Detection of plant growth regulator (Zeatin Riboside) in rockweed by immunoassay

Byungchul Kim[1], Emily R. Lane[2], George Seaver[3] and Titan Fan[1]

[1]Beacon Analytical Systems, Inc, Saco, ME, USA

[2]Department of Biotechnology, Endicott College, Beverly, MA, USA

[3]Ocean Organics, Waldoboro, ME, USA

Abstract

The use of seaweed extracts as biostimulants in agriculture and horticulture is well established. The beneficial effects of seaweed extracts include improved seed germination, seedling establishment, flowering, fruit and crop yield, enhanced resistance to disease and stress. It is believed that those beneficial effects of seaweed fertilizer are due to cytokinins (plant growth regulators) in the extract. Cytokinins have been identified in some seaweed extracts containing *t*Z (trans Zeatin), *t*ZR(trans Zeatin Riboside), DHZ (dihydrozeatin), DHZR (dihydrozeatin riboside), and iP (isopentenyl adenine). A competitive enzyme-linked immunosorbent assay (ELISA) for the detection of Zeatin Riboside (*t*ZR) was developed. The sensitivity of this assay is 0.3 ppb and the assay range is 0.3 to 30 ppb in buffer solution. This prototype kit uses rabbit polyclonal antibodies against *t*ZR. Using this kit, Zeatin Riboside content was determined in freshly harvested Rockweed from the coast of Maine and commercial seaweed extracts provided from Ocean Organics. The extraction efficiency of three different solvents was compared. The effect of drying process on the concentration of *t*ZR in rockweed was also studied.

Introduction

Cytokinins are substances recognized by their ability to induce cell division and other growth regulating functions in plants. Zeatin Riboside is a type of cytokinin found in plant cells, and more abundantly in seaweed. The benefits of using seaweed extract in agriculture and horticulture may be accredited to the presence of cytokinins such as Zeatin Riboside. It has been known that incorporating cytokinins and Zeatin Riboside in a fertilizer has a positive effect on the growth of plants subject to environmental stress. In seaweed, plant growth regulating substances are found in such small quantities that they are usually measured in parts per million (ppm) or parts per billion (ppb). Using even a minuscule amount of these plant growth regulators in extracts and fertilizers can help the development of roots and buds, speed up the process of cell division, delay senescence, and increase resistance to disease and environmental stress. Plant growth regulators in seaweeds have been determined by bioassay and colorimetric methods. But, these assays provide only gross estimates. Other analytical methods such as HPLC (high performance liquid chromatography) and GC (gas chromatography) have been used to analyze individual constituent. However, tedious sample clean-up procedure makes those analytical methods less attractive. In this study, a competitive ELISA was developed and used to determine Zeatin Riboside content in rockweed (*Ascophyllum nodosum*) extracts. Different treatments and extraction methods were compared to determine which yielded the highest quantity of Zeatin Riboside. Also, commercially available seaweed extract (Ocean Organics, Waldoboro, ME) was tested as well.

Figure 1. Rockweed (*Ascophyllum nodosum*) and commercial extracts.

Methodology

Polyclonal antibody production:

In the development of our assay, Zeatin Riboside was coupled to a modified protein carrier, which served as an immunogen, and horse radish peroxidase (HRP) which served as the enzyme conjugate. The antibody was produced through the immunization of New Zealand white rabbits. These rabbits were shaved and injections were administered intradermally on the back. The serum was collected monthly.

Assay performance:

Sheep anti-rabbit antibody was coated onto a 96-well microtiter plate.
The assay range is from 0.3 ppb to 30 ppb. The total assay run time is around one hour.

Assay protocol:

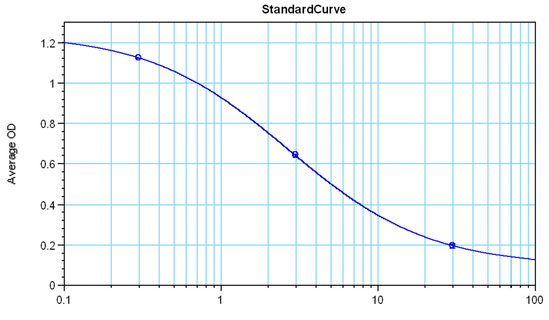
50 µl of HRP conjugate was added to each well followed by 50 µl of calibrator or sample extract. Lastly 50 µl of antibody solution was added to the wells and mixed gently for 30 seconds and incubated for 30 minutes. The well contents were then decanted and washed 4 times with PBST wash solution. Substrate (100 µl) was added to the wells and incubated for 30 minutes.
The reaction was stopped with 100 µl of Stop solution and the contents of the wells were read at 450nm-650nm.

Rockweed treatments:

• Rockweed (50 g) was sun dried for 0, 2, 4 and 6 hours. Before grinding with 100 mL of water, the exact amount of water that was lost by drying was added to compensate. Centrifuged for 10 min at 7,000 rpm. The supernatant was re-centrifuged for 5 min at 12,000 rpm. The supernatant was diluted and tested.

• Rockweed homogenate (25 g rockweed + 50 mL water) was mixed with 25 mL of 0.2 N KOH or 0.2 N HCl. Let it sit for 2 hr. Control was water addition. After the treatment, centrifuged for 10 min at 7,000 rpm. With the supernatant, pHs were adjusted to neutral (pH 7). Centrifuged again for 5 min at 12,000 rpm. The supernatant was diluted and tested.

Results



4-parameter curve fit: Y = (A – D)/(1 + (X/C)^B) + D

A	B	C	D	R^2
1.24	0.973	2.72	0.092	1

IC 50: 3.2 ppb, IC 85: 0.5 ppb

Figure 2. Zeatin Riboside standard curve.

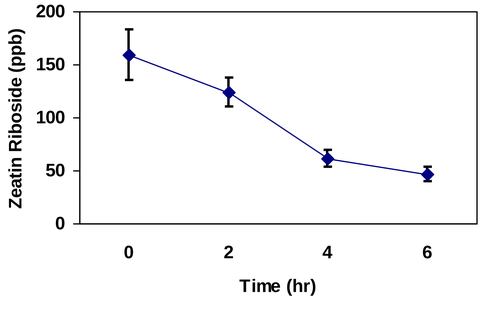


Figure 3. Effect of drying on Zeatin Riboside content.

Table 1 Zeatin Riboside content in rockweed extracted by different pHs and in commercial product.

Rockweed	ZR content (ppb)
KOH (pH 10.8)	143.37 ± 3.78
H_20 (pH 5.4)	301.64 ± 9.62
HCl (pH 2.4)	185.46 ± 7.81
Commercial product	400.07 ± 37.59

Discussion

An immunoassay to determine Zeatin Riboside from seaweed (or product) was developed by Beacon Analytical Systems Inc. This assay has a good sensitivity (0.3 ppb) and broad working range (0.3 – 30 ppb). Using this assay kit, local grown rockweeds (*Ascophyllum nodosum*) were analyzed with various treatments such as drying and pH difference. Also, commercially available seaweed extract (Ocean Organics, ME) sold as fertilizer was assayed as well.

ZR content decreases over the drying time. This might be either actual decrease by the drying effect or poor extraction due to the hardness of rockweed. Since the rockweed gets hardness during drying, it would be difficult to break rockweed cells and release ZR.

Although many seaweed extractions are conducted by using alkaline solution, our result indicates that water extraction is better than alkaline or acidic extraction. However, this could be changed if higher concentration of alkaline or acidic solution is used. Also, there are several other parameters that can affect ZR extraction such as temperature and pressure. ZR content in commercial seaweed extract was determined (around 400 ppb). However, high standard deviation was observed. We noticed that precise pipetting is difficult because of the viscosity of concentrated extract solution. It is difficult to give same volume consistently.

An immunoassay developed for Zeatin Riboside analysis was successfully investigated. The next step for this assay kit would be evaluation study to establish the relationship between bioassay for whole cytokinin analysis and immunoassay for Zeatin Riboside only so that this simple immunoassay can be a good screening method that represents and estimates whole cytokinins content in seaweed or commercial extract saving seaweed extract processors time and money for their quality control purpose.

References

Skoog, F. and D. J. Armstrong. 1970. Cytokinins. Annual review of plant physiology, 21, 359-384.

Crouch, I. J. and J. van Staden. 1993. Evidence for the presence of plant growth regulators in commercial seaweed products. Plant Growth Regulation, 13, 21-29.

Zhang, X. and E. H. Ervin. 2008. Impact of seaweed extract-based cytokinins and zeatin riboside on creeping bentgrass heat tolerance. Crop Science, 48, 364-370.