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STUDY ON BACTERIAL ENDOPHYTES OF OCIMUM SANCTUM FROM TWO DIFFERENT AGRO - CROPPING SYSTEMS

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ABSTRACT

Endophytes are helpful bacteria that dwell inside the intercellular spaces of these plants. They take up residence in the spaces between the cells. In order to successfully examine the biological relevance of endophytes, it is essential to keep in mind that these organisms must be able to collaborate with their host plants and must also have the ability to live inside of them. After the leaves of Ocimum sanctum were submitted to surface sterilisation, it was discovered that they contained four endophytic bacteria. In order to distinguish these bacteria, the labels OS-9, OS-10, OS-11, and OS-12 were granted. These isolated bacteria were tested against a variety of phytopathogenic microorganisms using a dual-culture setup. The microorganisms that were tested against these bacteria were Colletotrichum lindemuthianum, Fusarium solani, Alternaria solani, Sclerotium rolfsii, and Rhizoctonia solani at the same time. The strain OS-9 was demonstrated to be effective against R. solani, A. solani, F. solani, and C. lindemuthianum, according to these findings. On the other hand, strain OS-11 had the opposite effect when it came to A. solani. Endophytes have captured the interest of ecologists, chemists, and researchers due to the fact that they remain within the healthy tissues of the host plant without imposing any symptoms on the plant. This is due to the fact that endophytes provide a wide range of potential applications within the realm of biotechnology in the scientific community. According to the findings of a great number of research, endophytes can be discovered in virtually every plant.

Keywords:-endophytes, bacteria.

INTRODUCTION

Bacteria are found in almost every habitat, and they provide services that are not only necessary for the continuation of life but also necessary from an ecological point of view. bacteria are present in practically every ecosystem. There is a presence of bacteria in almost every habitat that people interact with. There is a significant possibility that will be discovered in almost any ecosystem one can imagine. They are the principal decomposers of the complex components of plant detritus, such as cellulose and lignin, and as a result, they play a key part in the process of recycling nutrients in all terrestrial settings. This is because they possess the ability to break down these components. Due to the fact that these organisms are the principal decomposers of plant materials, this is the situation that has arisen. It is mostly due to the fact that they are the principal organisms that are responsible for the breakdown of plant matter why this is the case. That are saprotrophic are an important component of the nutrient cycle because they are heterotrophic organisms that devour opportunistic species. This makes them a crucial component of the cycle. As a consequence of this, they comprise an important part of the cycle. To add insult to injury, as a direct result of this, they comprise

an essential component of the system. Because carbon is stored in the tissues of plants, including wood and other plant tissues, it is feasible to achieve this goal. This is because carbon is stored in the tissues of plants.

OBJECTIVES

To study the endophytes.

To study identification of bacteria in the end of the body.

The endophytes

Endophytic microorganisms, my dear interlocutor, are a fascinating group of microbes that exhibit the remarkable behaviour of investing their entire life cycle, or at least a portion thereof, in the colonisation of the hale and hearty tissues of the host plant. This colonisation can occur either within the cells themselves, known as intracellular colonisation, or in the spaces between the cells, aptly referred to as intercellular colonisation. Endophytes, which can be encountered in both terrestrial and aquatic environments, are known to inhabit both soil and water. Both the soil and the water possess the inherent capacity to serve as potential habitats for endophytes. The potential habitats for endophytes encompass both the terrestrial medium of soil and the aquatic medium of water within the surrounding environment. According to Rodrigues, it is he who is attributed with the distinction of being the individual responsible for the initial utilisation of the term "endophyte" in the year 1866. The credit for the creation of De Bary is attributed to De Bary. The nomenclature initially employed to designate all organisms that were unearthed within the anatomical structures of plants was the same as the one presently under discussion. In an effort to establish a clear distinction from epiphytes, which are organisms that thrive upon the external surfaces of plants, the aforementioned action was undertaken. The etymology of the term "endophyte" can be traced back to its Greek origins. Specifically, it is a compound word derived from the Greek terms "endon," denoting the internal or inner aspect, and "phyton," which pertains to the plant kingdom. The term "endophyte," which derives from either of these two terms, corresponds to the literal translation of the sentence in question. The term "endophyte" was initially employed to denote the amalgamation of these two concepts. Throughout various contexts, the term in question has been employed to describe a multitude of diverse scenarios. Based on the established definition, the term in question encompasses a variety of organisms, including bacteria, fungi, and actinomycetes, which reside within the plant's tissues without manifesting any apparent indications of disease.

According to Feller, the all-encompassing connotation of the term pertains to the incorporation of insects within its definition. Based on the findings of Saikkonen et al. and Bacon and White, it has been observed that endophytic fungi, which are characterised by their ability to inhabit plant tissues without inducing any noticeable symptoms, have been identified across a wide range of plant species. This phenomenon can be attributed to the fact that endophytic microorganisms do not elicit any discernible symptoms. The finding was produced by researchers hailing from both of these esteemed institutions. Wilson, in his pioneering work, was the individual who initially employed the term to denote an assemblage of bacteria and fungi that possess the remarkable ability to endure within the plant tissues for the entirety or a portion of their life cycle, all the while remaining devoid of any discernible indications or symptoms. The aforementioned phrase was initially employed with the intention of denoting the assemblage. Nevertheless, it is worth noting that certain mycologists to restrict the application of the designation "endophyte" exclusively to those fungi

that inhabit host organisms in the guise of mycelium cells. According to their perspective, it is postulated that this particular approach represents the sole method to adequately expound upon the intricacies of endophytes. However, it is important to note that this assertion stands in opposition to the broader and more general interpretation of the term "endophyte." The term "endophytes" was subsequently expanded upon by Hyde and Soytong, esteemed scholars who also played a pivotal role in highlighting the notion initially articulated by Petrini. Hyde and Soytong, esteemed scholars in their field, can be attributed to the advancement and subsequent establishment of the aforementioned concept. The statement posits that endophytes encompass all organisms that reside within plant organs and possess the ability to establish colonisation within internal plant tissues without manifesting any discernible detriment to the host. Endophytes, as specialised organisms, establish their residence within the various organs of plants. The term "endophytes" pertains to multicellular organisms that reside within the anatomical structures of plants.

Endophytes of various categories

The flip side of the coin is that there are a considerable number of endophytic of ocimum sanctumthat are members of the ascomycetes or the mitosporic fungi that they produce. The fungi in question are referred to asbacterial endophytic. There are two different kinds of bacteria that may be discovered among these bacterial species. These are the coelomycetes and the hyphomycetes. Basidiomycetes are considered to be endophytic; however, other species, such as zygomycetes, are only seen seldom. It is important to note that basidiomycetes are categorised as endophytic fungi. These are some extra points of interest to consider. The group of endophytic bacteria includes basidiomycetes, which are also included in this group. Basidiomycetes are one of the groups that make up this group. The existence of endophytes has been found in plants that have grown naturally in forests that are either tropical, subtropical, or temperate. These woods are often found in tropical regions. As a result of the fact that these forests are classed as tropical, subtropical, and temperate, the species that are found inside them are classified in a manner that is distinct from one another. The plants that are classified as herbaceous are the ones that are included in this category. These plants have the potential to be found in a broad variety of habitats, such as xerophytic environments severe arctic alpine conditions mesic temperate woodlands, and tropical forests. It is very probable that they are found in all of these ecosystems. This is only a small selection of the possible locations.

A plethora of angiosperms and gymnosperms, including but not limited to tropical palms, broad-leaved trees, estuarine plants, diverse herbaceous, deciduous, and evergreen perennials, as well as marine algae, lichens, mosses, ferns, and an extensive array of other plant taxa, have been found to possess endophytic bacteria. There are a plethora of mechanisms that are in existence for the interaction between endophytes and their respective host plants.

According to the results of research that was conducted out by Petrini and , endophyte interactions have been observed from plant hosts inhabiting temperate zones. The presence of these connections between the two variables has been shown by a number of investigations that have been carried out. On the other hand, Rodrigues and Petrini point out that the endophytic variety of tropical plants has not been given the same level of attention as the plant diversity that can be found across the tropics. This is due to the fact that the endophytic variety of tropical plants is not as famous as other types of plants. The endophytes that are indigenous to tropical regions have only been the subject of a small number of scientific discoveries and investigations up to this point.

Plants used in medicine

Joseph and Priya indicate that in the twenty years that have gone since the beginning of the twenty-first century, there has been a significant increase in the number of complementary and alternative therapies that have gained popularity. This is something that has occurred. The usage of medications that are derived from natural sources has a significant influence on the treatment and prevention of human illnesses. This is because these pharmaceuticals are more effective than synthetic ones. This is the case since these drugs are obtained from natural sources, which leads to the aforementioned situation. Over sixty-one percent of the novel medications that were generated between the yearswere derived from natural components. This is a significant portion of the total. In terms of percentage, this figure was somewhat higher than sixty-one percent. According to Cragg and Newman , these medications have shown a high degree of efficacy, notably in the treatment of infectious diseases while also being effective in the treatment of cancer and other conditions. Furthermore, Joseph and Priya indicate that more than ninety percent of the vocabulary that is documented in the medical literature of India is obtained from plant sources. There is a significant amount of plant-based terminology. The study conducted by the writers is the source of this information. According to Joseph et al. (2015), there has been a growing amount of interest in the investigation of a significant number of uniquely occurring bioactive chemicals that are created from plants. These compounds are formed from plants. This concentration has been steadily increasing over time. Taking this into consideration, it is important to point out that the rate at which active new chemical entities are being found has been slowing down over the course of the last several year .

Identification of bacteria in the end of the body

The identification of bacterial isolates was accomplished through the examination of various morphological characteristics, including the shape of the culture colony or hyphae, the features exhibited by the spores, and the reproductive structures present. The utilisation of conventional identification guides was employed for this particular objective. In light of the seminal works by Barnett and Hunter (1998) and Nagamani et al. (2006), subsequent investigations in Chapter Three employed molecular techniques to validate the identities of several noteworthy groups. The isolated of ocimum sanctum, regrettably, failed to manifest any discernible indications of sporulation and, quite disappointingly, persisted in a state of sterility throughout the entirety of the aforementioned process.

The sterile ocimum sanctum were cultivated on different mediums, including malt extract agar (MEA) and 2% agar (consisting of two grammes of agar and one hundred millilitres of water). Subsequently, an assessment was conducted to determine their ability to sporulate. Following the aforementioned procedure, the slides were meticulously prepared by being mounted in lactophenol and subsequently sealed with DPX. Every single experiment was conducted at least twice. The cultures lacking the ability to produce reproductive structures and undergo sporulation were designated as mycelia sterilia. The aforementioned cultures were subsequently categorised into multiple morphospecies, predicated upon the cultural attributes they exhibited.

The isolating of the endomyelitis fascia

To mitigate desiccation of the botanical specimens, the collection process involved the utilisation of either paper bags or perforated poly bags. Upon the completion of the sample collection process, it was deemed

necessary to subject the samples to a meticulous rinsing procedure utilising a continuous flow of water emanating from the tap. One of the paramount facets pertaining to the investigation of endophytes involves the imperative task of ascertaining the veracity of the fungi that have been isolated, specifically in terms of their genuine origin from within the host organism under scrutiny. In their respective studies, Arnold et al. (2000) and Nithya and Muthumary (2011) undertook an investigation wherein the samples were meticulously sectioned into segments ranging from 2 millimetres to 5 millimetres in length.

The aforementioned segments underwent surface sterilisation through immersion in a solution of 0.5% sodium hypochlorite for a duration of two minutes, followed by immersion in 70% ethanol for an additional two minutes. Subsequent to this step, the specimens underwent a thorough rinsing process utilising sterile water, followed by a period of desiccation on a sterile surface, all while maintaining aseptic conditions. After the elimination of any excess water from the botanical specimens, they were subjected to a desiccation process within a controlled laminar airflow chamber, wherein they were carefully placed upon sterile blotting paper sheets. The segments that had undergone surface sterilisation were subsequently placed onto Petri plates containing Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and Water Agar medium (WA). The Petri plates were effectively sealed using Parafilm, a commonly employed material for this purpose. Subsequently, the sealed dishes were carefully transferred to an incubator, where they were maintained at ambient temperature. The objective of this incubation period was to facilitate the emergence of bacterial growth, which was the desired outcome of the experiment.

To effectively track the progression of endophytic ofocimum sanctum, it was imperative to conduct regular examinations of the plant segments, as depicted in Figure 2.1. Upon the emergence of the hyphal tips from the plated segments, they were expeditiously transferred onto a Potato Dextrose Agar (PDA) slant, where they were subsequently maintained at a temperature of 4 degrees Celsius. The endophytes exhibited sporulation within a span of several weeks, specifically under temperatures ranging from 18 to 21 degrees Celsius. This sporulation occurred regardless of the presence of light, as it was observed both in the absence of light and under direct sunlight. The medium used for this observation was a 1% malt extract agar (MEA) solution. Throughout the operations that were conducted, a commendable degree of sterility was diligently upheld. Through careful examination and analysis of the morphological and cultural characteristics, including form, colour, and size, we were able to identify and classify the isolated of ocimum sanctum. The aforementioned task was successfully executed through a meticulous examination of the cultural expansion. In order to induce sporulation, it is imperative to transfer non-sporulating isolates onto a 2% agar medium. This particular medium is composed of 2 grammes of agar-agar meticulously dissolved in 100 millilitres of water.

Both the isolating and identifying of endophytic bacterial structures

The diligent implementation of aseptic techniques yielded a commendable outcome, as evidenced by the acquisition of a grand total of 335 distinct bacterial isolates. In the pursuit of ascertaining the identity of the bacteria that were subjected to separation, it became imperative to duly consider not only the colony morphology but also various other cultural factors. The aforementioned criteria encompassed the dimensions, morphology, and pigmentation of the spores. The dataset consisted of a grand total of 335 isolates, with the noteworthy observation that sporulation was observed in a substantial proportion of 270 of these isolates. The 60 remaining isolates have been duly verified as non-sporulating bacteria. The 335 endophytic bacterial isolates, which were obtained from 37 medicinal plants, have been categorised into 24

unique taxa. This classification was determined by analysing the characteristics of their culture colonies and/or reproductive structures. The aforementioned characteristics were employed in order to ascertain the classification of the isolates. The subsequent paragraphs contain comprehensive details regarding each taxon that has been identified solely based on its morphology.

Acremonium sclerotigenum, as described by W. Gams

The presence of mycelium that exhibits a fluffy and white morphology confers advantageous attributes to the expeditious growth and proliferation of colonies on the medium known as malt extract agar (MEA). The colonies achieved a diameter of five centimetres within a mere seven-day period subsequent to their establishment. One of the distinguishing characteristics of Acremonium sclerotiginum, in comparison to other fungal species, is the presence of elongated awl-shaped phialides. These phialides produce cylindrical, single-celled conidia that tend to aggregate in slimy clusters at the apex of each phialide. Additionally, these conidia possess a basal septum.

Keissl'sfrench name for alternariaalternata.

Over the span of one week, under ambient conditions, colonies cultured on MEA media exhibited a discernible growth, attaining a diameter of precisely 6.0 centimetres. Typically, conidiophores and conidia exhibit a hue reminiscent of medium golden brown. The conidiophores, as commonly observed, exhibit a rather uncomplicated morphology. They tend to possess a linear or curved structure, with a range of one to three septa present. In terms of dimensions, they measure up to fifty micrometres in length and three to six micrometres in width. Additionally, these conidiophores are characterised by the presence of one or more apical conidial pores. The conidia, commonly known as spores, exhibit a variety of shapes, including ovoid, obclavate, obpyriform, and occasionally ellipsoidal. These structures possess a prominent basal pore and may or may not possess a short conical or cylindrical apical beak, which does not exceed one third of the length of the conidium. The conidia are of a medium brown hue and possess a smooth outer wall. Furthermore, they display slight constriction at the three to eight transverse septa. In the lower portion of the conidium, one or two longitudinal septa can be observed. The formation of conidia occurs in elongated chains that often exhibit branching patterns.

CONCLUSION

When it comes to the treatment of a variety of illnesses, there is an immediate need to discover secondary metabolites that are produced from natural sources. The endophyte of ocimum sanctumhas been recognised as an essential source for the creation of secondary metabolites, and it has also been recognised as the model for determining the metabolic route of those compounds that are originated spontaneously. The studies that were discussed before indicate that endophytes of ocimum sanctumplay a significant part in the production of pharmaceutically significant chemicals that are comparable to the molecules that their host produces. On the other hand, there is still a long way to go before the commercial manufacturing of the naturally derived substance could be considered successful. With the help of this review, we have attempted to throw some light on the relationship between desert plants and fungal endophytes. The Thar Desert in Rajasthan is recognised for its dry habitat, which is a rich repository of ethnomedicinal plants. These plants are employed not only as a herbal medication, but also as a possible source of gum, resin, essential oils, tannin, and other substances. A multitude of pharmaceutically significant chemicals, including alkaloids, peptides,

flavonoids, phenolics, taxol, camptothecin, and others, have been identified from the ethnomedicinal plants of the Thar Desert, according to various pieces of published literature. As a result of the fact that the development of antibiotic resistance in human pathogenic bacteria has become a significant challenge for researchers, there is a significant need for the identification of innovative therapeutic molecules that are obtained from natural sources. Because endophytic of ocimum sanctum have been shown to possess a wide range of characteristics, including antibacterial, anti-diabetic, anti-cancer, anti-tumor, anti-fungal, anti-malarial, antioxidant, antiviral, and immunomodulatory activities, endophytic microflora has the potential to be an excellent alternative source of chemicals that are of significant importance in the pharmaceutical industry. The findings of these investigations, which investigate the part that endophytes of ocimum sanctumplay in the manufacture of bioactive compounds, provide the groundwork for more research that will be focused towards the studies that will scale up their production. The production of endophytic microflora-derived pharmaceutically important compounds may be scaled up in the future if an attempt is made to have an advanced molecular level and genetic level research to identify the regulatory gene of the biosynthetic pathway of metabolite construction. This would also improve our understanding of endophytic biodiversity for the benefit of human welfare.

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