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Ecological Studies on Pathogen of Melon Hairy Root

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Abstract
Several factors related to the growth and survival of pathogen of melon hairy root, *A. rhizogenes* were examined. The concerned bacteria could increase in number in sterilized soils at 20 and 30°C, and survive for a long time in naturally infested soils. The bacteria grew in soils at pH 4-8, and the optimum growth took place in the range of pH 6-8. The bacteria could grow when they infected sterilized soils with a density of 10^6 cells/g of dry soil with a water content of 20-100% of water holding capacity. However, in case where 10^3 cells/g of dry soils were infected, the bacteria died after the incubation for 7 days at a water content of 20% of the maximum water holding capacity. The bacteria did not change in number when they infected sterilized soils, while they decreased gradually in the non-sterilized soils, when their density was at the level of 10^8 cells/g of dry soil. Their density eventually reached a level of 10^4 cells/g of dry soil after the incubation for 250 days at 30°C.

Discipline: Plant diseases
Additional key words: *Agrobacterium rhizogenes*, melon, soil-borne disease

Introduction

An outbreak of hairy root disease of muskmelon cultivated under a greenhouse condition was observed in 1987, which disease was the first incidence on that crop in Japan. The causal bacteria isolated from the diseased roots and soils were identified as *Agrobacterium rhizogenes* biovar 1 after the comprehensive analyses on their pathological, physiological and biochemical properties[1,4].

Only limited data were then made available on pathological aspects of that disease, however, and on its ecology in particular. It was therefore of urgent necessity for its effective control to provide information on the critical factors affecting the growth and survival of the pathogen of the disease. The present paper accounts for the results of a series of the experiments pertaining to the behavior of the pathogen of melon hairy root in soils.

Effects of temperature on the growth of the pathogenic bacteria

A comparison of the growth of the bacteria infected to the sterilized soils (Andosols) was made among the various temperatures, under which they were exposed. For the purpose of detecting crown gall bacteria, *Agrobacterium tumefaciens*, similar to melon hairy root bacteria from soils, several selective media were employed[2]. Among those media, a DIM medium was chosen for the present study to isolate *agrobacteria* containing the melon hairy root bacteria from soils. This choice was made mainly on the basis of selectivity and colony characters[5].

The bacteria infected to the sterilized soils with a density of 10^4 cells/g of dry soil could increase in number under the temperatures of 20 and 30°C, and reached the level of 10^7 cells/g of dry soil in 3 to 5 days after the incubation, while they declined to zero, or the undeterminable level, after the

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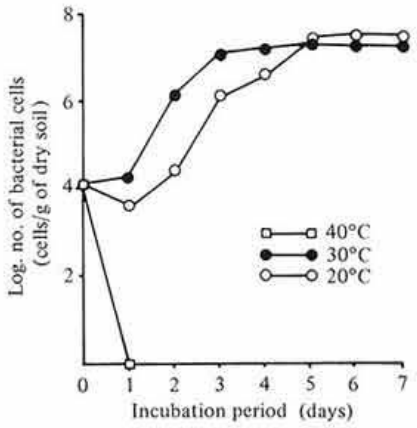

Fig. 1. Growth of *A. rhizogenes* in sterilized soils under various temperatures

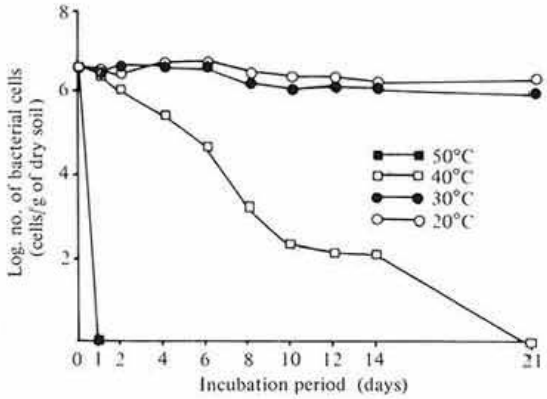

Fig. 2. Survival of *A. rhizogenes* in naturally infested soils under various temperatures

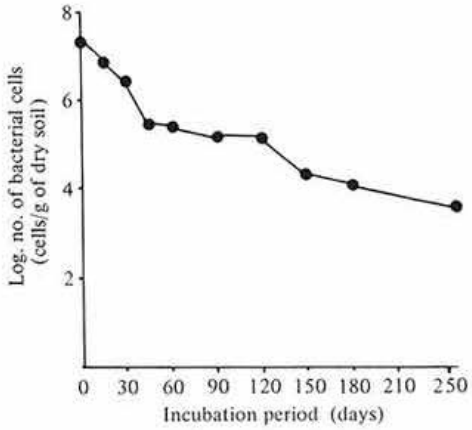

Fig. 3. Survival of *Agrobacteria* in naturally infested soils at 30°C

incubation for 1 day at 40°C (Fig. 1).

The *agrobacteria* did not show any change in number during the period of 21 days, when the field soils naturally infested were stored at 20 and 30°C. The number of the bacteria decreased rapidly to the undeterminable level in 21 days after storage at 40°C, and in 1 day at 50°C (Fig. 2).

The naturally infested field soils stored under the temperature of 30°C were subjected to investigations in regard to the pathogenicity of the *agrobacteria* isolated in the soils. The pathogenicity was examined with needle prick inoculation to tomato seedlings. The infested soils contained the *agrobacteria* at the level of 10^7 cells/g of dry soil at the initial stage. The *agrobacteria* gradually decreased in number to the level of 10^6 cells/g of dry soil in 30 days after the storage, 10^4 cells in 150 days, and 9×10^3 cells in 250 days (Fig. 3). It was recognized that a majority of the *agrobacteria* isolated from the soils were still pathogenic even after they were stored for 250 days at 30°C.

Effects of pH on the growth of the pathogenic bacteria

Effects of pH degree on the growth of the bacteria in soils were examined at 30°C. The artificially infested soils contained the bacteria at a level of 10^4 cells/g of dry soil. Those bacteria increased in number, and reached the level of 10^9 cells in 3 to 4 days after incubation in the soils of pH 7 and 8, 10^8 cells in 4 to 5 days in the soils of pH 6, and 10^7 cells in 4 to 5 days in the soils of pH 4 and 5 (Fig. 4).

Effects of water content on the growth of the pathogenic bacteria

In order to investigate the effects of water content on the growth of the bacteria, the following five levels of water contents were examined: 20, 40, 60, 80 and 100% of the maximum water holding capacity with dried soils of Andosols.

In the artificially infested soils containing the bacteria at the level of 10^6 cells/g of dry soil, they increased when the water contents were 40% or higher, and reached the level of 10^8 cells in 3 to 4 days after incubation under the temperature of 30°C. Under the condition of 20% water content, the bacteria decreased rapidly to the level of 10^{3-4} cells/g of

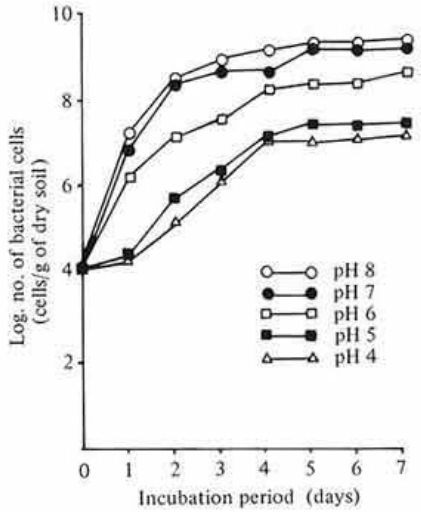

Fig. 4. Growth of *A. rhizogenes* in sterilized soils under various pH conditions at 30°C

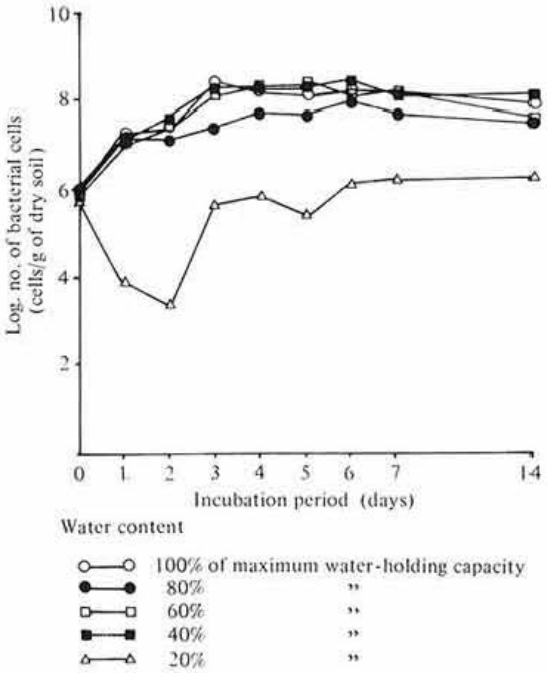

Fig. 5. Growth of *A. rhizogenes* in sterilized soils under various water contents at 30°C

dry soil in 1 to 2 days after incubation, followed by the restoration to the original level of cell number in 3 days with a slight increase in 6 days and beyond (Fig. 5).

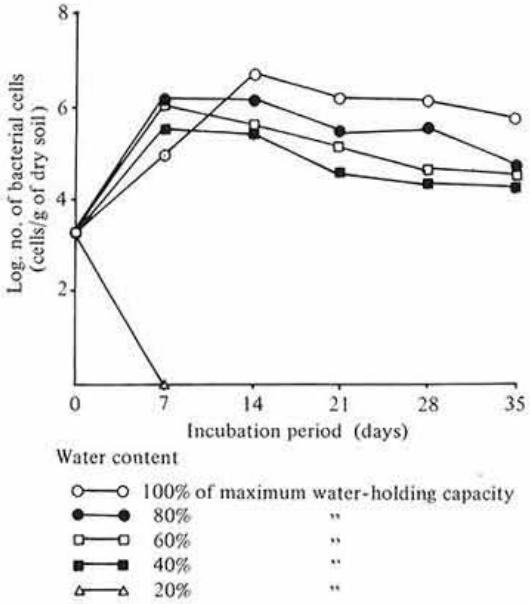

Fig. 6. Survival of *A. rhizogenes* in sterilized soils under various water contents at 30°C

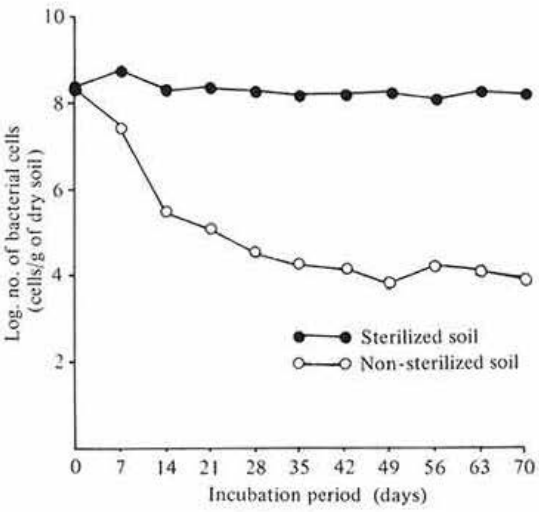

Fig. 7. Survival of *agrobacteria* in sterilized and non-sterilized soils under 30°C

In case where the soils were artificially infested with a bacterial density of 10^3 cells/g of dry soil, the bacteria increased in number under the water content of 40% and higher of the maximum water-holding capacity, and reached the level of 10^6 cells in 7–14 days after incubation. The number of the bacteria gradually decreased to the level of 10^5 cells

in 35 days. When the water content was 20%, however, the bacterial density decreased rapidly to the undeterminable level in 7 days after incubation (Fig. 6).

Effects of soil microorganisms on the growth and survival of the pathogenic bacteria

The implication of the presence of microorganisms in soils for the growth and survival of the pathogenic bacteria was examined in the soils with sterilized and non-sterilized treatments. The soils were maintained at water content of 60% under the temperature of 30°C. The soils used for this experiment contained a concentration of bacteria with a density of 37×10^6, actinomycetes with 40×10^5, and fungus with 64×10^3 cells/g of dry soil, respectively. These microorganisms were isolated with the albumine agar and rose bengal media method. At the beginning of incubation, *agrobacteria* had a concentration of 23×10^3 cells/g of dry soil, which was counted through the DIM medium.

Number of the bacteria infected in the sterilized soil was constant for 70 days after incubation. However, the *agrobacteria* in the non-sterilized soil gradually decreased in number, and reached the level of 10^4 cells/g of dry soil in 35–42 days after incubation (Fig. 7).

Conclusions

The above results of the present study indicate that the pathogenic bacteria of hairy root grow well in the sterilized soils in the absence of a host plant and survive for more than 250 days in the naturally infested field soils. This fact suggests that the naturally infected soils themselves be one of the major infection sources of this disease. In addition, the other survey showed that the bacteria grew in irrigation water as well as in suspension of various kinds of soils[3]. Muskmelon plants are intensively cultivated for commercial use about 4 times a year in a greenhouse. In so doing, soil sterilization is vital for controlling the other soil-borne diseases such as fusarium wilt and gummy stem blight. It is therefore important to prevent the pathogen survived in the infested plants and soils from coming in the sterilized field soils in the course of cultural operations.

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