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学 位 論 文 要 旨

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題目: Isolation and evaluation of salt tolerant strains induced by ethyl methanesulfonate in ectomycorrhizal fungus *Rhizopogon roseolus*
（Ethyl methanesulfonateによって誘導された外生菌根菌ショウロの耐塩性菌株系統の分離と評価に関する研究）

Rhizopogon roseolus (Corda) Th. M. Fr. (*R. rubescens* Tul. & Tul.), a hypogeous basidiomycete also known as “shoro” in Japan, is an important ectomycorrhizal symbiont of Pinaceae. Recent studies suggest that ectomycorrhizal fungi play an important role in the protection of host roots from environmental stressors such as drought, heavy metals and excessive salt. However, no mutagenesis study on the selection of halophilic ectomycorrhizal strains of *R. roseolus* has been performed. Moreover, there is no study on characterizing their salt tolerance in soil and on their specificity against stress responses such as drought and high temperature.

Here, to induce mutations, I treated two types of specimen, basidiospores, and homokaryotic mycelial fragments of *R. roseolus*, with ethyl methanesulfonate (EMS), which is a widely used chemical mutagen, and subsequently evaluated salt-tolerance on 300 mM salt agar plates. Among the strains recovered from EMS-treated basidiospores, I isolated halophilic strains that showed a more vigorous mycelial growth in medium containing 300 mM NaCl, compared to that of the strains derived from untreated basidiospores. The salt-sensitive strains could also provide useful characteristics for subsequent research in which the resistant mechanism against salt stress will be revealed. EMS treatment expanded the variations in salt tolerance ability of these strains derived from homokaryotic mycelial fragments of *R. roseolus*. By crossbreeding system, the heterokaryotic hybrid strains showed a higher tolerance and stability in NaCl stress than homokaryotic ones. Hybrids strains which were crossed with halophilic mutants showed a higher relative growth in medium containing 300 mM NaCl.

I used wild sandy soil to investigate the growth of *R. roseolus* strains in soil, and comparatively analyzed the correlation of mycelial growth between in soil substrate and one agar medium. All the strains grew well in sandy soil without a host plant and on the surface of the soil, several hyphae aggregated to form a mycelial colony. The quantitative assay revealed that, a significant positive correlation ($p < 0.05$) had been observed between the mycelial growth of *R. roseolus* in soil substrate and on agar medium. In saline culture, the mycelial growth in 100% seawater salinity soil were positive correlation ($p < 0.05$) with the mycelial growth on 100% seawater salinity agar medium. These results suggest that mycelium of *R. roseolus* seems more sensitive with salt in soil than on agar, and sandy soil is useful to characterize salt tolerant ability of *R.*

roseolus in soil substrate.

Finally, to investigate specificity against stress response, I have examined effect of drought stress and heat stress on salt halophilic strain of *R. roseoluse*. I used three types of solutions to adjust the water stress, seawater, glycerol containing water or polyethylene glycol (PEG) 6000 containing water to evaluate the stress tolerant of *R. roseolus* against three environments factors, viz., ion stress, non-ion osmotic stress and drought stress. As the results, in same water potential, the strains in salt and glycerol exhibited a completely opposite pattern of growth. While the strain that was tolerant salt stress was sensitive to glycerol, the salt sensitive strain H9 exhibited a vigorous mycelial growth in glycerol treatment than other strains. Almost strains of *R. roseolus* exhibited a vigorous mycelial growth on PEG-treated plates than control. Moreover, all *R. roseolus* strains contributed to promote drought tolerance ability of seedlings of *Pinus thunbergii*. While 66.7% of seedlings without inoculation became yellow, 100% of the seedlings inoculated with hybrid strains H1, H7 and H9 still kept in green. In salt stress, 50% of the seedlings inoculated with halophilic strain H1 still kept the green leaves. In contrast, 100% of the seedlings inoculated with salt-sensitive strain H9 have changed to yellowing. Yellowing was also observed in all of the seedlings without inoculation. When mycelium of *R. roseolus* were treated with high temperature at 38 °C for 3 days, the highest relative growth rate (77%) was observed in the strain H1, indicating that strain H1 is tolerant against not only salt stress but also high temperature stress.

These results in this study suggest that the combination of EMS mutant and the crossbreeding system could be an effective method to obtain halophilic strains of *R. roseolus*. The strains showing non-specific stress tolerant would be obtained by evaluating its potential in sandy soil, drought, temperature, and inoculation into host plants. Hybrid strain H1 recovered in this study considered to possess a stable halophilic ability, vigorous mycelia growth, and non-specific tolerant characteristics against drought and high temperature. Furthermore, the strain H1 will be expected as useful bio-fertilizer that could support *P. thunbergii* adapting to various natural environments.