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Antimicrobial activity of endophytes isolated from *Eucalyptus alba* and *Ziziphus nummularia*

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Abstract
Endophytic bacteria seem to be distributed in most plant species and have attracted increasing attention as they are efficient producers of antimicrobial agents and benefits plant in the biocontrol process against pathogens that are inhibiting plant growth by causing various diseases. In present study, antimicrobial activity was checked of the endophytes isolated from the *Eucalyptus alba* and *Ziziphus nummularia*. Antimicrobial activity included antifungal activity against *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii* and antibacterial activity against *Agrobacterium tumefaciens, Burkholderia gladioli* and *Erwinia amylovora.* Result of the present work showed that the endophytes do inhibit the growth of the pathogenic fungi and pathogenic bacteria and so to some extent contribute in the biocontrol process of the plants against the pathogens. Endophytes existing in plants have a wide range of antimicrobial strains, which are the important potential sources of antimicrobial substances. So it can be suggested from the result that the bacterial endophytes of *E. alba* and *Z. nummularia* could be a potential source for antimicrobial substances for biotechnological application.

Keywords: *Eucalyptus alba, Ziziphus nummularia,* antifungal activity, antibacterial activity, biocontrol

1. Introduction
Microbes that inhabit asymptomatically in the living tissues of plants without causing any substantive negative effect are known as endophytic microbes (Bacon *et al.*, 2000) [2]. Each plant species that exists on earth is considered to be a host for one or more endophytes (Anu and Priscilla, 2013) [1]. Endophytic bacteria seem to be distributed in most plant species and have been isolated from roots, leaves, and stems, and a few from flowers, fruits, and seeds (Lodewyckx *et al.* 2002) [7]. Endophytic bacteria may accompaniment certain metabolic properties, such as promoting plant growth, controlling soil-borne pathogens, or helping host plant to defeat stress responses to environmental abuse (Mastretta *et al.* 2006; Taghavi *et al.* 2007; Ryan *et al.* 2008) [8, 18, 10]. Plant communities in arid habitat are controlled by the interaction between biotic and physicochemical components of the desert matrix (Read, 1998) [9]. Interactions with microbes appear crucial in obtaining inorganic nutrients or growth-influencing substances (Bahig *et al.*, 2013). Generally, plant growing in unique environmental settings having special ethnobotanical uses having extreme age or interesting endemic locations possess novel endophytic microorganisms which can supply new leads (Bahig *et al.*, 2013).
It is the need of hour to search for new antimicrobial agents because infectious diseases are still a global problem because of the development and spread of drug resistant pathogens (Anu and Priscilla, 2013) [1]. Major active compounds used in medicines are obtained from microbes (Anu and Priscilla, 2013) [1]. Recently, many known as well as new endophytic bioactive metabolites, possessing a wide variety of biological activities as antibiotic, antiviral, anticancer, antiinflammatory, antioxidant, etc., have been identified (Strobel and Daisy 2003) [14, 16]. Endophytes existing in plants have a wide range of antimicrobial strains, which are the important potential sources of antimicrobial substances (Strobel 2003) [14, 16]. In present study which was performed at Department of Biosciences in Saurashtra University at Rajkot (Gujarat) India, antimicrobial activity of endophytes isolated from *Eucalyptus alba* and *Ziziphus nummularia* was checked, in which antibacterial activity against plant pathogenic bacteria *Agrobacterium tumefaciens* causes crown-gall, *Burkholderia gladioli* causes scab and *Erwinia amylovora* causes fire blight while antifungal against plant pathogenic fungi *Fusarium oxysporum* causes panama, *Rhizoctonia solani* causes damping off and *Sclerotium rolfsii* causes southern blight was checked. The study of endophytic bacteria is a challenging field of research even though the attempts to use endophytic bacteria for the production of bioactive compounds have been promising (Anu and Priscilla, 2013) [1].

2. Methodology
Antimicrobial activity was performed of the endophytic cultures isolated from *E. alba* and *Z. nummularia* to check whether they can able to inhibit the growth of the pathogenic fungi and pathogenic bacteria or not.

2.1 Antifungal activity
Antifungal activity (Coombs *et al.*, 2004) [4] against *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii* were performed. Potato dextrose agar plates were prepared on which pathogenic fungus was inoculated and other half plate on which endophytic culture was inoculated. These plates were incubated at 28ºC for 72 hours. After incubation these plates were checked for the inhibition of pathogenic fungi which decides the antifungal capacity of endophytic cultures.

2.2 Antibacterial activity
Antibacterial activity was performed (Sessitsch *et al.*, 2004) [13] against *Agrobacterium tumefaciens, Burkholderia gladioli* and *Erwinia amylovora***.** LB agar plates were prepared on which pathogenic bacterium was inoculated and in well endophytic culture was inoculated. These plates were incubated at 28ºC for 72 hours. After incubation these plates were checked for the

inhibition of pathogenic bacteria which decides the antibacterial capacity of endophytic cultures.

3. Result & discussion

Table 1 shows the result obtained from the antifungal tests of endophytes isolated from *E. alba* and *Z. nummularia* which was performed against different plant pathogenic fungi. Table 2 shows the result obtained from the antibacterial tests of endophytes isolated from *E. alba* and *Z. nummularia* which was performed against different plant pathogenic bacteria.

Antimicrobial activity results showed that endophytic cultures were able to inhibit growth of some pathogen while failed to do so in case of some pathogens. So it can be suggested from the result that the bacterial endophytes isolated from *E. alba* and *Z. nummularia* could be a potential source for antimicrobial substances for biotechnological application and helps in biocontrol process of plant against various harmful pathogens which may prove to be beneficial in the growth of plants. It has been earlier reported that some of the endophytic bacteria belonged to members of diverse genera namely *Pseudomonas*, *Burkholderia* and *Bacillus* (Lodewyckx *et al.*, 2002) [7]. These genera are well-known for production of their secondary metabolites namely antibiotics, anticancer agent like Taxol (Strobel *et al.*, 1993) [17] antiviral compound such as Cytonic acid A (Guo *et al.*, 2000) [5] and Cytonic acid B, insecticidal agent like Oocydin (Strobel *et al.*, 2004) [15] and some immune suppressant agents. *Bacillus pumilus* INR7 is an endophytic bacterium that has been commercialized as a biological control product against soilborne pathogens as well as foliar pathogens by direct antagonism and induction of systemic resistance (Jeong *et al.*, 2014) [6]. In wheat, *B. pumilus* also induces plant resistance to Take-all (*Gaeumannomyces graminis*), a fungal disease which can significantly damage wheat crops (Sari, E., *et al.* 2007) [11]. *Bacillus anthracis* is a member of the *Bacillus cereus* group species. The diversity of bacterial endophytes associated with ginseng plants of varying age levels in Korea was investigated. Fifty-one colonies were isolated from the interior of ginseng stems. Isolates E-I-3 (*Bacillus megaterium*), E-I-4 (*Micrococcus luteus*), E-I-8 (*Bacillus cereus*), and E-I-20 (*Lysinibacillus sp.*) were positive for most of the plant growth promoting traits, indicating their role in growth promotion of ginseng (Vendan *et al.* 2010) [19].

Table 1: Result of antifungal activity of endophytic cultures isolated from experimental plants

Endophytic culture	Endophyte isolated from	*Fusarium oxysporium*	*Rhizoctonia solani*	*Sclerotium rolfsii*
Bacillus anthracis	Seed of *E. alba*	-	+	+
Bacillus pumilus	Root of *E. alba*	+	-	+
Lysinibacillus macroides	Seed of *Z. nummularia*	-	+	-

Table 2: Result of antibacterial activity of endophytic cultures isolated from experimental plants

Endophytic culture	Endophyte isolated from	*Agrobacterium tumefaciens*	*Burkholderia gladioli*	*Erwinia amylovora*
Bacillus anthracis	Seed of *E. alba*	-	+	+
Bacillus pumilus	Root of *E. alba*	+	+	-
Lysinibacillus macroides	Seed of *Z. nummularia*	-	+	+

[Note: - (+) shows positive result - inhibited bacteria/fungi growth, (-) shows negative result - not inhibited bacteria/fungi growth.**]**

4. Conclusion

Each plant species that exists on earth is considered to be a host for one or more endophytes. Endophytes existing in plants have a wide range of antimicrobial strains, which are the important potential sources of antimicrobial substances. It can be concluded from the present study that, as bacterial endophytic cultures of *E. alba* and *Z. nummularia* were able to inhibit growth of some pathogen, they could be a potential source for antimicrobial substances for biotechnological application and also help in biocontrol process of plant against various harmful pathogens which may prove to be beneficial in the promotion of plant growth, immunity and increase the resistance to arid and saline conditions which is not only of economic importance but also counted as contribution to the agriculture field.

5. References

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