Indian Journal of Advanced Chemistry (IJAC)
ISSN: 2582-8975 (Online), Volume-1 Issue-1, April 2021

Mass Spectrometric Analysis and Antibiotic Activity of Endophytes from Juniperus Virginiana

Steven Fortucci, Christine E. MacTaylor

***Abstract*—Endophytes are becoming a major source of novel-use products, showing prevalent worth throughout problem areas involving pharmaceuticals as well as agricultural problems of bacterial, fungal, and pest control. Samples from the Eastern Red Cedar, Juniperus virginiana, were examined for novel fungal endophytes. Endophytes were tested for antibiotic resistance and studied for potential biofuel precursors and agricultural usess. Two natural product compounds with potential industrial applications were identified and antibacterial bioactivity was demonstrated through endophytic fungi originating from the twig stems of the tree.**

***Index Terms*—Endophyte, Gas Chromatography-Mass Spectrometry [GC-MS], Antibacterial Bioactivity.**

I. INTRODUCTION

Activity of endophytic species has largely been assessed as internal maintenance via defensive systems for their host plant against pathogenic invaders. Likewise, there has also been work to show endophytes attributing to oxidative stress relief under strenuous physical conditions [1]. Other functional qualities include a support role towards the host, having been found to increase tolerance towards environmental factors such as metals and pollutants in the soil [2]. Other contributions to host plants, such as nitrogen fixation, have been observed among endophytic populations of bacteria [1]. The difficulty in studying endophytes lies in the variability of the endophytic species themselves, as they can be fungal, archaeal, or bacterial. Among each different class of microorganism are a nearly uncountable number of variations of extant species. The study of fungal endophytic members itself has proven tedious due to the inconsistent nature of the group. Extracting DNA from fungus becomes much harder without a proper identification of the organism [3] and with the vast amounts of unknown variations among species of endophytic fungi, genomic studies are expansive and difficult to generalize upon. In order to better cope with varying plant-endophyte relations, model plant organisms have been used to attain a better understanding of the different types of possible interactions [4].

Manuscript received on 28 March 2021 | Revised Manuscript received on 03 April 2021 | Manuscript Accepted on 15 April 2021 | Manuscript published on 30 April 2021.

***** Correspondence Author

Steven Fortucci*, is a recent graduate of Salem State University, Salem, MA, USA with a B.S. in Biology and a minor in Chemistry.

Christine E. MacTaylor, Ph.D., is a professor in the Department of Chemistry and Physics at Salem State University, Salem, MA USA. cmactaylor@salemstate.edu

The majority of what is to be learned about the lifestyles and metabolites of most endophytes is largely unknown. However, this has not hindered their usefulness as sources of industrial enzymatic tools, novel sources of antibiotics [5], anti-cancer agents, as well as sources of novel biodiesel precursors [6]. Among many species of plants, some have unique endophytes, found only within those very plants. Endophytes of the plant examined in this article, *Juniperus virginiana*, have had unique *Bacillus* strains isolated, found to have antitermitic qualities [7]. These trees span the entire eastern seaboard of North America, being found from Texas to Quebec, and throughout the central United States. There are two subspecies of Eastern Red Cedar, and the trees are known to make hybrids between the two. [8]. Due to the high potential for usefulness of endophytic compounds, specifically compounds of eradicating termites, and nearly endless supply of endophyte variations on the planet, further studies of this immensely useful and broad field could harbor many future benefits across a very wide spectrum of areas.

This research focuses on novel molecules identified using Gas Chromatography-Mass Spectrometry of extracted *Juniperus Virginiana* endophyte metabolites. In addition to potential industrial uses medical applications are also tested to determine antibacterial bioactivity.

II. MATERIALS AND METHODS

A. Sampling and Plating procedures

Plant samples of an Eastern Red Cedar, *Juniperus virginiana*, were taken from Choat Farm in Danvers, MA, USA (42° 35' 52.9" N, 70° 56' 01.1" W). Bleach and ethanol were used to sterilize the outer layer of plant tissue. Samples were taken from the twig stem, leaves, and leaf tips. The cedar was notably affected by Apple Rust, *Gymnosporangium juniperi-virginiae*, a fungal infection common among Eastern Red Cedars. Samples of the reproductive growths of the apple rust were taken and plated. Initial plating was done on plain agar. After 24 hours of growth on agar, samples were moved to potato dextrose agar. Growth on potato dextrose agar was then used to start cultures in potato dextrose broth to be used for extraction and use in mass spectrometric and antibacterial bioactivity analysis.

B. Endophyte Metabolite Extraction

Potato Dextrose broth from each sample was poured through cheese cloth into a separation funnel. Extractions using ethyl acetate were performed in triplicate.

The ethyl acetate was then removed using a rotary evaporator leaving behind the extracted metabolites for further testing.

C. Mass Spectrometry

GC-MS samples were run on a Shimadzu instrument with a 1 µL split injection. Temperature programming conditions increased linearly from 40°C to 200°C with temperatures held at the beginning and ending of each run. The total run time was 24.0 minutes. GC-MS samples were analyzed against the NIST database with GC-MS Postrun Analysis software.

D. Antibacterial Bioactivity

Individual nutrient agar plates were each inoculated with *Escherichia coli* and *Staphylococcus aureus*. Extracted endophyte metabolite samples and the solid fungal endophytic growth from each sample were placed in corners of the inoculated plates to test for their bioactivity against gram-positive and gram-negative bacteria respectively.

III. RESULTS AND DISCUSSION

Two major components identified in the GC-MS Postrun Analysis software indicated a 94% match of 2,5-Furandione, and an 88% match for Ethanal 2, 5-Furandione is the acid anhydride form of succinic acid which has numerous applications in industry. It is not only a dietary supplement it is also used as an acid regulator in foods and pharmaceuticals [9], [10].

Ethanal, or Acetaldehyde has many uses with a number of patents associated with it; however, one of particular interest is the production of acetaldehyde oxime as an intermediate used in the production of pesticides [11]. It is likely that the endophyte is producing some form of protection in the form of a natural pesticide. The push towards greener, natural products with less waste makes any form of naturally occurring pesticide of great interest. The identification of Ethanal in the eondphytic metabolite is not conclusive evidence of a natural pesticide is being produced but it indicates the potential for one that should be investigated further.

Antibacterial bioactivity was found in one specific sample. The solid fungal endophyte originating from a twig stem of the cedar tree sample showed strong activity against *E. Coli* but not against *Staphylococcus*. An area of inhibition surrounding the twig stem endophyte extending out three times the diameter of the sample was observed. This indicates a strong bioactivity against gram-negative bacteria. The twig stem endophyte shows potential as a naturally occurring antibiotic. The specific compound or compounds being produced by the endophyte that are responsible for the antibacterial properties could be determined for potential novel pharmaceutical applications.

It is important to note again that this particular tree was infected by Apple Rust, a common fungal infection. This may be important because it is possible that the endophytes present in this tree demonstrated certain properties because of the presence of this infection. Endophytes typically act to protect their host plant in some way, which is why they often have anti-fungal, or anti-bacterial, or pesticidal properties. An Eastern Red Cedar tree that does not have Apple Rust may not present with the same endophytes as a tree that does contain this common fungal infestation.

IV. CONCLUSIONS

The *Juniperus Virginiana* endophytic fungi show several novel applications. While the field of endophyte research is growing rapidly, few researchers are searching volatile metabolite extracts to identify specific components using GC-MS. With this method two compounds not known to be produced by Jniperus Virginiana endophytes were identified. Both 2, 5- Furandone and Ethanal were identified and each one has potential applications in industry from pesticide production to pharmaceutical and food acid regulators. The use of a naturally produced pesticide would be of particular interest and is worthy of further investigation.

In addition to the two identified metabolite components, a strong gram-negative anti-bacterial response was seen in one of the fungal endophytes. This indicates a potential source for a novel antibiotic that could be obtained from a natural product readily grown in the laboratory.

Future in-depth pharmaceutical research should be performed to discern the specific compounds responsible for the antibacterial bioactivity. Side by side studies on trees with and without Apple Rust should also be carried out to determine if the presence of this fungus affects the endophytes that are present or the anti-bacterial properties of the endophytes. In the never-ending quest to find new antibiotics to which bacterium have not yet built up a resistance, fungal endophytes are likely to play a key role. This work indicates that in the search for gram-negative antibiotics, the *Juniperus Virginiana* should be investigated further.

ACKNOWLEDGMENT

The authors would like to thank Mr. Dakota Hamill for his guidance in this project. Funding for this project was support through the Department of Chemistry and Physics at Salem State University, Salem, MA. USA.

REFERENCES

1. White J, Torres M, Johnson H, Irizarry I. 2014. “A functional view of plant microbiomes: endosymbiotic systems that enhance plant growth and survival. “ *Advances in Endophytic Research*. 425-429.
2. Torres M, White J, Zhang X, Hinton D, Bacon C. 2012. “Endophyte-mediated adjustments in host morphology and physiology and effects on host fitness traits in grasses.” *Fungal Ecology* 5(3): 322-330.
3. Fredricks D, Smith C, Meier A. 2005. “Comparison of six DNA extraction methods for recovery of fungal DNA as assessed by quantitative PCR”. *Journal of Clinical Microbiology*. 43(10): 5122-5128.
4. Garcia E, Alonso A, Platas G, Sacristan S. 2013. “The endophytic mycobiota of *Arabidopsis thaliana.” Fungal Diversity*. 60(1): 71-89.
5. Suryanarayanan T. 2013. “Endophyte research: going beyond isolation and metabolite documentation.” *Fungal Ecology*. 6(6): 561-568.
6. Santos-Fo, Florisvaldo C, Fill T, Nakamura J, Monteiro M, Rodrigues-Fo E. 2011. “Endophytic fungi as a source of biofuel precursors.” *Journal of Microbiology and Biotechnology*. 21(7): 728-733.
7. Zhao K, Liu J, Li Z, Chang Z, Shi P, Ping W, Zhou D. 2011. “*Bacillus subtilis* subspecies *virginiana*, a new subspecies of antitermitic compound-producing endophytic bacteria isolated from *Juniperus virginiana*. “ *Journal of Economic Entemology* 104(5): 1502-1508.

8. Lawson E. *Juniperus virginiana* L. "Eastern Red Cedar." Northeastern Area State and Private Forestry. http://www.na.fs.fed.us/pubs/silvics_manual/Volume_1/juniperus/virginiana.htm
9. Zeikus, J. G.; Jain, M. K.; Elankovan, P. 1999. "Biotechnology of succinic acid production and markets for derived industrial products". *Applied Microbiology and Biotechnology* **51** (5): 545.
10. Rutesh H. Dave, 2008 "Overview of pharmaceutical excipients used in tablets and capsules" *Drug Topics:Voice of the* Pharmacist, http://drugtopics.modernmedicine.com/drug-topics/news/modernmedicine/modern-medicine-news/overview-pharmaceutical-excipients-used-tablets
11. John H Bonfield, Stephen E. Belsky, Donald Pickens 1982. US Pat. US4323706 A "Production ofacetaldehyde oixme"