Production of Disease-Resistant Tissue Culture Seedlings Using Endophytic Actinomycetes©

Hitoshi Kunoh, Tomio Nishimura, Sachiko Hasegawa, Akane Meguro, and Tadao Fujimori
Akatsuka Orchid Co. Ltd., 1868-3 Takanoo-cho, Tsu, Mie 514-2293, Japan

In the mid 1990s several researchers found that a number of actinomycetes inhabit a wide range of plants as either symbionts or parasites. Since then, endophytic actinomycetes have been attractive sources of novel antibiotics and growth regulators of other organisms. Actinomycetes, especially *Streptomyces* species, isolated from the rhizosphere have proven to be excellent biocontrol agents of soilborne plant pathogens. Such an effective activity is largely dependent on secondary metabolites produced by these organisms.

These earlier reports led us to assume that if a useful endophytic actinomycete isolated from a field-grown plant can successfully colonize tissue-cultured seedlings of the plant, the seedlings could become resistant to various plant pathogens. Because tissue culture flasks are usually axenic, such a novel technique should allow this actinomycete to colonize easily its host plant without competition and/or antagonism by any other microbes.

Twenty-five strains of endophytic actinomycetes were isolated from roots, stems, and leaves of field-grown rhododendron. One strain, R-5, that was identified as *S. galbus* was selected as a candidate strain. This strain does not adversely affect growth of the rhododendron seedlings, has a broad antimicrobial spectrum, grows actively on the multiplication medium for tissue culture of rhododendron, and produces two major antibiotics—actinomycin X_2 and fungichromin.

When mycelial suspension of R-5 was spread on the multiplication medium in a glass flask with rhododendron seedlings, mycelia of R-5 grew, sporulated to form powdery colonies on the medium surface within 2–3 days, and colonized inside the plants within 7 days. The seedling on the multiplication medium that was previously treated with R-5 were shown to be resistant to diseases caused by *Pestalotiopsis sydowiana* and *Phytophthora cinnamomi*, major air-borne and soilborne diseases of rhododendron, respectively. Promising protective effects were obtained when R-5 was applied in the tissue culture flasks or directly into soil.

First we assumed that antibiotics produced by R-5 might induce resistance in the seedlings. However, morphological, biochemical, and molecular studies revealed that resistance induced by inhabiting R-5 (acceleration of papilla formation, phytoalexin production, and signal transduction through the jasmonate pathway) might be more directly associated with the disease resistance.

Our in vivo test proved that R-5 was an excellent biocontrol agent to produce disease-resistant seedlings of flowering pot plants for practical purposes. This technique was patented in Japan [P3629212 ('04.12.07)] and U.S.A. [US 6,544,511 B2 ('03.04.08)].