NORTHERN ILLINOIS UNIVERSITY

Therapeutics for Coral Bleaching:

insight from a novel triterpenoid metabolite

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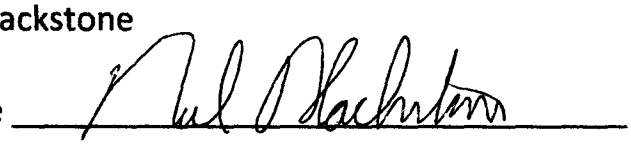
Faculty Approval Signature 

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ABSTRACT (100-200 WORDS):

The focus of the work will be to use therapeutics try to fix and/or prevent coral bleaching. Bleaching is when the coral releases its colored zoozanthellae symbionts, causing a white color appearance. This is a stress response for the coral and is caused mainly by an increase in temperature. This is a problem because once coral bleaching starts it can continue even without the presence of the stressors. If the coral does manage to survive this period it will take a long time for the symbionts to grow back to their original proportions in the coral. This topic is of interest because coral reefs are disappearing every day and conservation of coral reefs has been a hot topic in recent scientific discussions. This paper looks into the use of therapeutics in preventing coral bleaching and helping with recovery when a bleaching event occurs.

Therapeutics for Coral Bleaching: insight from a novel triterpenoid metabolite

Introduction:

To develop therapeutics for coral bleaching there needs to be a method in which results can be obtained quickly and efficiently so that experiments can be done in a timely manner. One way in which to do this is to use a rapid assay method. While this method does not necessarily demonstrate all of the complex phenomena that occur during the experiment it is a starting point to examining results. Also because this can be done fairly quickly it is very useful the in the laboratory setting.

By using rapid assays short term effects of the therapeutics can be measured through studying growth outcomes and physiology of the stress response. While the rapid assay says nothing about the long term effects of the therapeutics it can shed light on the effects in the short term which can then give rise to more complex experiments, much in the way human drugs are tested.

The goal of these experiments is to test whether or not the rapid assay technique can be affectively used in the determining potential of therapeutics, specifically avicins, in coral bleaching.

Background:

Avicins are therapeutics that are derived from the plant *Acacia Victoria*. Avicins are triterpene electrophiles and are used in the regulation of innate stress responses in cells. Avicins have been shown to induce the stress response in human cells. In humans research has

been done with avicins as an anticancer drug. It is useful in cancer therapy because it causes apoptosis in cancerous cells while causing minimal perturbation in other, healthy, cells.

Coral bleaching occurs when environmental stressors, such increases in temperature and light, cause the zooxanthellae photosynthetic symbionts of the coral to be lost due to an innate stress response. When coral bleaching occurs the photosynthetic symbionts within the coral are expelled. The loss of these symbionts causes the coral to have a white colored appearance. This white "bleached" looking color of the coral is due to the stress response of losing their symbionts. Symbionts are expelled in one of three ways: expelled out the mouth, are absorbed in the tissue or are detached and go back into the stolon.

Coral bleaching is a problem because once coral bleaching starts it can continue even without the presence of the stressors. If the coral manages to survive this period of symbiont loss it could take a long time for the symbiotic protozoans to grow back to their original proportions in the coral. Sometimes the loss of the symbionts can be more than the coral can handle and death will result.

Coral bleaching is proposed to be caused by many factors. In a recent research review Wooldridge has proposed that bleaching is caused by redox related activity. CO_2 is used by the algal symbionts in the dark reaction (Calvin-Benson Cycle) of photosynthesis, which occurs in the stroma. O2 and (CH2O)n, products of the light and dark reaction respectively, are released back into the host coral for use.

When biophysical factors cause the demand for CO2 within the cell to exceed the CO2 supply of the host, photosynthetic reactions are altered. The lack of CO2 causes the dark reaction of photosynthesis to not occur; this in turn causes an increase in NADPH and ATP in

the cell, by products of the light reaction that is used in the dark reaction. These increases, and the continual production of oxygen by the light reaction cause increase reactive oxygen species (ROS) in the host. ROS can create free radicals which will cause oxidative stress to the cells and other parts of the host. The way in which ROS are combated is through antioxidants that the host cell possesses as well as programmed cell death. If the host is able to combat the increase in ROS

When the host cannot combat the ROS, something else is needed to assist it. This is where avicins come in. Avicins have antioxidant properties that help diminish ROS through their ability to decrease NADPH and increase O2 uptake. Avicins also help in programmed cell death helping the host to eliminate the harmful ROS cells while leaving the other healthy cells mostly unperturbed.

Method:

First clavulariid species A was grown on cover slips in the laboratory. Regular up keep of the clavulariid species A was done until it was big enough to be used for the experiment. Then when the explants were big enough to be used images were taken of the entire colony. Then for the control group DMSO was added, for the treated group DMSO and avicins were added. The explants were then put in a 32 degree Celsius incubator for 12-14 hours, overnight, so they could begin the bleaching process. After 12-14 hours they were filmed again, this time of the gastro-vascular system of polyps and stolons. Pictures were also taken of the whole colonies once again. Then they were put back into the tank so that they could grow for another 36 hours and then the colonies were imaged again to see if they have regressed at all.

After the images have been collected different data sets were collected from the images. These measurements included: growth of the stolons of the colonies, attachment and detachment rates of the symbionts as well as the number of symbionts in the tissue and in the flow.

Preliminary results:

For the growth of the colonies there was a trend with the avicin treated colonies regressing more after the first 12 hours but recovery quicker than the control colonies after 36 hours (figure 1). However this data is not significant because we only have a sample size of four. For the number of symbionts (figure 2) there were less symbionts in the avicins treated colonies. For the attachment detachment rates there were more detaching than attaching in the control (figure 3).

Ultimately what the data suggests thus far is that pretreatment would be the best in controlling the effects of coral bleaching by helping the host to expel the least tolerable symbionts while keeping tolerable ones, therefore allowing it to recover quicker from the bleaching process.

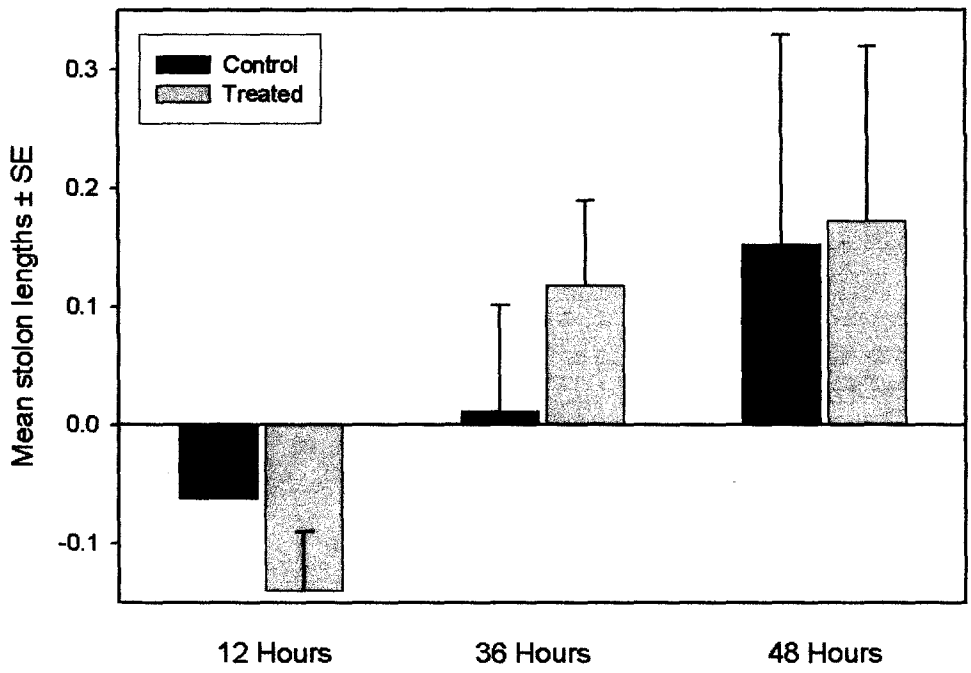

Figure 1: mean stolon lengths (plus standard error) of control and treated colonies 12, 36 and 48 hours after treatment.

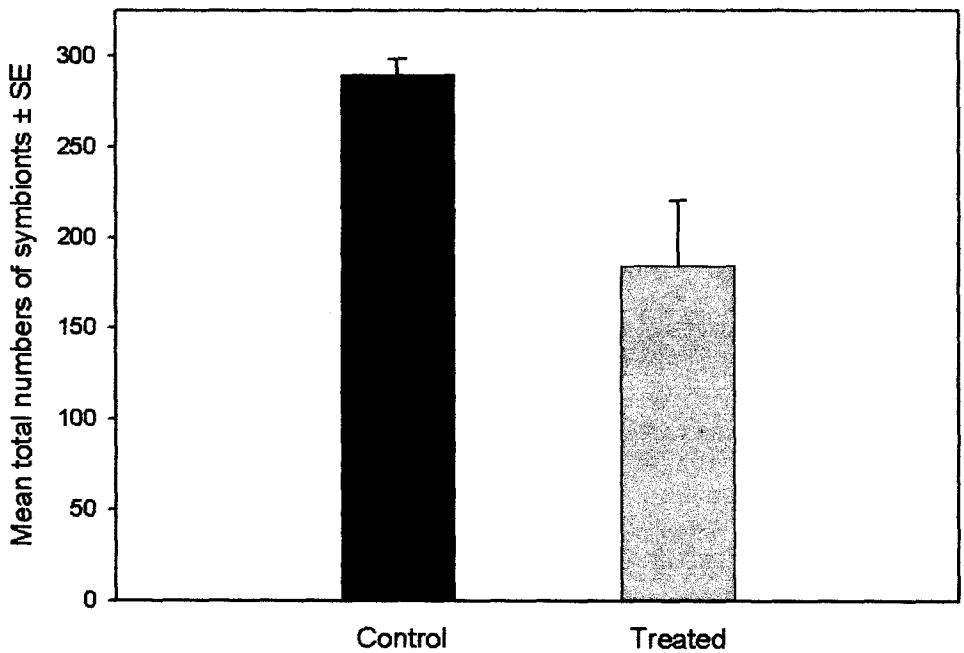

Figure 2: mean total number of symbionts plus standard error in both control and treated colonies.

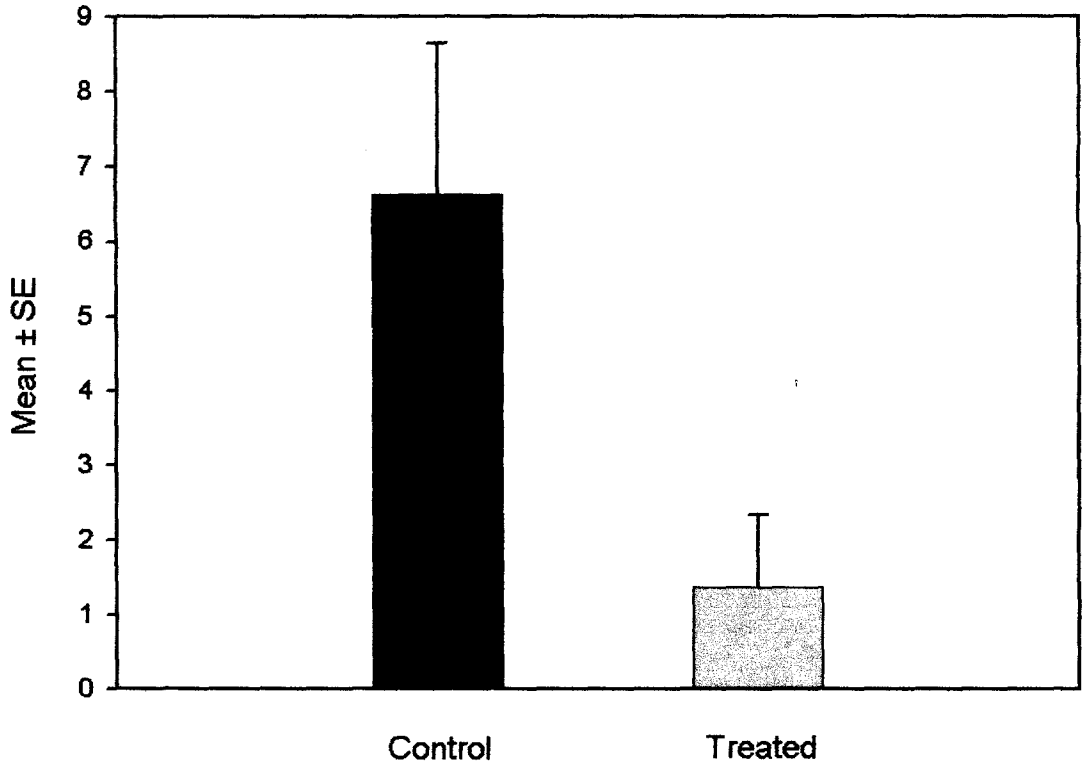

Figure 3: attachment and detachment rate of control and treated colonies. Single attachment and detachment cancel each other out.

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