

Characterization of Volatile Secondary Metabolites from Marine *Trichoderma viride*

Uma S.*¹ and Jeevan P.²

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Key words: GC-MS, Metabolites, *Trichoderma*, Volatile

Fungi are very successive soil inhabitant, with high plasticity and capacity to adopt to adverse condition [1]. The marine mycota is represented by lower fungi (*Haplomastigo mycotina* and *Diplomastigomycotina*) and higher fungi (Ascomycotina, Basidiomycotina, and Deuteromycotina). The estimated coastal isolated fungi was about 1500 species. This number seem to be low according to the number of estimated terrestrial fungi, which was estimated around 250,000 species. Several bioactive like cytoglobosins and halovirs were isolated from marine fungi. Thus, it was proved that numerous marine fungi with remarkable structures and ability to produce several bioactive compounds which are used for the production of biofertilizers [2].

Trichoderma spp. are present in nearly all types of soil and other diverse habitats. In relation to other fungi in soil, these are the most prevalent fungi belonging to the genus *Trichoderma* under Deuteromycotina. This genus comprises large number of fungal strains like *T. asperellum*, *Trichoderma atroviride*, *T. harzianum*, *T. hamatum*, *T. koningi*, *T. virens* and *T. viride*, which are widely used as bio-control agents of plant diseases and in addition these are found effective in increasing plant growth and development [3-5]. *Trichoderma* strains exhibit biocontrol activity against fungal phytopathogens either indirectly, by competing for nutrients and space, modifying the environmental conditions, promoting plant growth and plant defensive mechanisms and antibiosis, or directly by mechanisms such as mycoparasitism. The *Trichoderma* may act synergistically by indirect and direct mechanisms. The mechanisms in the biocontrol process may vary according to the *Trichoderma* strain, their antagonized fungus, the crop plant, and the environmental conditions, including nutrient availability, pH, temperature, and iron concentration [6]. *Trichoderma*

species have many characteristics that make them of significant interest to the research community. It was reported that *Trichoderma* isolates involve in the production of natural products or secondary metabolites. These secondary metabolites of volatile or non-volatile nature, often have obscure or unknown functions that are of considerable importance to human kind in medical, industrial or agricultural applications. Secondary metabolic compounds appear as intermediate or end products of heterogenous metabolic pathways and belong to various structural classes such as mono- and sesquiterpenes, ketones, lactones, alcohols and esters compounds [7-8]. Compounds such as pyrones [9], anthraquinone, butanolide [10], cyclopentyl isocyanide, isonitrine-type compounds and peptaibols [11-13] which have been reported to play vital role in managing the plant pathogens like *Gau- mannomyces graminis* var. *tritici* [14], *Rhizoctonia solani* and *Fusarium oxysporum* sp. *lycoersici* [15] and *Phytophthora* [16]. It was reported that the production of volatile secondary metabolites varies between different *Trichoderma* strains. Thus, in the present study marine *Trichoderma viride* strain was characterised for volatile secondary metabolites through gas chromatography - mass spectrometry (GC-MS).

Liquid culture of *Trichoderma viride* (ATJJC1) strain was analyzed for the presence of secondary metabolites by using chromatographic analysis followed by Mass spectrometry for the identification of separated components. Procedure followed for extraction of secondary metabolites was adopted from Siddiquee *et al.* [17] with few modifications.

Trichoderma viride (ATJJC1) strain was grown on potato dextrose broth (PDB) at 25±1°C for 25 days. Culture filtrate was extracted by straining through muslin cloth. Metabolites were extracted by solvent extraction method into hexane in the ratio of 1: 1 (v/v). Solvent (hexane) was evaporated from the solution using rotary evaporator with a rotor speed of 120 rpm at 400C until the residues were visible. Obtained residues were re-suspended in solvent (acetone) for further characterization by GC-MS. GS-MS analysis was performed in GCMS-QP2010 Plus ultra. The column temperature settings were programmed to begin with 800C for 2 minutes, followed by an increase at a rate of

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Article

Taxonomic and Functional Distribution of Bacterial Communities in Domestic and Hospital Wastewater System: Implications for Public and Environmental Health

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Abstract: The discharge of untreated hospital and domestic wastewater into receiving water bodies is still a prevalent practice in developing countries. Unfortunately, because of an ever-increasing population of people who are perennially under medication, these wastewaters contain residues of antibiotics and other antimicrobials as well as microbial shedding, the direct and indirect effects of which include the dissemination of antibiotic resistance genes and an increase in the evolution of antibiotic-resistant bacteria that pose a threat to public and environmental health. This study assessed the taxonomic and functional profiles of bacterial communities, as well as the antibiotic concentrations in untreated domestic wastewater (DWW) and hospital wastewater (HWW), using high-throughput sequencing analysis and solid-phase extraction coupled to Ultra-high-performance liquid chromatography Mass Spectrometry (UHPLC-MS/MS) analysis, respectively. The physicochemical qualities of both wastewater systems were also determined. The mean concentration of antibiotics and the concentrations of Cl⁻, F⁻ and PO₄³⁻ were higher in HWW samples than in DWW samples. The phylum *Firmicutes* was dominant in DWW with a sequence coverage of 59.61% while *Proteobacteria* was dominant in HWW samples with a sequence coverage of 86.32%. At genus level, the genus *Exiguobacterium* (20.65%) and *Roseomonas* (67.41%) were predominant in DWW and HWW samples, respectively. Several pathogenic or opportunistic bacterial genera were detected in HWW (*Enterococcus*, *Pseudomonas* and *Vibrio*) and DWW (*Clostridium*, *Klebsiella*, *Corynebacterium*, *Bordetella*, *Staphylococcus* and *Rhodococcus*) samples. Functional prediction analysis indicated the presence of beta-lactam resistance, cationic antimicrobial peptide (CAMP) resistance and vancomycin resistance genes in HWW samples. The presence of these antibiotic resistance genes and cassettes were positively correlated with the presence of pathogens. These findings show the risk posed to public and environmental health by the discharge of untreated domestic and hospital wastewaters into environmental water bodies.

Keywords: antimicrobials; antibiotic resistance genes; untreated wastewater; public health; environmental health

1. Introduction

Wastewater originates from various anthropogenic sources including mining and agricultural activities, as well as domestic, industrial, and hospital effluents [1,2]. Typically, domestic wastewater (DWW) is characterised by high amounts of organic load that provides suitable substrate to the growth and/or survival of a wide range of microorganisms including bacteria, viruses and protozoa [3]. Compared to DWW effluents, hospital wastewater (HWW) effluents contain, in addition to organic load, high concentrations of



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Association of Agriculture Occupational Exposure With Diabetes and Cardiovascular Risk Factors in South Indian Villages: REDSI Study

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There has been a huge increase in diabetes and its associated cardiovascular complications over the last decade, predominantly in the middle- and low-income countries. In these countries, the majority live in rural areas. The Rural Epidemiology of Diabetes in South India (REDSI) study was aimed to analyze the prevalence of diabetes, cardiovascular risk factors, and its complications in rural farming and non-farming villages in Tamil Nadu, South India. A research survey on the prevalence of self-reported diabetes, cardiovascular risk factors (age, sex, obesity, hypertension, hypercholesterolemia, alcohol and tobacco use) and agricultural occupational exposure was executed among 106,111 people from 61 villages in the state of Tamil Nadu, South India, during 2015–2018. Overall, we observed a diabetes prevalence of 11.9% in rural South India. A nearly two-fold higher prevalence of diabetes was observed among the farming community (15.0%) compared to that among the non-farming population (8.7%). Logistic regression analyses revealed a strong association with agrochemical



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Biodiesel Production of *Microseira woelli* Isolated from Fresh Water Pond

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ABSTRACT

Algal biofuel or algal oil is an alternative to liquid fossil fuels that uses algae as the source of energy and they are considered as a replacement for biofuel sources such as corn and sugarcane. This study presents the isolation of algal strain from fresh water ponds in Pudukkottai District, Tamil Nadu, India. The algal strain was confirmed as *Microseira woelli* after PCR amplification and sequencing of the 16s rRNA gene of the DNA isolated from the species. The structure of the strain was observed using a trinocular microscope. Biodiesel was produced from *M. woelli* by lipid extraction and allowed for the process of trans-esterification. The biodiesel production of was *M. woelli* was confirmed by GC-MS analysis which showed high quantity of methyl ester compounds.

Keywords: Algae, *Microseira woelli*, biodiesel, 16s rRNA gene, trans-esterification, GC-MS

INTRODUCTION

The energy crisis and greenhouse gas emissions throughout the world have driven the search for alternative and eco-friendly renewable energy sources (Passoth et al. 2014). Biodiesel derived from biomass is an emerging source of fuel, and the global application of biodiesel in the transport sector was tremendously increasing (Valentine et al. 2012) and Kim et al. 2004). The Microalgae biofuel is identified as one of the renewable energy sources for sustainable development, having the potential to replace fossil fuels (Klutz et al. 2017) and Gnansounou 2010). Microalgae biofuel was devoid of the major drawbacks associated with oil crops and lignocelluloses-based biofuels. They are economically viable and cost-competitive which require minimal water use and mitigate atmospheric CO₂ (Glithero et al. 2015). However, biofuels are currently mainly produced from so-called first-generation substrates such as sugar cane, wheat grain, or vegetable oils (Sims et al. 2010) and (Townsend et al. 2017), i.e. resources that also can be used as human food. This use of food crops has been criticized due to potential food versus fuel competition (Jorgensen et al. 2018), (Panoutsou et al. 2017) and (Kasting, 2013). Therefore, substantial

research has been conducted to establish biofuel production. Microalgae can be a rich source of carbon compounds, which can be utilized in biofuels, health supplements, pharmaceuticals, and cosmetics (Kadam et al. 2000). Microalgae produce a wide range of bioproducts, including polysaccharides, lipids, pigments, proteins, vitamins, bioactive compounds, and antioxidants (Talebniya et al. 2010). Cyanobacteria possess certain properties which have entitled them to be one of the most promising feedstock for bioenergy generation (Tishler et al. 2015) i.e., They contain considerable amounts of lipids, which are mainly present in the thylakoid membranes, possess higher photosynthetic levels and growth rates compared to other algae and higher plants and cyanobacteria grows easily with basic nutritional requirements; they can survive if supplied with air [N₂ (nitrogen-fixing strains) and CO₂], water, and mineral salts (especially phosphorous-containing salts) with light as the only energy source.

The accumulation of lipids in algae occurs when the organism is under stress (e.g. nutrient deprivation) and in the stationary growth phase (Passoth et al. 2013). Another secondary advantage is that cyanobacteria, being prokaryotes, can much more readily be genetically engineered to enhance the production of biofuels as opposed to eukaryotic algae

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Dual Culture Effect of Marine Trichoderma Recorded from Athirampattinum Against Plant Fungi Obtained from Thirukalapatti Village Spinach Cultivating Field, Sivagangai, Tamil Nadu

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ABSTRACT

It was reported that yield of the spinach reduced due to the plant disease. *Fusarium* wilt, consider to be a soil borne disease which highly influence the production of crops. Outbreak of *fusarium* diseases causes major economic loss on crops throughout the world. *Fusarium oxysporum*, *Rhizoctonia solani*, *Pythium aphanidermatum*, *Fusarium culmorum*, *Gaeumannomyces graminis*, *Sclerotium rolfsii*, *Phytophthora cactorum* are some of plant pathogens that cause severe diseases in various cash crops. It was reported that *fusarium oxysporum* species "Spinacia" proven to be the most dreadful disease of the crop spinach. *Fusarium* species also causes damping-off, root rot, and discoloration of both vascular system of seedlings and mature plants. To overcome this situation biocontrol methods should be followed. It was reported that *Trichoderma* species – are recorded to an effective biocontrol agent that act against pathogens such as *Fusarium oxysporum*, *Rhizoctonia solani*, *Pythium aphanidermatum*, *Fusarium culmorum*, *Gaeumannomyces graminis*, *Sclerotium rolfsii*, *Phytophthora cactorum*, *Botrytis cinerea* and *Alternaria* species effectively. Thus, *Trichoderma* species isolated from the coastal area Athirampattinum was tested against the fungal pathogens which are isolated from the spinach cultivating field of Thirukalapatti Village to overcome the disease and to found the potentiality of marine *Trichoderma* (Antagonistic) by dual culture method which was recognized to be a viable alternative method to manage plant diseases.

Key words: Spinach, Marine fungi, Dual culture, Antagonistic fungi

Fungi are very successive soil inhabitant, with high plasticity and capacity to adopt to adverse condition [1]. Soil fungi play an important role as major decomposer in the soil ecosystem. They decompose the soil components by producing a wide variety of extracellular enzymes [2]. There are about 75000 species of soil fungi in the world. Fungi are one of the dominant group presents in soil, which strongly influence ecosystem structure and functioning and thus plays a key role in many ecological services. Soil borne plant "Pathogenic fungi" cause a variety of disease such as rot (stem, root, crown), damping-off and wilts. Therefore, there is a growing interest in designing biocontrol agents, its

biological functioning to overcome the crop diseases and also an integrated disease management approach, including the use of disease-resistant cultivars, crop rotation, care full irrigation and organic fungicides to produce a high-quality product of crops [3].

The marine mycota is represented by lower fungi (*Haplomastigo mycotina* and *Diplomastigomycotina*) and higher fungi (*Ascocomycotina*, *Basidiomycotina*, and *Deuteromycotina*). The estimated coastal isolated fungi was about 1500 species. This number seem to be low according to the number of estimated terrestrial fungi, which was estimated around 250,000 species. Several bioactive like cytoglobosins and halovirs were isolated from marine fungi. Thus, it was proved that numerous marine fungi with remarkable structures and ability to produce several bioactive compounds which are used for the production of biofertilizers [4].

MATERIALS AND METHODS

1. a.) Collection of infected leaf Sample (*Spinach oleracea*)



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Phytochemical and Pharmaceutical Importance of *Ipomoea staphylina* Roem. and Schult: A Medicinal Review

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ABSTRACT

Plant based remedies are best alternative to allopathic medicine. They have been contributed significantly to rural livelihoods. Apart from the traditional healers used herbal medicine, many people are involved in the collection and trading of medicinal plants. This has been resulted an increasing demand for herbal remedies worldwide leading to enhance new drugs. The bioactivity of natural compounds is associated with the effects of various phytochemicals such as alkaloids, tannins, cardiac glycosides, terpenoids, saponins, flavonoids etc. In this study the medicinal plant *Ipomoea staphylina* was reviewed. This plant was widely used for the treatment of various diseases like cancer, diabetes, inflammation, oxidative stress, pathogenic infection etc. reported by various authors. Moreover, this plant was used by many tribal people for the treatment of many diseases included antidote. Thus the plant might be useful for discovery of new natural drugs for many diseases after detailed study.

Key words: Ethnobotanical, Phytochemistry, Bioactive compounds, *Ipomoea staphylina*, Diabetes and Cancer.

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INTRODUCTION

Plants have been a valuable source of natural products for maintaining human health, especially, in the last decade with more intensive studies for natural therapies.^[1] Medicinal herbs are widely used with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant-based products for the prevention and cure of different human diseases. It has been recorded that 80% of the world's population has fidelity in traditional medicine, particularly plant-based drugs for their primary health care.^[2] India is also one of the mega biodiversity countries in the world.

The total number of plant species of groups recorded from India is 45000. Of these seed-bearing account for nearly 15,000-18,000. In India, more than 1000 species were used in several countries in the traditional system of medicine viz. Ayurveda, Siddha, and Unani which has survived through 3000 years mainly using plant-based drugs. The ancient texts like Rigveda (4500 – 1600 B.C) and Athrvana Veda mention the use of several plants as medicine. The books on Ayurvedic medicine such as Charaka Samhita and Sushruta Samhita referred to the use of more than 700 herbs.^[1]

Classification and Taxonomy

Kingdom: Plantae
Division: Tracheophyta
Class: Magnoliopsida
Order: Solanales
Superorder: Asteranae
Family: Convolvulaceae
Genus: *Ipomoea*
Species: *staphylina* Roem. and Schult

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EFFECT OF INTEGRATION OF GEOFUNGI WITH COMMERCIAL FUNGICIDES
AGAINST *FUSARIUM OXYSPORUM*

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ABSTRACT

Fungal plant pathogen are among the most important factors that cause serious losses to agricultural produce biological control of plant diseases including fungal pathogens has been considered as viable alternative method to chemical control. In plant pathology, the term bio control applies to the uses of microbial antagonists to suppress disease, these interactions and significantly affect plant health in various ways. In the present investigation suggested that the preliminary screening of fungal population isolated from soils. Totally 10 species of fungal population were identified and conformed. The geo fungi like *Aspergillus flavus*, *A.fumigatus*, *A.terreus*, *A.niger*, *A.oryzae*, *A.luchensis*, *Curvularia lanata*, *Penicillium* sp., *Memnonilla* sp. and *Trichoderma viride* were recorded from the soil. The maximum zone of inhibition by *Trichoderma viride* showed promising activity in the pathogenic fungi *Fusarium oxysporum* when compared with other fungi by dual culture experiments.

In the culture filtrate of geo fungi *Aspergillus flavus*, *A.fumigatus*, *A.terreus*, *A.niger*, *Penicillium* sp. and *Trichoderma viride* of 100, 200, 300 and 400µl concentration were treated against *F.oxysporum* by *in vitro* method, the fungal culture filtrates of 100µl concentration has excellent zone of inhibition found to be recorded in the following order like *Trichoderma viride*, *penicillium* sp., *Aspergillus niger*, *A.terreus*, *A.flavus*, *A. fumigatus* were observed respectively. No zone of inhibition 400µl concentration in the culture filtrate of all fungi against *F.oxysporum* and efficacy of different pesticides agromax, fytolan, monoepzole, chiarpyrifes, nexconazole of different concentration of 100,200,300,400, and 500 ppm were treated against *F.oxysporum* respectively. Among the different fungicides, chiarpyrifes insecticides were excellent anti pathogenic properties against *F.oxysporum* and zone of inhibition were also analyzed and recorded. The biocontrol products of plant diseases is bright and promising and with the glowing demand for bio control products among the powers, it is possible to use the biological control as an effective strategy to manage plant diseases, increase yield, protect the environment.

Keyword: Dual culture, culture filtrate, fungicide

INTRODUCTION

Biological control of plant pathogens through fungi has been considered as a potential tool for management of post- harvest diseases in recent years and search for potential bio-agents (Balai and Singh, 2013). *Trichoderma* sp. are now the most common fungal bio-control agent that have been comprehensively studied and deployed throughout the world. Plant diseases caused by a variety of fungi may cause significant losses on agricultural crops. All plants are attacked by some kinds of fungi, and each of parasitic fungi can attack one or many kinds of plants. More than 10,000 sp. of fungi cause disease in plants. Brinjal (*Solanum melongina* L.) is grown as a vegetable crop in India and the plant is affected by various fungal diseases which in turn produces low crop yield. Pathogens being soil borne, causes a huge problem in controlling the disease. Soil borne diseases are difficult to control and seed treatment with fungicides has low impact. The chemical pesticides have been known to cause various environmental and health problems. Intensive use of fungicides for the control of diseases has resulted in the accumulation of toxins to human beings as well as to the environment.

The chemical pesticides have been increasing effects of these chemical residues found in eatables, plant growers are being challenged to maintain the plant health with reduced input from agricultural chemicals. Microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents,

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