

Production improvement of nori aquaculture using biostimulants

Mahiko ABE*^{1, †}, Chizuru TARA*², Shinya FUJIKI*², Shusaku KAWASAKI*³,
and Noboru MURASE*¹

Abstract: Nori aquaculture is one of the most significant fisheries industries in Japan. However, the production of dried nori sheets has continuously been decreasing since 2000's due to high water temperature in an early aquaculture period (Oct. to Nov.), oligotrophication throughout the season and predation by herbivorous fish and birds. The genetic diversity of nori strains used for aquaculture is low, making it difficult to breed new beneficial strains against the climate change. So, we focused on “biostimulants,” which have been known to give various plants some tolerance to abiotic stresses. In a series of our experiments, amino acids were evaluated as biostimulants for nori culture. We conducted three experiments based on the scheme of land plants using biostimulants: 1) screening of amino acids that are effective for the growth and survival of axenic nori protoplasts, 2) laboratory culture experiments in the presence of bacteria, and 3) field aquaculture trials. Moreover, prior to the field trials, we confirmed the effectiveness of a method of soaking the conchospores in tank seawater where each amino acid was dissolved to avoid using large amounts of amino acids when they are directly added into the area of aquaculture practice. As a result of the screening test, nori protoplasts treated with arginine or ornithine showed rapid growth. Under the condition of bacteria's existence, these two amino acids enhanced the adherence strength of nori thalli just by once soaking for 15 h. In the field trials, soaking conchospores in arginine or ornithine for 24 h enhanced not only the adherence strength but also the growth of nori blades, which increased the production of nori sheets by 1.1 to 1.3 times. We clarified that arginine and ornithine are the useful biostimulants in nori aquaculture, especially for enhancing the adherence strength and growth in an early aquaculture period and increasing the nori production.

Key words: adherence strength, amino acids, high water temperature, *Pyropia yezoensis*, soaking treatment

Introduction

High water temperature caused by the global climate change has influenced the nori aquaculture in an early period of cultivation in Japan (October to November), and oligotrophication occurred in a late aquaculture period (January to March) also affected the productibility. Recently, high water temperature and oligotrophication have become the most serious problems throughout the aquaculture period, while the predation by herbivorous fish and birds is becoming

another great issue in nori aquaculture.

Breeding has been carrying out for the improvement of production and settlement of some environment-induced troubles occurred in nori aquaculture for a long time. However, the genetic diversity of nori strains used for aquaculture is low, making it difficult to breed new beneficial strains against the climate change. It is necessary to find or develop novel techniques for stable production of nori. So, we focused on “biostimulants” (BS), which have been known to give various plants some tolerance to abiotic stresses. European

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*1 National Fisheries University, Japan Fisheries Research and Education Agency, Shimonoseki, Yamaguchi 759-6595, Japan

*2 AJINOMOTO CO. INC., Chuo, Tokyo 104-8315, Japan

*3 Hyogo Nori Institute, Hyogo Prefectural Federation of Fisheries Co-operative Associations, Akashi, Hyogo 674-0093, Japan

† Email: abemahi at fish-u.ac.jp

Biostimulants Industry Council (EBIC) defined plant BS as “a product which stimulates plant nutrition processes independently of the product’s nutrient content, with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: nutrient use efficiency, tolerance to abiotic stress, quality traits, or the availability of confined nutrients in soil rhizosphere.” Moreover, EBIC also described as “biostimulant components include microorganisms, plant and algae extracts, amino acids, humic substances, mineral salts and some chemicals with biostimulant properties. Unlike fertilizers, which directly supply nutrients to plants, biostimulants stimulate the plant’s own processes to better utilize nutrients and water” (<https://biostimulants.eu/plant-biostimulants/>).

Fig.1 shows a conceptual diagram of the harvest yield in land plants. The potential yield, which is 100%, could decrease to 20% due to various biotic and abiotic stresses in the process of growth from seedling to mature plant (Yamauchi and Kawai 2020). Application of pesticide and fertilizer, for example, can increase the yield to 40%, and further use of BS can increase the yield by 1.2 times. There are plenty of BS materials for land plants, e.g., seaweed extracts, polyphenols, humic substances, amino acids, bacteria and so on as above mentioned. For application of BS to macro algae, there are several studies reported recently (Abe *et al.* 2021a, 2021b, 2022; Han *et al.* 2022; Hurtado and Critchley 2018; Umanzor *et al.* 2019, 2020, 2022). Land plants could continuously be supplied with BS materials in an economical way at the right timing during the cultivation. On the other hand, for seaweed aquaculture, we have to consider how to use BS effectively due to disperse of BS when they are applied to the coastal area of nori farm. In this paper, we introduce a novel efficient method for using BS in nori aquaculture.

Short Materials and Methods

Experimental procedure to use BS for nori aquaculture

In the case of experiments on land plants, the effects of BS are evaluated by three steps. The first step is screening of candidate materials under an axenic culture condition. The second step is effect confirmation of BS selected in the first step under the presence of bacteria. The third step is the final effect confirmation of BS in a field trial. We carried out our experiments following these procedures.

1. Screening of beneficial amino acids

We used 18 amino acids as candidate BS and carried out the screening of beneficial amino acids (1 mM) using axenic protoplasts of nori *Neopyropia yezoensis* (*Pyropia yezoensis*, Abe *et al.* 2021a, 2021b).

2. Effect of soaking conchospores in closed amino acid-dissolved seawater

To use BS efficiently, it was thought that the artificial BS treatment should be conducted in a closed system on land rather than their application in coastal nori farms. In the practical nori aquaculture, there is a process of soaking the net with nori seedlings being attached using land apparatus for 5 to 15 h in approximately 5 ton tank filled with clear seawater to enhance the adherence of conchospores. In this context, we tested the effect of soaking the net in tank seawater with amino acid being dissolved (Abe *et al.* 2022). Vinylon yarns with conchospores were soaked in seawater containing arginine (Arg) or ornithine (Orn) (1 mM) for 10 min, 1 h and 15 h. Then, we carried out an ordinal laboratory culture for about 2 months. We measured the adherence strength of young nori thalli to the nori nets in each treatment time.

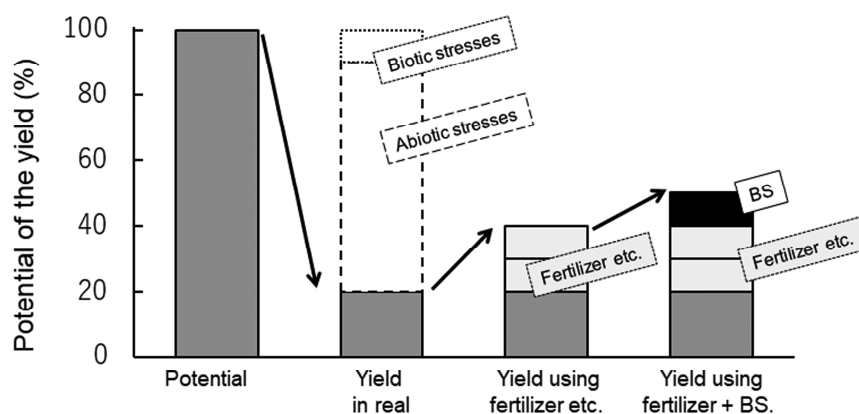


Fig.1 A conceptual diagram of the harvest yield in land plants. This figure was redrawn by the authors referencing that in Yamauchi and Kawai (2020)

3. Field culture trial

We conducted field culture trials over three years. After seedling by a common method on land, nori nets with conchospores being attached were soaked in seawater containing Arg or Orn (1mM) for 24 h. Then, nori farmers carried out an ordinal nori aquaculture in their own farms until harvest.

Results and Discussion

1. Amino acids beneficial to axenic nori protoplast

Fig.2 shows the results of survival rate (a) and thallus area (b) of axenic nori protoplasts cultured in a medium supplemented with each amino acid. Survival rate of nori protoplasts without

amino acids (Control) was $57.1 \pm 5.7\%$. The rates treated with each amino acid were at levels similar to or less than that of Control. On the other hand, thallus area of Control was $1237.5 \pm 222.3 \mu\text{m}^2$. The areas treated with Arg, glutamine (Gln), histidine (His), Orn and phenylalanine (Phe) were larger compared with that of Control. In terms of survival and growth, Arg and Orn were selected as effective BS for nori culture.

2. Effect of soaking conchospores in closed seawater containing Arg or Orn

Fig.3 shows the results of adherence strength measurement in each treatment time (a, 10 min; b, 1 h; c, 15 h). Soaking nori conchospores for 10 min did not change the adherence strength

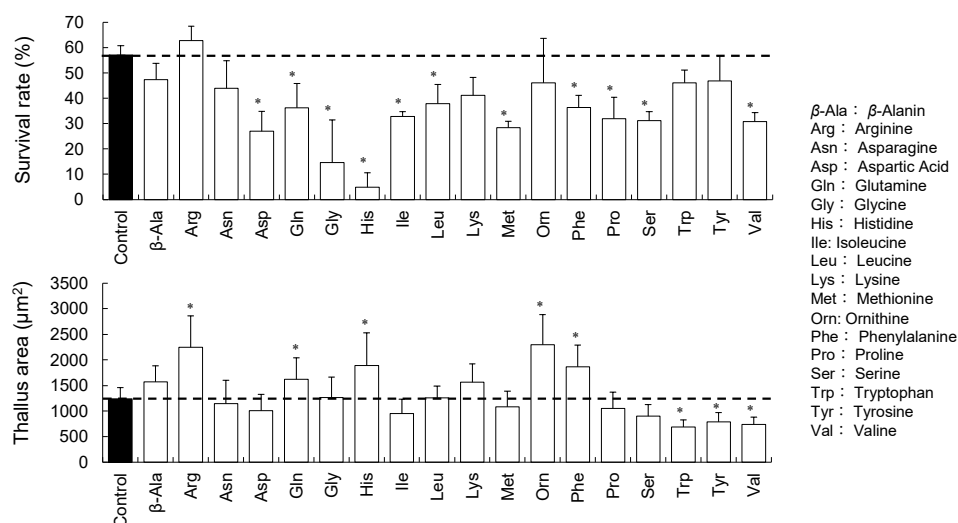


Fig.2 The results of survival rate (a) and thallus area (b) of axenic nori protoplasts previously treated with amino acids

Dotted lines indicate the average value of Control. An asterisk indicates a significant difference relative to the value of Control ($P < 0.05$).

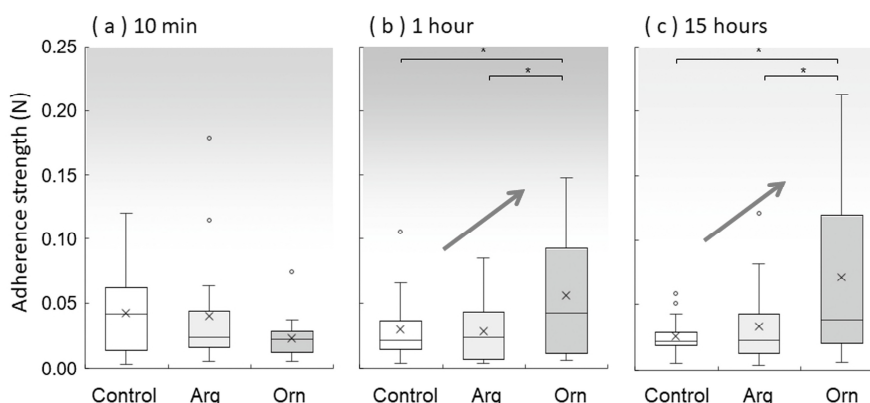


Fig.3 The results of adherence strength of nori thalli previously treated with arginine or ornithine in each treatment time (a, 10 min; b, 1 h; c, 15 h)

An asterisk indicates a significant difference between the groups ($P < 0.05$).

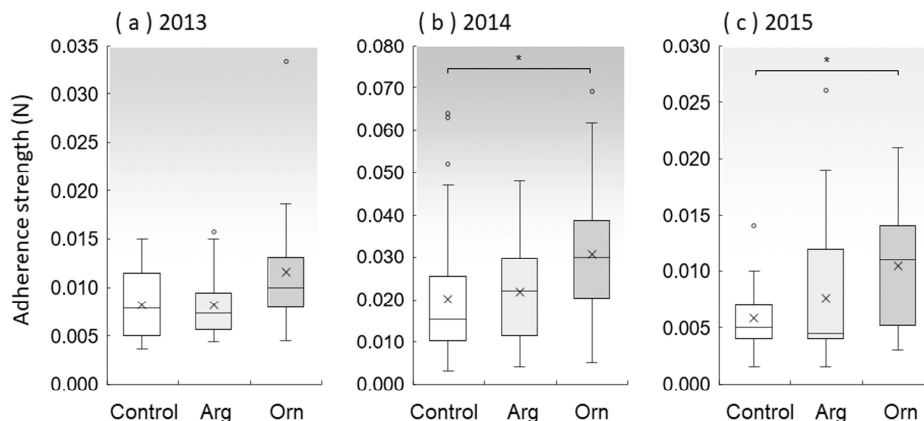


Fig.4 The results of adherence strength of nori blades previously treated with arginine or ornithine in each year of the field trials (a, 2013; b, 2014; c, 2015)

An asterisk indicates a significant difference between the groups by Tukey’s multiple comparison test ($P < 0.05$).



Fig.5 The photographs of nori blades of each soaking treatment taken in the field trial (Nov. 2013)

of nori thalli among treatments. However, 1 h and 15 h of soaking, especially soaking in Orn, enhanced the adherence strength of thalli. These results suggest that soaking treatment with Arg or Orn dissolved in seawater after seedling has positive effects on nori culture.

3. Production improvement in field culture trials

Fig.4 shows the results of adherence strength measurement in each treatment in each year (a, 2013; b, 2014; c, 2015). In the field trials, soaking conchospores in Arg or Orn for 24 h enhanced the adherence strength of nori thalli in each year as found in the laboratory experiment. Fig.5 shows the photographs of nori blades in each soaking treatment group taken in Nov. 2013. In the Arg and Orn treatments, thalli grew better compared with that of Control. Additionally, Fig.6 shows representative daily yields of nori sheets in the 2015 season. The result shows that soaking nori conchospores in Arg or Orn for 24 h could increase the nori sheet production by 1.1 to 1.3 times.

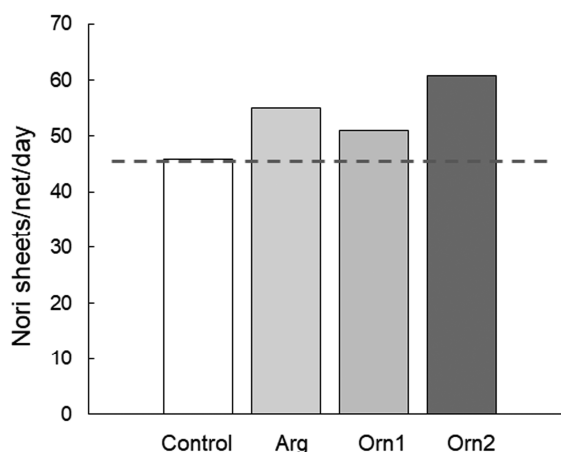


Fig.6 Representative daily yields of nori sheets in the 2015 season

Orn1 and Orn2 indicate the production in two different areas with the same ornithine soaking treatment. Dotted line indicates the average value of Control.

4. Potential of BS in seaweed aquaculture

It is important to use suitable materials specific to a given plant or seaweed as BS at the correct time. In the previous studies that examined the effects of BS for seaweeds, Han *et al.* (2022) and Umanzor *et al.* (2022) reported nori soaked in a medium containing brown alga extracts as BS for 10 days enhanced the tolerance for higher water temperature. Moreover, the brown alga extracts have another useful effects on the improvement of growth and vegetative propagation of nori. However, soaking or dipping of seaweeds as a temporal treatment of BS has only limited effects compared with a constant application of BS to land plants throughout the cultivation.

On the other hand, most of seaweed aquaculture are carried out in coastal areas, and it is practically difficult to soak or dip immature seedlings in seawater containing BS for a long time like 10 days. Moreover, we have to consider how to use BS effectively for seaweed aquaculture because BS materials are soon spread out by the water currents in the ocean. In this paper, we introduced a novel method of soaking conchospores in closed seawater containing Arg or Orn immediately after seedling of nori (Abe *et al.* 2022). This method can be carried out on land with a limited amount of BS (Arg or Orn) and to be incorporated in the ordinal process for seedling in Japanese nori aquaculture. It should be noted that repeated soaking was not necessary in this treatment, although it took 24 h. It is interesting to note that these two amino acids, Arg and Orn, were effective for conchospores, while application of Arg or Orn to adult nori thalli were ineffective (unpublished). This means that we have to use Arg or Orn at the correct time during nori cultivation.

As previously described, suitable materials should be used as BS at the correct time specific to a given plant or seaweed. The previous and present studies have shown that BS have a lot of potential for enhancement of environmental tolerance and production improvement in seaweed aquaculture. However, the number of BS materials, the effectiveness of which has been elucidated so far, are not enough for seaweeds. Thus, it is necessary to find another BS materials beneficial for seaweed culture.

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