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COMPARATIVE AND FUNCTIONAL BIO - PROSPECTION OF BACTERIAL ENDOPHYTES OF OCIMUM SANCTUM FROM TWO DIFFERENT AGRO - CROPPING SYSTEMS

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ABSTRACT

These plants contain beneficial microorganisms known as endophytes, which live within the intercellular spaces of the species. These organisms make their homes in the gaps that exist between the cells. In order to properly investigate the biological significance of endophytes, it is vital to keep in mind that these organisms must be able to work together with their host plants and must also be able to exist inside of them. This would be a prerequisite for conducting a successful investigation. Ocimum sanctum leaves were subjected to surface sterilisation, and the results of this process revealed that the leaves harbored four endophytic bacteria. The designations OS-9, OS-10, OS-11, and OS-12 were assigned to these bacteria so that they could be distinguished from one another. Through the utilization of a dual-culture setup, these isolated bacteria were examined for their ability to combat a wide range of phytopathogenic microbes. At the same time, the microorganisms that were examined for their ability to combat these bacteria were Colletotrichum lindemuthianum, Fusarium solani, Alternaria solani, Sclerotium rolfsii, and Rhizoctonia solani. These data indicate that the strain OS-9 was successful in combating R. solani, A. solani, F. solani, and C. lindemuthianum. This was proved through the test results. Instead, strain OS-11 had the opposite effect on A. solani. This was the case when it came to the organism. Endophytes have piqued the curiosity of ecologists, chemists, and researchers due to the fact that they are able to survive within the healthy tissues of the host plant without causing any symptoms to be displayed by the plant. The reason for this is that endophytes offer a diverse variety of possible applications within the field of biotechnology, which is something that the scientific community is already familiar with. There are endophytes that can be found in nearly every plant, as indicated by the findings of a large number of studies.

Keywords:- *endophytes, bacteria.*

INTRODUCTION

There are bacteria in practically every environment, and they perform services that are not only essential for the maintenance of life, but also essential from an ecological point of view. Bacteria are found in almost every habitat. There is evidence of the presence of bacteria in virtually every habitat. On the other hand, germs can be found in virtually every environment that people come into contact with. There exists a substantial potential that will be found in virtually every ecosystem that one can conceive of. This is because they are the primary decomposers of the complex components of plant detritus, such as cellulose and lignin, and as a consequence, they play an important role in the process of recycling nutrients in all terrestrial environments. This is due to the fact that they have the capability of disassembling these components separately. As a result of the fact that these organisms are the primary decomposers of plant materials, this circumstance has come about as a result. This is mostly owing to the fact that they are the primary organisms that are accountable for the decomposition of plant materials, which is the reason why this is the case. That are saprotrophic are an essential part of the nutrient cycle because they are heterotrophic organisms that consume opportunistic species. This makes them an essential component of biodiversity. On because of this, they constitute an essential part of the cycle. Consequently, they constitute a significant component of the cycle as a result of this. Furthermore, as a direct consequence of this, they constitute an integral component of the system, which adds a further insult to injury. It is possible to accomplish this objective due to the fact that carbon is stored in the tissues of plants, including wood and other plant tissues. Carbon is deposited in the tissues of plants, which is the reason for this phenomenon.

OBJECTIVES

1. To study the endophytes.
2. To study identification of bacteria in the end of the body.

The endophytes

My dear interlocutor, endophytic microorganisms are a fascinating group of microbes that demonstrate the amazing behavior of investing their whole life cycle, or at least a portion thereof, in the colonization of the healthy and robust tissues of the host plant. This is a wonderful behavior that gives endophytic microbes their unique characteristics. On the other hand, this colonization can take place either inside the cells themselves, which is referred to as intracellular colonization, or in the spaces between the cells, which is appropriately referred to as intercellular colonization. Endophytes are known to live in both soil and water, and they can be found in both terrestrial and aquatic environments thanks to their ability to adapt to their surroundings. Both the soil and the water have the intrinsic capacity to serve as possible habitats for endophytes. This capacity is present in both environments. The potential habitats for endophytes include both the terrestrial medium of soil and the aquatic medium of water that are present in the environment that surrounds them. According to Rodrigues, it is he who is attributed with the distinction of being the man responsible for the inaugural utilization of the term "endophyte" in the year 1866. The conception of De Bary is credited to De Bary, who is also the one who got the credit. The terminology that was initially used to designate all of the creatures that were discovered within the anatomical structures of plants was identical to the one that is currently being discussed. The aforementioned action was carried out in an effort to make a definite distinction from epiphytes, which are organisms that flourish on the surfaces of plants that are exposed to the environment. The term "endophyte" may be traced back to its Greek roots, which is where its etymology can be investigated. To be more specific, it is a compound word that is formed from the Greek words "endon," which refers to the internal or inner aspect, and "phyton," which refers to the plant world. There is a direct correspondence between the literal translation of the line in question and the term "endophyte," which is derived from one of these two additional terms. When these two ideas were first combined, the name "endophyte" was used to describe the resulting entity. A wide variety of different situations have been described using the term in issue, which has been used in a variety of different different settings. In accordance with the definition that has been defined, the term in question comprises a wide range of organisms, including as bacteria, fungi, and actinomycetes, that are found within the tissues of the plant without exhibiting any obvious signs of disease.

As far as Feller is concerned, the all-encompassing sense of the phrase is connected to the fact that it includes insects as part of its definition. According to the results of Saikkonen et al. and Bacon and White, it has been discovered that endophytic fungi, which are distinguished by their capacity to inhabit plant tissues without generating any obvious symptoms, have been identified across a wide variety of plant species. This is known as endophytic fungi. It is possible that this phenomena is due to the fact that endophytic microbes do not cause any symptoms that may be identified. Researchers associated with each of these prestigious institutions were responsible for the discovery that was made. Wilson, in his pioneering work, was the individual who first used the term to refer to a collection of bacteria and fungi that have the remarkable ability to survive 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. This was the way that the term was initially used. In the beginning, the phrase that was mentioned above was used with the goal of referring to the collection. However, it is important to note that some mycologists limit the use of the term "endophyte" to only those fungi that live inside of host species in the form of mycelium cells. This is something that should be taken into consideration. Their point of view is that this particular strategy is the only method that can sufficiently elaborate on the complexities of endophytes. This is the hypothesis that they have put out. On the other hand, it is essential to point out that this assumption is in direct contrast to the more expansive and all-encompassing understanding of the term "endophyte." Hyde and Soytong, two highly regarded academics, were the ones who extended the meaning of the term "endophytes" in the years that followed. They were also instrumental in bringing attention to the concept that Petrini had previously stated. Both Hyde and Soytong, who are well regarded academics in their respective fields, are responsible for the development of the notion that was discussed earlier and its subsequent establishment. The statement asserts that endophytes encompass all organisms that dwell within plant organs and possess the ability to establish colonization within interior plant tissues without displaying any visible damage to the host. Endophytes are specialized organisms that develop their nests within the numerous organs of plants for which they are responsible. For the purpose of this discussion, the term "endophytes" refers to multicellular organisms that are found within the anatomical structures of plants.

Endophytes of various categories

On the other hand, there are a significant number of endophytic organisms that are found in Ocimum sanctum. These endophytes are either members of the ascomycetes or the mitosporic fungus that they produce. The fungus in question are known as bacterial endophytes inside the bacterial community. Among these bacterial species, there are two distinct types of bacteria that have the potential to be found. The coelomycetes and the hyphomycetes are the organisms in question. It is generally accepted that basidiomycetes are endophytic; however, some species, such as zygomycetes, are only observed in relatively infrequent instances. Endophytic fungi are the category that basidiomycetes fall under, which is a crucial fact to keep in mind. These are some additional details that should be taken into consideration. Within the category of endophytic bacteria, basidiomycetes are also included. This category also encompasses other types of bacteria. One of the groupings that are included in this category is the basidiomycetes taxonomic group. Endophytes have been discovered in plants that have grown naturally in forests that are either tropical, subtropical, or temperate. These woods have been tested for their presence of endophytes. In tropical places, you can frequently find these types of woods. Because these forests are defined as tropical, subtropical, and temperate, the species that are found within them are classified in a manner that is distinct from one another. This is because these forests are classified according to their climate. In this group, the plants that are included are those that are classed as herbaceous according to the classification system. These plant species have the ability to be found in a wide range of habitats, including xerophytic settings, severe arctic alpine conditions, mesic temperate woodlands, and tropical forests, among others. It is quite likely that this species can be found in each and every one of these environments. This is but a small selection of the various locales that could be chosen.

There are a wide variety of angiosperms and gymnosperms that have been discovered to contain endophytic bacteria. These include, but are 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 a wide variety of other plant populations. In order to facilitate the interaction that occurs between endophytes and the plants that serve as their hosts, there are a multitude of mechanisms that are now in existence.

Interactions between endophytes and plant hosts that are found in temperate regions have been noticed, as indicated by the findings of study that was carried out by Petrini and. Numerous research that have been carried out have demonstrated that these linkages between the two variables are there. These connections have been presented as evidence. However, 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 something that has been a subject of contention between the two groups. This is because the endophytic variety of tropical plants is not as well-known as other kinds of plants. This is the reason for this. The endophytes that are native to tropical climates have only been the focus of a limited number of scientific discoveries and inquiries up to this point in time to this point.

Plants used in medicine

Joseph and Priya state that in the twenty years that have passed since the beginning of the twenty-first century, there has been a considerable growth in the number of complementary and alternative therapies that have acquired popularity. This is something that has occurred. The occurrence of this is something that has done. It is important to note that the utilization of pharmaceuticals that are generated from natural sources has a substantial impact on the treatment and prevention of illnesses that concern humans. This is due to the fact that these botanical medicines are more efficient than their synthetic counterparts. In light of the fact that these pharmaceuticals are derived from natural sources, the situation described above brings about the aforementioned circumstance. More than sixty-one percent of the unique drugs that were developed throughout the years were derived from natural components. these medications were developed. Within the whole sum, this constitutes a sizeable chunk. When expressed as a percentage, this number was slightly higher than sixty-one percent. According to Cragg and Newman, these drugs have demonstrated a significant degree of success, particularly in the treatment of infectious disorders. In addition, they have been found to be effective in the treatment of cancer and other conditions. Additionally, Joseph and Priya state that more than ninety percent of the vocabulary that is documented in the medical literature of India is taken from plant sources. This information is according to the findings of the two individuals. There is a substantial number of terminology concerned with plant-based foods. This information was obtained from the study that was carried out by the authors. There has been an increasing degree of interest in the examination of a considerable number of bioactive compounds that are formed from plants, as stated by

Joseph et al. (2015). These chemicals are created from plants. Plants are the source of these biochemical substances. For a considerable amount of time, this concentration has been progressively growing. After taking all of this into consideration, it is essential to highlight the fact that the rate at which active new chemical entities are being discovered has been decreasing over the course of the past few years.

Identification of bacteria in the end of the body

The identification of bacterial isolates was performed by analyzing a variety of morphological traits. These criteria included the shape of the culture colony or hyphae, the characteristics displayed by the spores, and the reproductive structures that were present. For the purpose of accomplishing this particular objective, the utilization of traditional identifying guides was utilized. Following the seminal works of Barnett and Hunter (1998) and Nagamani et al. (2006), additional investigations in Chapter Three utilized molecular techniques to validate the identities of several significant groups. These investigations were carried out in light of the aforementioned works. The isolates of ocimum sanctum, regrettably, failed to display any obvious indicators of sporulation and, rather disappointingly, maintained in a condition of sterility throughout the whole of the aforementioned process.

Several alternative mediums were used to develop the sterile ocimum sanctum. These mediums included malt extract agar (MEA) and 2% agar, which was composed of two grammes of agar and one hundred millilitres of water. Consequently, an evaluation was carried out in order to ascertain whether or not they were capable of sporulation. By mounting the slides in lactophenol and then sealing them with DPX, the slides were methodically prepared in accordance with the procedure that was described before. At least two separate experiments were carried out for each and every one! The cultures that were unable to generate reproductive structures and go through the process of sporulation were referred to as mycelia sterilia. Following that, the aforementioned cultures were classified into a number of different morphospecies, with each morphospecies being determined by the cultural characteristics that they displayed.

The isolating of the endomyelitis fascia

During the process of collecting the botanical specimens, either paper bags or perforated poly bags were used. This was done to prevent the specimens from drying out. Following the completion of the technique for collecting samples, it was determined that it was necessary to expose the samples to a rigorous rinsing operation that made use of a continuous flow of water coming from the tap. The research of endophytes involves a number of important aspects, one of the most important of which is the task of determining the authenticity of the fungi that have been isolated, particularly with regard to the fact that they originated from within the host organism that is being investigated. Arnold et al. (2000) and Nithya and Muthumary (2011) conducted an inquiry in which the samples were methodically sectioned into segments ranging in length from 2 millimeters to 5 millimetres. This was done in the context of their respective research.

The aforementioned segments were subjected to surface sterilisation by first being submerged in a solution of 0.5% sodium hypochlorite for a period of two minutes, and then being submerged in ethanol at a concentration of 70% for an additional two minutes. Following this stage, the specimens were subjected to a comprehensive rinsing procedure that was carried out with the use of sterile water. This was then followed by a time of desiccation on a sterile surface, all of which was carried out while the conditions were kept aseptic. Immediately following the removal of any surplus water from the botanical specimens, they were put through a desiccation procedure within a controlled laminar airflow room. During this process, the specimens were carefully placed on sterile blotting paper sheets. After the segments had been subjected to surface sterilization, they were subsequently positioned on Petri plates that contained Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and Water Agar medium (WA). It was possible to successfully seal the Petri plates by utilizing Parafilm, which is a material that is frequently used for this purpose. After that, the dishes that had been sealed were carefully moved into an incubator, where they were kept at the temperature that was considered acceptable for the environment. The incubation period was designed to assist the appearance of bacterial growth, which was the expected outcome of the experiment. The purpose of this incubation period was accomplished.

To successfully monitor the development of endophytic ocimum sanctum, it was necessary to carry out routine inspections of the plant segments, as shown in Figure 2.1. This was done in order to prevent any potential complications. Upon the emergence of the hyphal tips from the plated segments, they were swiftly moved onto a Potato Dextrose Agar (PDA) slant, where they were thereafter maintained at a temperature of 4 degrees Celsius. Endophytes displayed sporulation throughout a span of several weeks, notably under temperatures ranging from 18 to 21 degrees Celsius. This occurred under the conditions of the experiment. The sporulation that was observed took place regardless of whether or not there was light present, since it was observed both in the absence of light and in the presence of direct sunshine. Malt extract agar (MEA) solution containing one percent was the medium that was utilized for this discovery. The level of sterility that was maintained throughout the operations that were carried out was commendable and was maintained with great care. We were able to identify and categorize the isolated ocimum sanctum by conducting a thorough investigation and analysis of the morphological and cultural characteristics, which included the form, color, and size of the organism. An in-depth analysis of the cultural expansion was the key to successfully completing the work that was indicated earlier. For the purpose of inducing sporulation, it is absolutely necessary to transfer non-sporulating isolates onto a medium consisting of 2% agar. This particular medium is made up of two grams of agar-agar that has been carefully dissolved in one hundred milliliters of water.

Both the isolating and identifying of endophytic bacterial structures

A noteworthy result was achieved as a result of the meticulous use of aseptic procedures, which was demonstrated by the acquisition of a total of 335 different bacterial isolates. In the process of determining the identity of the bacteria that were separated, it became necessary to take into account not only the colony shape but also a variety of other cultural variables. This was done in order to ensure that the correct identification was achieved. The size, shape, and coloration of the spores were all important aspects that were considered in the aforementioned criteria. It is important to highlight that sporulation was found in a significant proportion of 270 of the isolates that were included in the dataset, which comprised of a grand total of 335 isolates. All sixty of the remaining isolates have been adequately confirmed to be bacteria that do not produce spores. Three hundred and thirty-five endophytic bacterial isolates, which were collected from thirty-seven different medicinal plants, have been classified into twenty-four distinct taxa. Through an examination of the properties of their culture colonies and/or reproductive structures, this categorization was arrived at. In order to determine the classification of the isolates, the features that were discussed before were utilized. Detailed information regarding each taxon that has been recognized purely on the basis of its morphology is provided in the paragraphs that follow.

Acremonium sclerotigenum, as described by W. Gams

The presence of mycelium that has a fluffy and white appearance provides advantageous features to the rapid growth and proliferation of colonies on the medium known as malt extract agar (MEA). These colonies can be found on the media. Seven days after their creation, the colonies had already reached a diameter of five centimeters. This was accomplished within a short amount of time. When compared to other species of fungi, Acremonium sclerotiginum is distinguished by the presence of elongated awl-shaped phialides. This is one of the defining characteristics of this species. Conidia that are cylindrical and composed of a single cell are produced by these phialides. These conidia have a tendency to form slimy clusters at the center of each phialide. Additionally, these conidia have a septum that is located at the base.

Keissl's french name for alternaria alternata.

A visible growth was observed in colonies that were cultivated on MEA media over the course of one week, and the colonies reached a diameter of precisely 6.0 centimeters. This development occurred under ambient circumstances. The color of conidiophores and conidia is typically described as having a hue that is comparable to a medium golden brown. As is usually noticed, the conidiophores have a morphology that is not overly complicated. They have a tendency to have a structure that is either linear or curved, and they can comprise anywhere from one to three septa. The length of these objects can reach up to fifty micrometers, and their width can range anywhere from three to six micrometers. Furthermore, these conidiophores are distinguished by the presence of one or more apical conidial pores, which are included in their characteristics. There are many other shapes that the conidia, which are more generally referred to as spores, can take. Some of these shapes include ovoid, obclavate, obpyriform, and even ellipsoidal. These structures include a conspicuous basal pore and may or may not have a short conical or cylindrical apical beak, which does not surpass one third of the length of the conidium. Additionally, the coneidium does not exceed one third of its length. The conidia have a smooth exterior wall and range in color from a medium brown to a lighter brown. Additionally, they exhibit a small constriction at the three to eight transverse septa located in their bodies. There are one or two longitudinal septa that can be seen in the lower region of the conidium. The production of conidia takes place in chains that are elongated and frequently have branching patterns.

CONCLUSION

The discovery of secondary metabolites that are formed from natural sources is an urgent necessity in the field of medicine, as it pertains to the treatment of a wide range of disorders. Both the endophyte of Ocimum sanctum, which has been recognized as a crucial source for the production of secondary metabolites, and the model for establishing the metabolic route of those chemicals that are created spontaneously, have been recognized as being essential to the process. The investigations that were described earlier reveal that endophytes of Ocimum sanctum play a substantial role in the creation of pharmaceutically relevant chemicals that are comparable to the molecules that their host generates. This shows that endophytes are responsible for the production of these chemicals. On the other hand, there is still a significant amount of work to be done before the commercial production of the naturally derived material can be regarded as a successful endeavor. We have made an effort to shed some light on the interaction that exists between desert plants and fungal endophytes with the assistance of this review. The Thar Desert in Rajasthan is well-known for its dry environment, which is associated with a large number of plants that are used for traditional medicine. Not only are these plants used as a herbal medicine, but they are also utilized as a potential source of gum, resin, essential oils, tannin, and a variety of other chemicals. The ethnomedicinal plants of the Thar Desert have been shown to have a wide variety of chemicals that have important use in the pharmaceutical industry. These substances include alkaloids, peptides, flavonoids, phenolics, taxol, camptothecin, and many others. This information has been established through a variety of published publications. There is a major demand for the identification of novel therapeutic compounds that are obtained from natural sources. This is because the development of antibiotic resistance in human pathogenic bacteria has become a huge issue for researchers. As a result of this, there is a significant need for the identification of these molecules. Endophytic microflora has the potential to be an excellent alternative source of chemicals that are of significant importance in the pharmaceutical industry. Ocimum sanctum endophytes 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. They have also been shown to possess immunomodulatory properties. The outcomes of these investigations, which study the role that endophytes of ocimum sanctum play in the creation of bioactive compounds, provide the framework for more research that will be focused on studies that will scale up their production. These studies will determine how to increase the production of bioactive compounds. The creation of pharmaceutically significant compounds that are obtained from endophytic microflora has the potential to be scaled up in the future if an effort is made to conduct research at both the molecular and genetic levels in order to uncover the regulatory gene of the biosynthetic pathway of metabolite construction. With this, our understanding of the biodiversity of endophytes would also be improved, which would be beneficial to the welfare of humans.

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