

## Bacterial Endophytes of *Ocimum Sanctum*: A Functional BioProspection from Cropping Systems

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### ABSTRACT

Endophytes are helpful organisms that live inter-cellularly inside the plants. Cooperation of endophytes with the host plants and their capacity inside their host are imperative to address biological significance of endophyte. Four endophytic microbes OS-9, OS-10, OS-11, and OS-12 were isolated from surface sterilized leaves of *Ocimum sanctum*. These isolated microorganisms were tested on a dual-culture against different phytopathogenic microorganisms such as *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium solani*, *Alternaria solani*, and *Colletotrichum lindemuthianum*. Of these, strain OS-9 was observed effective against *R. solani*, *A. solani*, *F. solani*, and *C. lindemuthianum* while OS-11 present a reverse effect against *A. solani*. The growth promoting potential of these endophytes were assessed in the greenhouse by vaccinated seeds of *O. sanctum*. Treatment with endophytes OS-10 and OS-11 showed promising potential as indicated a relatively high dry weight under the treatment. Strains OS-10 and OS-11 were represented by two genotypes OS Purple and CIM-Angana and it was observed that both the genotypes fundamentally improved new herbage yield (t/ha). These bacterial endophytes found to have significant impact of the essential oil productivity especially in cultivar OS Purple and ended up in a higher oil yields. Strain OS-11 is identified as *Bacillus subtilis*.

**KEYWORDS:** Endophytes, Cropping Systems

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### I. INTRODUCTION

Tulsi (*Ocimum sanctum*) belongs to family Lamiaceae is known to have high therapeutic value. The essential spot of beginning of *O. sanctum* is accepted to be Central and West Africa. Most of the types of *Ocimum* develop all through the tropical and subtropical districts of the world. Tulsi leaves contain a dazzling yellow unstable oil, answered to have against bacterial properties and furthermore goes about as bug spray. Tulsi leaves are customarily utilized for getting help from normal cold, bronchitis, hack, and stomach related issues. The quantity of reports on microbes being confined from inside sound plant tissues considered as endophytes is fastly expanding. The interior tissues of the plants give a uniform and safe climate for the endophytes. This benefit imagines the utilization of endophytic microbes for more effective organic control of plant sicknesses. Although, the cooperation between endophytic microorganisms and their host plants isn't completely perceived, numerous strains have been displayed to advance plant development. Some of endophytes might be creating bioactive substances that may have materialness in medication. Thus the investigation of host colonizing microorganisms animating plant development either straight by delivering plant chemicals, supplement take-up and protection from the pressure conditions or in a roundabout way by changing the microbial equilibrium in the rhizosphere for these valuable microbial organic entities could open an intriguing space of examination in restorative plants where just divided data as far as endophyte happens.

The current investigation centers around the separation and portrayal of a portion of the endophytic microbes from *O. sanctum* for their yield-improving capacities both as far as herbage and oil yields.

### II. MATERIAL AND METHODS

#### Isolation of endophytes from plant

Healthy leaves were collected for the isolation of bacterial endophytes as described by Petrini (1991). The leaves were washed in running water. The petioles were paraffin-implanted and went through this battery of arrangements: sterile refined water for 1 moment, ethanol 70% for 1 moment, sodium hypochlorite 3% for 4 minutes, ethanol 70% for 30 seconds and sterile refined water for 6 minutes. The leaves were cut in parts that were subsequently developed for 20 days at 28°C in a potato-dextrose-agar medium or specific agar for actinomycete (AC) (Küster and Williams, 1964). To dispose of the epiphytic microorganisms of *V. divergens* leaves we utilized the decontamination convention of six stages (Bettioli, 2008), in medium AC added of Tetracycline (100 µg/mL) and Cycloheximide (50 µg/mL).

### **Endophytes identification**

An investigation dependent on a polyphasic approach incorporating ordered data, morphological qualities and the sequencing of the ITS1–5.8S–ITS2 of the rDNA or 16S was utilized, as portrayed by Gomes-Figueiredo et al. (2007). Secludes were at first recognized dependent on their minuscule and plainly visible qualities including their morphology and attributes when developed on the accompanying society media: PDA, cereal agar (OA) (20 g l<sup>-1</sup> oat, 20 g l<sup>-1</sup> glucose, 15 g l<sup>-1</sup> agar), malt extricate agar (MEA), and complete medium (CM) (Pontecorvo et al., 1953). Disconnects were hatched for 7 days at 22 or 280C and a 12 h light: 12 h dull photoperiod. The trial configuration was totally randomized with 3 repeats. States were broke down regarding their normal measurement (cm), the part of their lines, the viewpoint and hue of the mycelium, sporulation, mycelium qualities, the creation of acervuli, the tinge of the opposite of the Petri dish, the consistency and shading of the medium, and the size and hue of the conidia. A sum of 20 conidia from each culture medium were seen under light microscopy (x 1000 amplification) subsequent to being developed for 7, 14, and 21 days. Conidia were surveyed concerning their width and length and the length of the apical limbs. The shading of the middle cells was additionally recorded. For actinomycetes distinguishing proof, attributes of states were utilized, after development in AC medium. The disengages Gram-stained were seen under light microscopy (x 1000 amplification).

The growths segregates were arbitrarily chosen as morphotypes as indicated by Arnold et al. (2000), and the endophytes that introduced something like one of the concentrates with antimicrobial action were submitted to distinguishing proof utilizing ITS successions of the rDNA. DNA extraction followed technique depicted by Raeder and Broda (1985), adjusted by GlienkeBlanco et al. (2002). For the organisms, the groundworks V9G (De Hoog et al., 2003) and ITS4 (White et al., 1990) were utilized to enhance the ITS1-5.8S-ITS2 of the atomic ribosomal RNA, in the accompanying response combination (50 µl): 0,2 mM of each dNTP, 1X Tris/HCl, 1.5 mM MgCl<sub>2</sub>, 1.5 U Taq polymerase, 0.06 µM every preliminary and 50ng of DNA; the PCR was prepared in a Mastercycler Gradient (Eppendorf®) with the accompanying project: 94 °C for 2 min toward the beginning followed by 35 patterns of 94 °C for 30 s, 55 °C for 1 min and 72 °C for 1 min and a last expansion of 72 °C for 3 min. For the actinomycete the groundworks Sm6F (5'GGTGGCGAAGGCGGA 3') and Sm5R (5' GAACTGAGACCGGCTTTTTGA 3') were utilized to intensify the 16S rDNA. Intensification conditions followed Arzanlou et al. (2008) for the organisms and Monciardini et al. (2002) for the actinomycete. Amplicons were sequenced utilizing both PCR preliminaries and DYEnamic ET Dye Terminator Cycle Sequencing Kit for MegaBACE (Amersham Biosciences). Arrangements were physically adjusted utilizing Mega v. 5 programming (Kumar et al., 2004) by embeddings holes. The acquired successions were adjusted by existing arrangements at the data set NCBI however the BLASTn program. Phylogenetic examinations of the adjusted arrangement information were performed with PAUP (Phylogenetic Analysis Using Parsimony) v. 4.0b10 (Swofford, 1998).

### **ENDOPHYTES EXTRACTS**

Endophytes were chosen for the extraction of dynamic metabolites by aging. After the development in potato-dextrose-agar medium in Petri dishes for 7-14 days at 28°C, parts of the endophytes with a measurement of 10mm were taken out and planted in Erlenmeyers with 50mL and 100mL of the fluid medium Czapeck (Silva et al., 2004), MPE (Hamada et al., 1974) and malt separate stock (20 g l<sup>-1</sup> malt remove, 1 g l<sup>-1</sup> peptone, 20 g l<sup>-1</sup> glucose), and were brooded at 28°C at 120rpm. The 50mL societies were hatched for 24 hours, while the ones with 100mL of medium were developed for 7 days. After the foreordained period the mycelium was isolated of the metabolic medium by paper Whatman n°4 vacuum filtration and afterward put away. Either compound from the way of life and the ones held on the cell structures were removed with ethyl acetic acid derivation p. a. (EtOAc; Merck). Dissolvable vanishing was done utilizing a rota evaporator at 45°C. The last concentrate was gauged and weakened in methanol, methanolic separates (ME) at a centralization of 10 mg/mL (Corrado and Rodrigues, 2004). The fermentative fluid was lyophilized, gauged and furthermore weakened in ultrapure cleaned water, watery concentrates (AE) at centralization of 10 mg/mL.

### **CROPPING SYSTEM**

Ranchers deal with their harvests and fields through groupings of specialized demonstrations, which are associated since every one of these demonstrations has numerous and delayed consequences for the agroecosystem elements. Some random yield is chosen and overseen comparable to the previous and resulting crops, and a given specialized activity is chosen and executed corresponding to different strategies associated with the harvest the board. This demonstrates the idea of the cropping framework. Here, a cropping framework is considered as a bunch of the board methods applied to guaranteed, consistently treated rural regions; this can be a field, part of a field, or a gathering of fields (Sebillotte, 1990). Following definition, cropping systems – an agronomic thought – are parts of cultivating systems, which allude to

agronomic substances. They are recognized by an arrangement and/or a spatial blend of yields and the comparing specialized activity, including the actual harvests, yet additionally between crop periods with exposed soil or a plant cover. The noteworthy effect of green transformation that put India to 'bread-bin' status from 'asking bowl' inside a brief time of 10 years, predominantly accomplished by thorough act of the rice-wheat framework. Delayed development of the cropping framework represented a genuine test on maintainable creation with specific reference to usefulness, soil ripeness, and vermin issue, vigorous and monetary reasonability. In this part, an exertion has been made to survey the writing with regards to how extraordinary cropping systems do influence the efficiency, biology, economy and energetics for the pursuit of naturally strong, monetarily suitable and ecologically safe practical creations systems.

### **Effect of Cropping System on Productivity of Crops**

Worldwide populace has effectively expanded from 2.5 billion of every 1950 to its present 6.0 billion and is projected to be about 8.0 billion by 2025 (Evans, 1998). Quite a bit of this increment will happen in urban areas of the creating scene with metropolitan populaces multiplying inside 20 years. Rising per capita livelihoods of this urbanized populace will bring about huge expansion in the interest for food harvests, creature and vegetable protein and backwoods items. Extended expansion in human populace along with a further developed eating routine show that ebb and flow creation of harvests and trees should be raised considerably throughout the following not many years.

Expansion in horticultural creation in the past was a direct result of both extensification (modifying regular environment to produce items) and strengthening (delivering a greater amount of the ideal item per unit of space of land previously utilized for agribusiness) where cropping framework assumed a key part. Efficiency of yields in the monoculture has been expanded by presenting different harvests in the framework. Maize yield expanded presenting soybean in the framework by 5-20% over maize monoculture. In the USA under long haul test (LTE) during 1888-1996, yield of maize was lower than that of maize-oat and maize-oat-roughage crop arrangement. In any case, issues of late stagnation/decrease in yield with consistent contribution under LTE in rice-rice and rice-wheat cropping framework are of specific worry among researchers and ranchers. The potential reasons for this yield stagnation/decrease may be because of changes in biochemical and actual piece of soil natural matter, soil richness awkwardness because of steady decrease in the stock of plant supplements, imbalanced utilization of composts, shortage of surface water, ground water and quality water that worry environmental manageability of the framework.

Nonetheless, unique cropping systems have distinctive usefulness levels. Numerous specialists have thought about usefulness capability of various rice-based cropping systems of broadened eco-systems. Singh and Yadav (1988) considered execution of rice-based systems, for example, rice – wheat, rice – grain, rice – chickpeas, and rice – lentil, rice – safflower, rice – *Eruca sativa*, and rice – *Brassica juncea* in stream flood fields of Masodha (Uttar Pradesh). They noticed most noteworthy rice comparable yield in rice – wheat and rice – *Brassica juncea* cropping systems. Kundu (1990) revealed that all out financial yield of rice – potato – maize, rice – potato – jute groupings outperformed among ten rice-based cropping successions at terai zone of West Bengal. He additionally revealed that rice – potato – rice, rice – wheat – rice and rice – wheat – maize were likewise effective cropping arrangements. Joshi and Choudhuri (1993) assessed execution of rice – wheat – green gram, rice – gram – green gram, rice – dark gram – sunflower and rice – neglected – groundnut in order space of Bhandara (Maharashtra). They got higher grain efficiency from rice – neglected – rice and rice – decrepit – groundnut cropping arrangements. Khai et al. (1999) revealed most noteworthy rice identical yield from rice – toria - groundnut cropping framework among six rice-based cropping systems in Pantnagar (Uttaranchal). Parihar et al. (1999) from Bilaspur (Madhya Pradesh) saw that rice – nut was more useful than rice – rice and rice – wheat cropping systems. They detailed least yield from rice – mustard succession. Singh et al. (2002) thought about seven rice-based cropping systems in flood inclined circumstance of Eastern Uttar Pradesh. They announced that the rice – lentil – maize – cowpea (grub) succession gave the most elevated rice comparable yield, trailed by rice – wheat – maize – cowpea (grain). The least grain comparable yield was gotten in rice – sunflower - maize + cowpea (feed). They thought that high worth harvests like lentil and wheat and lower yield of sunflower were the purposes behind yield varieties. Presentation of potato into cropping framework expanded the efficiency of the cropping framework in numerous spaces because of better return of potato. Azad and Nabi (1984) noticed higher usefulness of jute – potato improved cropping design than conventional examples in Bangladesh. Roy et al. (1999) assessed the possibilities of rice – potato – rice in West Bengal over other rice based cropping systems as far as efficiency. Sharma and Goydani (1999) revealed higher potato identical yield in potato-based cropping systems like soybean – potato – okra, maize – potato – green gram over existing maize - wheat – cowpea cropping framework at Chindwara (Madhya Pradesh). Mukhopadhyay and Roy (2000) likewise noticed higher efficiency of potato based cropping successions over wheat – jute – rice grouping at Kalyani (West Bengal). They likewise detailed that potato – jute – rice succession was generally useful among four

groupings considered. Singh et al. (1979) assessed some maize-based cropping systems in Punjab and recorded the usefulness of the framework in the request for cowpea – maize – potato – wheat > moong – maize – potato – wheat > cowpea – maize – wheat > moong – maize – wheat > maize – wheat cropping systems. Xian and Lin (1995) revealed from China that green excrement – rice – rice and rapeseed – rice – rice systems had the most elevated grain efficiency followed by maize – rice and soybean + maize – rice systems. In the USA under extremely long haul try during 1888-1996, yield of maize – maize was lower than that of maize – oat and maize – oat – feed crop groupings (Susan and Wonder, 1998). Raskar et al. (2000) assessed the efficiency of soybean-based cropping groupings under watered conditions in Maharashtra. They revealed that all out grain comparable yield of soybean – chickpea was essentially higher followed by soybean – wheat and they additionally announced least efficiency in groundnut – wheat succession.

There are gives an account of impact of cropping framework on yield of segment crops. assessed five various cropping designs (potato – rice – rice, maize – rice – rice, groundnut – jute – rice, rice – jute – rice and rice – rice) at Central Rice Research Institute, Cuttack (Orissa). They revealed that the yield of kharif rice was most noteworthy in potato – rice – rice followed by maize – rice – rice and rice – rice cropping groupings. They likewise detailed that yield of summer rice was most noteworthy in potato – rice – rice followed by rice – jute – rice framework. Higher plant stature, basal breadth and yield of jute was seen in jute after groundnut than after rice in groundnut – jute – rice and rice – jute – rice cropping successions. Be that as it may, discovered no impact of cropping framework on dry land rice yield among rice – mungbean, rice – cowpea, rice – yam – maize, rice – sorghum – sorghum and rice – maize – cowpea cropping systems in Philippines. Maize and sorghum yield had been expanded by presenting soybean over their monoculture in the USA got higher seed and oil yield of mustard in green fertilizer – mustard followed by cowpea – mustard and green gram - mustard cropping systems. The yield improvement was basically because of expansion in yield parts like number of branches, siliqua and seed weight per plant under green fertilizer – mustard cropping framework. A diminishing pattern in jute and wheat yield was seen in a drawn out jute – rice -wheat cropping framework at Barrackpore (West Bengal). However, the yield of rice was not really influenced which may be because of enhanced soil through normal leaf concealing of recently developed jute crop saw that the rice yield and dry matter were essentially higher in rice – chickpea than rice – neglected framework on an alfisol and a vertisol in Raipur (Madhya Pradesh). The unfriendly impacts of creation systems with high outer information sources, particularly misfortunes of supplements from composts and excrements to conduits and gases to the air could be limited with the utilization of effective yields/assortments to build use proficiency of supplements and water in the method of expanding crop efficiency (Gregory et al., 2002).

### III. CONCLUSION

Modern day scientific research into tulsi demonstrates the many psychological and physiological benefits from consuming tulsi and provides a testament to the wisdom inherent in Hinduism and Ayurveda, which celebrates tulsi as a plant that can be worshipped, ingested, made into tea and used for medicinal and spiritual purposes within daily life. In providing a focus for ethical, sustainable and ecological farming practices that provides a livelihood for thousands of farmers, the cultivation of tulsi goes beyond providing benefits for individuals and households and begins to address broader social, economic and environmental issues.

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