On the Isolation and Cultivation of Intracellular Symbiotes of *Oryzaephilus surinamensis* L. (Cucujidae: Coleoptera)

Association of intracellular bacterium-like microorganisms with *Oryzaephilus surinamensis* L. was first discovered and studied by PIERANTONI[1]. Later KOCH[2] worked out their morphology, development and mode of transmission from one generation to the succeeding one. He proved that they are passed on to the progeny by means of ovariole infection in the early stages of development of egg in the female beetle. PANT and FRAENKEL[3] extended the studies to include the functional aspects of the symbiosis and concluded that the symbiotes may be supplying some of the accessory growth factors.

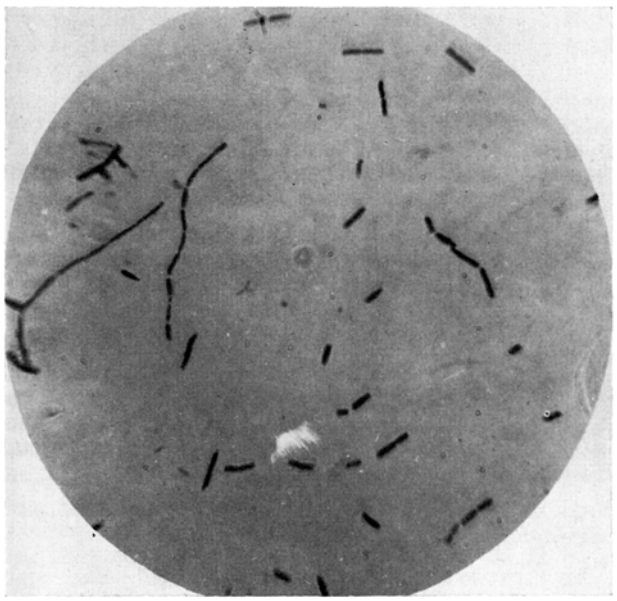

Fig. 2. – Photomicrograph of cultivated symbiotes of *O. surinamnesis*.

Some attempts are reported to have been made to cultivate the symbiotes outside the body of the insect in artificial media, but they were never successful[3]. We have now been able to cultivate successfully the bacterium-like symbiotes of *Oryzaephilus surinamensis* on lactosenutrient broth consisting of the following ingredients:

Beef extract	3·0 g
Peptone	5·0 g
Lactose	5·0 g
Distilled water	1000·0 ml
pH adjusted to 7–7·2	

To this medium was added the inoculum containing symbiotes obtained from eggs. The eggs were externally sterilized after the manner described by BEGG and SANG[4]. The sterilizing fluid was made by mixing together mercuric chloride 0·5 g, sodium chloride 6·5 g, hydrochloric acid 1·25 ml, absolute alcohol 500 ml and water to make 1000 ml. The eggs were treated in this fluid for 20 min followed by 35 min exposure to sterile 70% ethanol and then finally washed in sterile water for at least 5 min. In this way egg-surface could be sterilized without any ill effect on the eggs. A sterility test was always run by inoculating sterilized whole eggs as such in the nutrient medium and incubating at 37°C for 24 h after which an absence of contamination indicated the successful surface sterilization. Another batch of similarly treated eggs was macerated in sterile water and the suspension was transferred aseptically into the culture medium. After 24 h of incubation at 37°C, the culture medium was full of growing symbiotes of similar type as that found in the egg-smear (Fig. 1). The cultivated symbiotes are 2–4 μ × 2–3 μ in size and gram negative (Fig. 2). After 30 h of incubation, they start forming long chains.

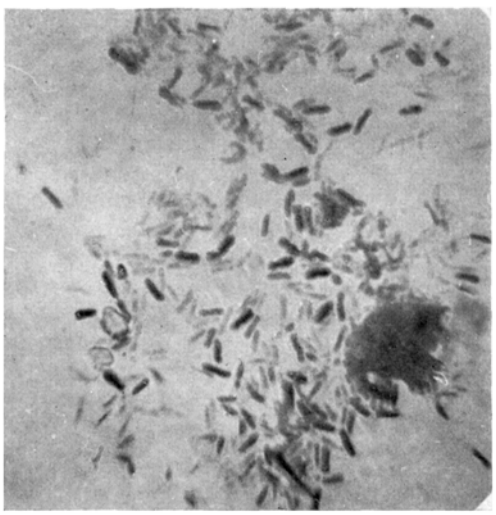

Fig. 1. – Photomicrograph of egg smear of *O. surinamensis* showing bacterium like symbiotes.

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N. C. PANT*, J. K. NAYAR, and Miss P. GUPTA

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Zusammenfassung

Bakterienähnliche Symbionten von Eiern der *O. surinamensis* sind auf künstlichem Laktose-Nährboden mit Erfolg gezüchtet worden. Die Zuchtformen sehen gleich aus wie die Symbionten der Eier.

[1] U. PIERANTONI, R. C. Accad. Lincei *9*, 451 (1929); Atti Accad. Sci. Fis. Mat. Napoli *18*, 1 (1930).
[2] A. KOCH, Z. Morph. Ökol. Tiere *23*, 389 (1931); *32*, 137 (1936).
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[4] M. BEGG and J. H. SANG, Science *112*, 11 (1950).

* Present address: Division of Entomology, Indian Agricultural Research Institute, New Delhi 12, India.