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Uma S. and Jeevan P.

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Short Communication

Characterization of Volatile Secondary Metabolites from Marine *Trichoderma viride*

Uma S.*[1] and Jeevan P.[2]

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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. koningii*, *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, ketons, lactones, alcohols and esters compounds [7-8]. Compounds such as pyrones [9], anthraquinone, butanolide [10], cyclo-pentyl isocyanide, isonitrine-type compounds and pep-taibols [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*f. sp. *lycoersici* [15] and *Phytohthora* [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

* **Uma S.**
✉ umasivagurunathan@gmail.com

[1-2] P. G. and Research, Department of Microbiology, J. J. College of Arts and Science, (Autonomous), Affiliated to Bharathidasan University, Pudukkottai - 622 422, Tamil Nadu, India

100C/min. till 2500C followed by final injection temperature of 2800C. The linear velocity of carrier gas was 40.5 cm/sec. Samples were injected by splitless mode with sampling time of 1 minute. The ionization for MS detection was performed with ion source temperature of 2300C and interface temperature of 2700C. Starting time for acquisition after injection was 5 min. and end time was 44.49 min. The detected compounds were identified by matching the electron impact spectra against the National institute of standards and technology (NIST) library.

Results of the present study revealed that *Trichoderma viride* (ATJJC1) strain produce many important secondary metabolic compounds. A total of 13 volatile compounds were detected from culture filtrate of *Trichoderma viride* (ATJJC1) strain which were further characterized after matching the electron impact spectra against NIST library. Major compounds identified were Cetene (1-Hexadecene) (RT-2.85), I-Iodo-2methyldodecane (RT-5.54) and 6-n-pentyl-2H-Pyon2one(6PAP) (RT-8.23). Apart from these compounds, secondary compounds like Tetradecane, 2,6,10-trimethyl, Tritetracontane, Decane,2-methyl, 2-methyldodecane, [1,1"-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-,methyl ester, 5-Octadecene, 2,5-Hexanedione,3,4-dihydroxy-3,4-dimethyl, Benzamide,3-amino-N-[4,5-dihydro-5-oxo-1-(2,4,6-trichloropheny. All these compounds may responsible for enhancing biocontrol activity of *Trichoderma viride* against *Fusarium oxysporum* and other plant pathogens.

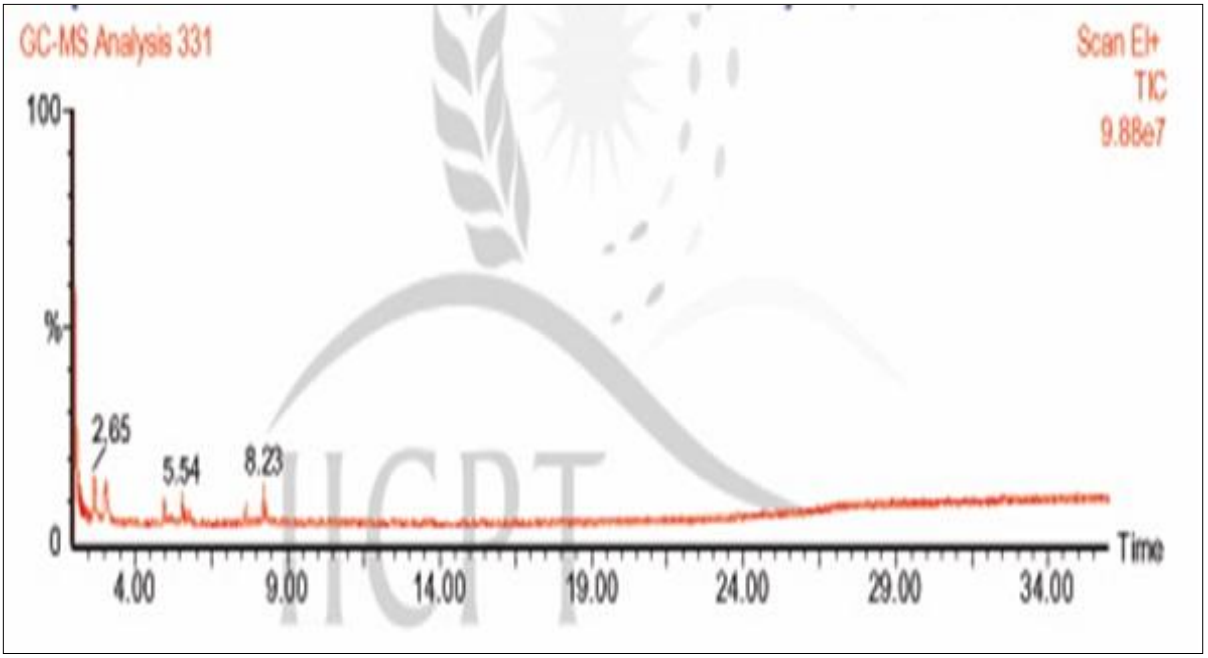

Fig 1 Gas chromatography and Mass spectrometry analysis of marine *Trichoderma viride* (ATJJC1)

Table 1 Characterization of bioactive compounds from *Trichoderma viride* by using GC-MS

RT	Name of the compounds	Molecular formula	MW (KDa)
2.85	1- Hexa decene (Cetene)	$C_{16}H_{32}$	224
3.89	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	240
5.54	I-Iodo-2Methyl-Ioundecene	$C_{13}H_{28}$	184
5.71	Tritetracontane	$C_{43}H_{88}$	604
8.23	6-n-Pentyl-2H-Pyron-2one(6PAP)	$C_{10}H_{14}O_2$	166
8.45	Decane, 2-methyl-	$C_{11}H_{24}$	156
10.45	Dodecane, 2-methyl-	$C_{13}H_{28}$	184
27.02	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	322
29.78	5-Octadecene, (E)-	$C_{18}H_{36}$	252
31.46	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	296
32.73	Tetratetracontane	$C_{44}H_{90}$	618
34.89	2,5-Hexanedione, 3,4-dihydroxy-3,4-dimethyl-	$C_8H_{14}O_4$	174
35.74	Benzamide, 3-amino-N-[4,5-dihydro-5-oxo-1-(2,4,6-trichloropheny	$C_{16}H_{11}C_{l3}N_4O_2$	396

RT- Retention time, MW-Molecular weight

CONCLUSION

From the study, it can be concluded that marine *Trichoderma viride* (ATJJC1) strain harbours many important volatile secondary metabolites that have been reported to perform diverse functions ranging from anti-pathogenic to plant growth promotion. Thus, this marine *Trichoderma viride* (ATJJC1) strain could be further studied for management of plant pathogens as well as to promote the biofertilizers production.

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