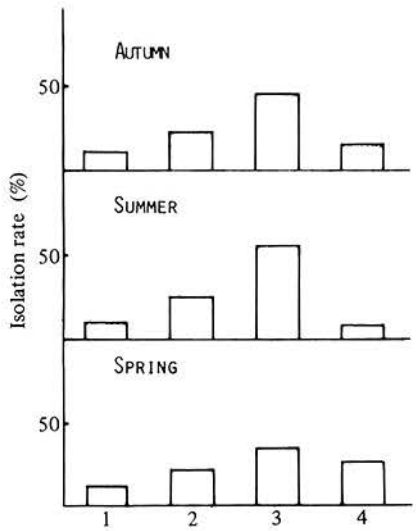



Fig. 1. Isolation of virus and septic bacteria from dead silkworms in cocoons shown in percent isolation
1: Nothing isolated
2: Only septic bacteria isolated
3: Both septic bacteria and virus isolated
4: Only virus isolated

worms are *Serratia*, *Pseudomonas*, *Aeromonas*, *Proteus*, *Streptococcus*, *Bacillus*, etc.[4,5].

Dead silkworms in cocoons occurred in the Ibaraki Prefecture in 1981 were collected, and virus detection (the ES method) and isolation of septic bacteria were carried out[3] (Table 1).

Virus was isolated from about 60% of the dead silkworms examined, and the septic bacteria were isolated from 60–80% of the samples. The septic bacteria were isolated from 70–80% of the silkworms infected with the virus. It suggests the mixed infection of virus and septic bacteria (Fig. 1). Of the septic bacteria isolated, *Serratia* was most abundant. It was isolated more in the summer and autumn silkworm rearing season.

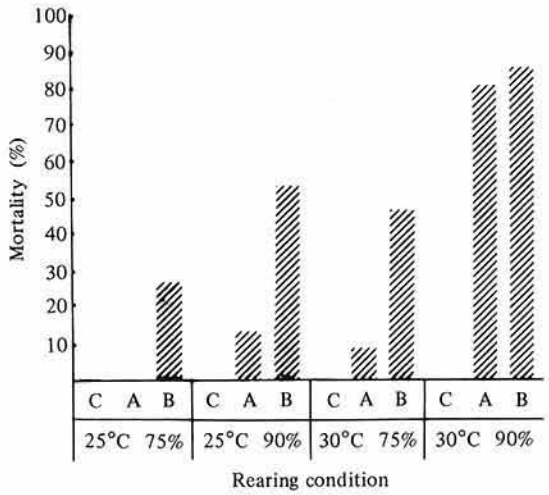



Fig. 2. Mortality of silkworm larvae by inoculation of *Serratia*
C: Non inoculation (control)
A: Oral inoculation of $10^7$/m*l*
B: Oral inoculation of $10^9$/m*l*

## Infection of septic bacteria to silkworms

The pathogenicity of these septic bacteria was confirmed by the occurrence of septicemia, when the bacteria was injected into the silkworm hemolymph. Of these septic bacteria, *Serratia* showed the highest pathogenicity: Injection of $6–8\times10^2$ bacteria caused a death within about 18 hr. The pathogenicity of *Streptococcus* and *Bacillus* was lower than other bacteria[5].

The pathogenicity of *Serratia* orally inoculated showed a high mortality at high humidity (Fig. 2), although the mortality varied with the amount of the bacteria added to feed.

## Silkworm rearing environment and behavior of bacteria

### *1) Temperature and humidity of rearing room*

When the temperature and humidity in the rearing room were kept at 25.5°C and 72.5% (R.H.), the temperature at the rearing beds (above and below the nets) was a little lower than the room temperature. On the other hand, the humidity above and below the nets was as high as about 90%, showing almost no variation during the daytime (Table 2).

### *2) Survival of Serratia in silkworm rearing vessels*

Mulberry leaves, to which *Serratia* suspension had been smeared, was supplied to feed the 5th instar larvae in silkworm rearing vessels. Remaining leaves were taken out after 4, 5, and 6 days for

**Table 2. Temperature and humidity conditions at rearing beds**

| | | Above the net* | | Below the net | |
|---|---|---|---|---|---|
| | Time | 10 : 00 | 17 : 00 | 10 : 00 | 17 : 00 |
| Temperature (°C) | A,B,C** | 24 | 24 | 23 | 23 |
| Humidity (%) | A | 90.0 | 81.0 | 88.5 | 89.5 |
| | B | 91.0 | 84.0 | 92.5 | 91.0 |
| | C | 89.0 | 82.5 | 89.5 | 83.0 |

* Bed-cleaning net
** A,B and C indicate both ends and the central part of a rearing bed, respectively.
Room condition was kept at 25.5 °C and 72.5% humidty.

examination. The number of *Serratia* as well as the total number of bacteria were increased under the humid condition (Fig. 3).

*3) Movement of bacteria on rearing beds*

Mulberry leaves, to which bacterial suspension of *Serratia* or *Streptococcus* had been spread, were placed on a site of rearing bed in each vessel. To these vessels, clean mulberry leaves were supplied two times a day. In one group of the vessel, silkworms were reared, while the other group was left without silkworms. After 4 days, distribution of the bacteria on the remaining leaves of each day was examined. The result showed that both *Serratia* and *Streptococcus* hardly diffused on the rearing bed left without silkworms, whereas in the vessels with silkworms both bacteria apparently showed wide spreading over the rearing bed (Fig. 4).

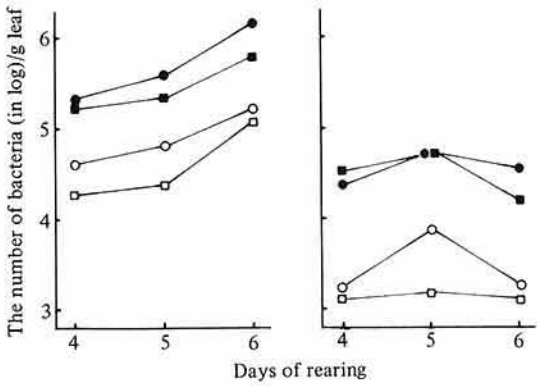



Fig. 3. The number of bacteria detected on mulberry leaves which were taken out from the rearing beds on which *Serratia*-inoculated silkworms were reared

Left: All bacteria, Right: *Serratia*

Rearing condition: ● 25°C 90% humidity
■ 30°C 90% humidity
○ 25°C 75% humidity
□ 30°C 70% humidity

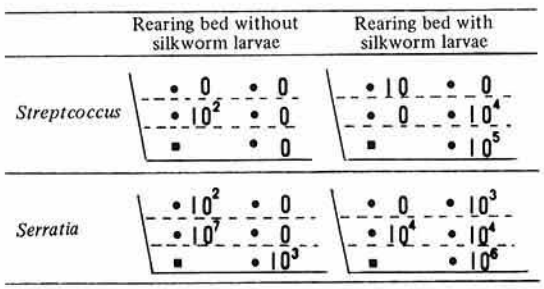



Fig. 4. The spread of bacteria over the rearing beds caused by growing silkworm larvae

● Sites for collecting bacteria
■ Sites where bacterial inoculation was made
— Silkworm rearing vessel
···· Bed cleaning net
Numerals: The number of bacteria/g mulberry leaf at each site.

*4) Entering of Serratia into cocoons and changes in the amount of bacteria inside cocoons*

The suspension of *Serratia* was smeared on body surface of silkworms 3–7 days after the ecdysis to the 5th instar, and after the cocooning-pupation, the number of the exuvium carrying *Serratia* was examined. The shorter the period from the application of the bacteria to the cocooning-pupation, the greater was the number, and this tendency showed no difference due to rearing temperature and humidity (Table 3). However, the total number of the bacteria in the cocoon shells was larger at higher humidity. High humidity favored the survival and movement of bacteria.

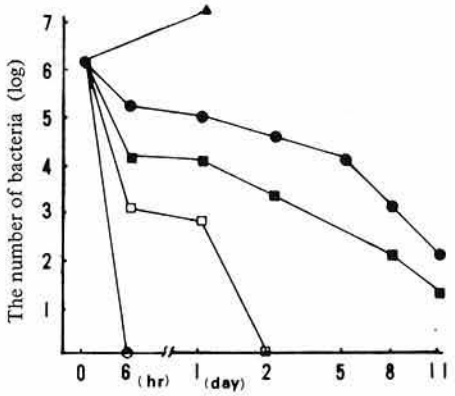
**Table 3. The number of exuvia carrying *Serratia***

| Days after ecdysis | Rearing condition (Temp. and R.H.) | | | |
|---|---|---|---|---|
| | 25 °C, 75% | | 28 °C, 90% | |
| | a) | b) | a) | b) |
| 3 | 40 | 20 | 20 | 20 |
| 4 | 50 | 40 | 40 | 20 |
| 5 | 50 | 40 | 40 | 20 |
| 6 | 70 | 60 | 50 | 60 |
| 7 | 100 | 90 | 100 | 100 |

Numerals in the table show the exuvia contaminated with *Serratia* in percentage of the exuvia examined (10 exuvia).
a), b): Replication

## Hemorrhage caused by a shock of falling and infection with *Serratia*

Pupae within 12 hr after pupation, and pupae at 2 days after pupation were subjected to the shock




▲—▲ Number of *Serratia* survived in 0.5% ag.NaCl solution
●—● Number of *Serratia* survived in humid condition
○—○ Number of *Serratia* survived in dry condition
■—■ Number of *Streptococcus* survived in humid condition
□—□ Number of *Streptococcus* survived in dry condition

Fig. 5. Resistibility of bacteria to drought

of falling. With the former, the falling from a height of 5 cm caused hemorrhage, while the latter bled by a 10 cm falling. The bleeding occurred from the head in both cases. When the *Serratia* suspension was applied to those bled pupae, the most of them died of septicemia.

## Dry condition and survival of bacteria

Suspension of *Serratia* or *Streptococcus* was applied to filter paper disks (8 mm in diameter) to be adsorbed by the disks. Then the disks were stored either in dry or humid condition. Both bacteria survived even for 11 days under humid condition, whereas under dry condition *Serratia* died after 6 hr, and *Streptococcus* after 2 days (Fig. 5). Thus, *Serratia* is apparently susceptible to dry condition.

## Discussion

From 60–80% of the dead silkworms in cocoons, which occurred in the Ibaraki Prefecture, septic bacteria were isolated, and it was made clear that silkworms died of septicemia are the cause of spoiled cocoons (with spoiled inside).

As to the infection route of septic bacteria into cocoons, a possibility considered was that the bacteria attached to larvae before cocooning-pupation were brought into cocoons with the larvae, and caused the infection after the cocooning, because silkworms in cocoons have no contact with the outside of the cocoons.

When *Serratia*, one of the septic bacteria, was smeared on the body surface of the 5th instar larvae before cocooning, *Serratia* was isolated from the exuvium in the cocoons. This fact furnishes a strong evidence that the bacteria existing in cocoons are the bacteria which adhered to the body surface of larvae and were introduced into cocoons with the larvae.

How long can the bacteria survive when they adhered on the body epidermis of larvae? It seems that no nutrient which can be used by the adhered bacteria is present on the epidermis. Since the relative humidity at the silkworm rearing beds was 90%, the survival period of the bacteria on the epidermis can be estimated (as far as separation of the bacteria from the epidermis is not considered) from that of the bacteria adsorbed on filterpaper disks under humid condition in Petri dishes. It was 11 days. As the period of the 5th instar is 7 days, it is highly possible that the bacteria adhered to larvae even at an early stage of the 5th instar were introduced into cocoons.

When *Serratia* was smeared to a site of each rearing bed, one with silkworm rearing and the other without silkworm rearing, the bacteria spread far widely over the rearing bed with silkworms, as compared with that without silkworms. This result indicates that silkworms themselves help bacterial contamination of rearing beds and the bacteria multiplied on the beds contaminate silkworms in turn.

From these experimental results it was made clear that when the rearing beds at the 5th instar stage are contaminated with the bacteria body surface of silkworms growing there was also contaminated, and the bacteria are brought into cocoons.

Septicemia of silkworms in cocoons is caused by septic bacteria introduced into cocoons by silkworms. In this case, the bacteria separated from the body surface of silkworms adhered to the inside of cocoonshells and the bacteria on exuvium serve as the infection source. These bacteria are known to easily multiply under humid condition[3]. Therefore, even though only a small amount was

introduced into cocoons, the bacteria may surely increase to the amount enough for infection. Under such a condition contaminated with the bacteria, injured or bleeding pupae are easily infected with the bacteria. Particularly, the bleeding is apt to occur even with a slight shock at the stage soon after pupation giving a high risk of septicemia infection.

Thus, it was made clear that the occurrence of silkworm septicemia is closely related to high humidity conditions. Particularly, septicemia occurring in cocoons is related to three important factors: the extent of contamination of silkworm body at the time of mounting with the pathogenic bacteria, humidity control after mounting, and handling of cocoons produced.

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