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

Toshikazu TAKAHAGI¹, Yoshikazu YAMAMOTO²*, Yasuhiro KINOSHITA³,
Shunji TAKESHITA¹ and Takuzo YAMADA¹

¹ Department of Science Education, Hyogo University of Teacher Education, Yashiro,
Hyogo 673-1415, Japan
² Department of Biological Production, Faculty of Bioresource Sciences, Akita Prefectural University,
Shimoshinjo-nakano, Akita 010-0195, Japan
³ Basic Research Department, Nippon Paint Co., Ltd., 4-1-15, Minamishinagawa, Shinagawa,
Tokyo 140-8675, Japan
⁴ Faculty of Education, Hiroshima University, 1-1-1, Kagamiyama, Higashi-Hiroshima,
Hiroshima 739-8524, Japan

*Corresponding author E-mail address: yyamamoto@akita-pu.ac.jp

Received 19 July 2001; accepted 19 November 2001

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. subbreviuclea 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. subbreviuclea (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 X 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](image-url)

**Fig. 1** Effects of NaCl concentration on induction of growth of mycobiont and photobiont of *Ramalina litoralis*, *R. crassa*, *R. subbrevissula*, *R. yasudae*, *R. sinensis* and *R. calicaris* var. *japonica* 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 no 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. littoralis* and *R. subbrevisculla*). 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).

Acknowledgements

We thank Prof. Isao Yoshimura of Kochi Gakuen College for identification of lichens and his useful comments in our research and also thank Dr. Hiromi Miyawaki of Saga University and Dr. Hideo Matsubara of Nippon Paint Co., Ltd. for collecting *Ramalina* species.

References


