

Inhibitory Effects of Sodium Chloride on Induction of Tissue Cultures of Lichens of *Ramalina* Species

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Abstract

Tissue cultures of six lichens of *Ramalina* species were induced on malt–yeast extract medium supplemented with various concentrations of sodium chloride. The growth of mycobionts and photobionts from thallus fragments inoculated was affected by the concentrations of NaCl. Inhibitory effects of NaCl on the growth of tissue cultures were not different between maritime and non-maritime species of tested *Ramalina* lichens.

Keywords: algae, fungus, lichen, *Ramalina*, sodium chloride, tissue culture.

Abbreviation

NaCl, sodium chloride.

Lichens are symbiotic associations of fungal (mycobiont) and algal (photobiont) partners and can grow in extreme environments such as seashores. Therefore, it is expected that they have stress tolerant properties such as salt tolerance. Symbionts can be separated and cultured from the lichen thallus and their salt tolerance properties have been studied. Ramkær (1978) and Takahagi *et al.* (2000) investigated effects of salinity on spore germination and hyphal penetration and indicated that discharged spores of many tested species germinated and their hyphae grew at 0.6 M NaCl. Watanabe *et al.* (1997) and Yamamoto *et al.* (2001) tested salt tolerance of cultured photobionts and mycobionts separately isolated from lichen thalli, respectively. The relationship between symbionts is expected to exist in salt tolerance, however, it is difficult to produce differentiated state in vitro culture. We previously established the method of induction of undifferentiated state (tissue culture) composed of fungal and algal symbionts from thallus fragments

(Yamamoto *et al.*, 1985) and also investigated the effects of environmental factors such as culture temperature and light on induction of tissue cultures (Yamamoto *et al.*, 1987). The influence of salinity under the undifferentiated state such as tissue culture in which fungal and algal symbionts of lichens existed has not been investigated. In the present paper, we studied the effect of NaCl concentration on induction of tissue cultures of lichens of *Ramalina* species and compared salt tolerant property between symbionts of tested species.

Ramalina species are fruticose lichens and are widespread all over the world. *R. crassa*, *R. litoralis* and *R. subbreviscula* grow on the rock at southern seashore of Japan and on the other hand, *R. calicaris* var. *japonica*, *R. sinensis* and *R. yasudae* grow on the rock or bark at inland of Japan. Specimens of *R. crassa* and *R. litoralis* were collected at Kushimoto seashore, Wakayama Pref., Japan. That of *R. subbreviscula* (no. HM95082408) was collected at Kamui peninsula, Hokkaido Pref., Japan. Those of *R. calicaris* var. *japonica* and *R. sinensis* were collected at the Tokyo University Forest in Hokkaido, Hokkaido Pref., Japan. That of *R. yasudae* was collected at Takeo shrine, Saga

Pref. Japan. After the collection, the specimens were stored at -20°C for 1 month and voucher specimens were deposited in the herbarium of Hyogo University of Teacher Education, Hyogo, Japan.

A fragment (*ca.* 1 cm in length) was cut off from the tip of a thallus of each specimen. According to the Yamamoto's method (Yamamoto *et al.*, 1985), each thallus fragment was homogenized in a mortar with sterilized water, and small fragments of 150 to 500 μm in size were selected by a two-filter system. One fragment composed of mycobiont and photobiont was picked up and inoculated onto an agar-plate of 5 ml malt-yeast extract medium (Ahmadjian, 1961) in the test tube, each of forty-nine fragments was inoculated as the same manner

and cultured at 15°C in the dark for 26 weeks. Projection of the filamentous mycobiont hyphae and green photobiont cells from the explants in each test tube were examined every week after inoculation under the dissecting microscope and each number of test tubes in which mycobiont or photobiont cells grew colonized was counted. Influence of the light for microscope observation was negligible. Colony formation rates (CFR = the number of test tubes with colony formation of mycobiont or photobiont $\times 100$ /the number of uncontaminated test tubes) were calculated.

Mycobiont and photobiont cells were induced to culture from explants of all of tested *Ramalina* species. Fig. 1 shows the inhibition effects of NaCl concentration on induction of growth of mycobiont

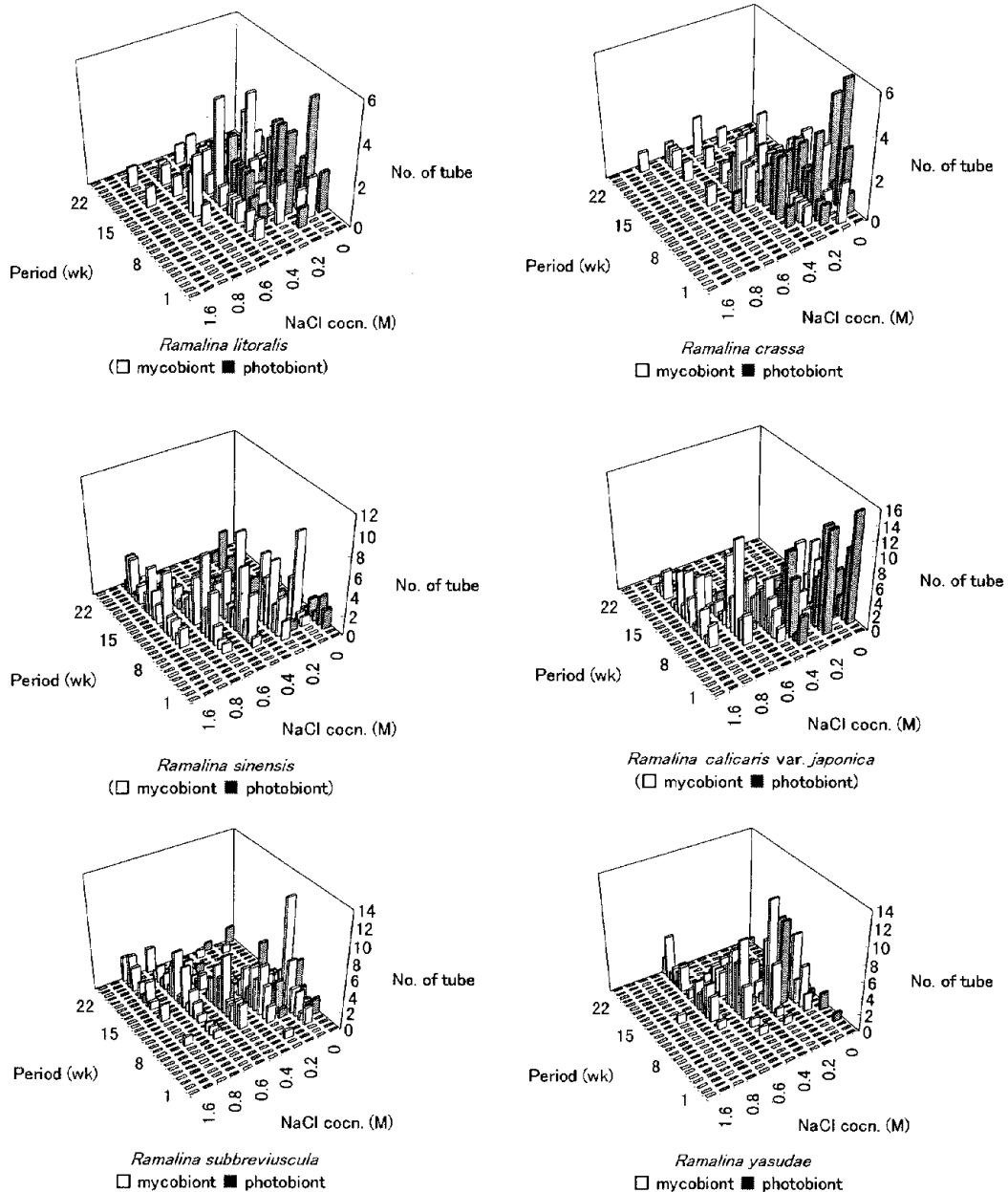


Fig. 1 Effects of NaCl concentration on induction of growth of mycobiont and photobiont of *Ramalina litoralis*, *R. crassa*, *R. subbreviscula*, *R. yasudae*, *R. sinensis* and *R. calicaris var. japonica* on malt-yeast extract medium at 15°C without light for 26 weeks.

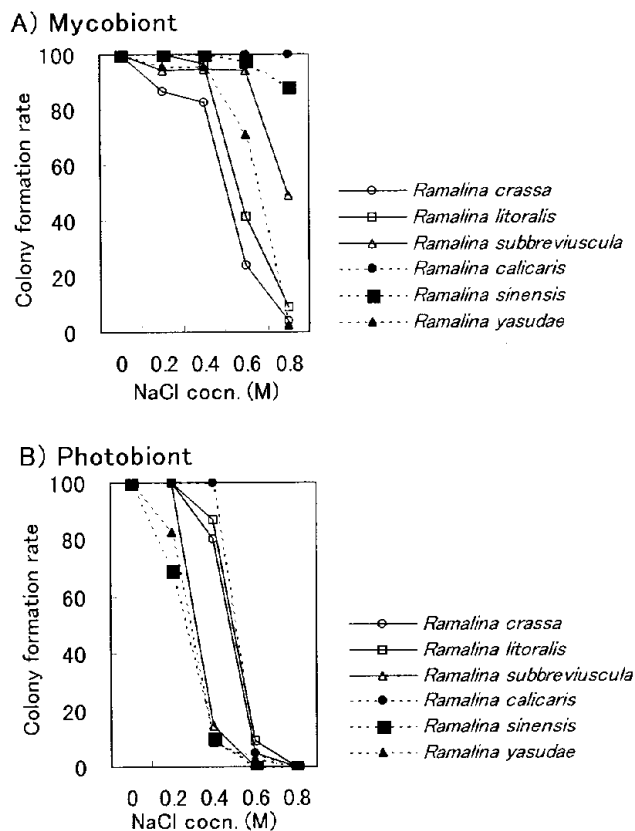


Fig. 2 Inhibition Effects of NaCl concentration on colony formation rates of mycobiont (A) and photobiont (B) of *Ramalina* species cultured on malt-yeast extract medium at 15 °C without light for 26 weeks.

and photobiont cells of lichen species. Photobiont cells of most tested species started to grow earlier than mycobiont hyphae, however they could not grow at higher concentrations of NaCl where mycobiont cells grew. Thus, mycobionts of *Ramalina* species indicate higher tolerance to NaCl than their photobionts.

All *Ramalina* mycobionts grew from thallus fragments of tested species even at 0.8 M NaCl. Judged from **Fig. 2A**, the mycobiont of *R. calicaris* var. *japonica* showed highest salt tolerance among them, since it showed 100% of CFR even at 0.8 M NaCl. Furthermore, there may be not difference on salt tolerant property of mycobionts between non-maritime (*R. calicaris* var. *japonica*, *R. sinensis* and *R. yasudae*) and maritime species (*R. crassa*, *R. litoralis* and *R. subbreviscula*). Takahagi *et al.* (2000) and Yamamoto *et al.* (2001) reported that effect of NaCl concentration on spore germination and mycobiont growth of tested species of lichens didn't depend on how far from seashores the lichens grew, respectively. Our result was coincided with the observations of Takahagi *et al.* and Yamamoto *et al.*

There has been no report on salt tolerance of

Ramalina photobionts. All photobionts except *R. sinensis* stated to grow at 0.6 M or lower concentrations of NaCl. As shown in **Fig. 2B**, photobionts of *Ramalina* species as well as mycobionts show a wide range of salt tolerant property. Among them, the photobiont of *R. calicaris* var. *japonica* showed best growth even at 0.4 M NaCl (**Fig. 2B**). Salt tolerant tendency of tested mycobionts was not related to that of corresponding photobionts. Watanabe *et al.* (1997) examined the salt tolerance property in photobionts from only marine and maritime lichens, but didn't do inland species and they showed that all photobionts from tested species grew well in the medium supplemented with ca. 0.5 M NaCl. However, we proved that photobionts as well as mycobionts have no difference on salt tolerant property between inland and seashore species. This indicates that cultured mycobionts and photobionts shows different salt tolerant properties from those in the natural condition (in the lichenized state).

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