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Short Communication

ANTIBACTERIAL ACTIVITY OF EDOPHYTES AGAINST OF *SALMONELLA TYPHII* AND *SALMONELLA TYPHIMURIUM*

Faraz Khan*[1], Rajesh Kumar Tenguria[1], Firoz Naem Khan[2], Kamal Uddin Zaidi[2]

[1]*Division of Microbiology, Department of Botany, Govt. Motilal Vigyan Mahavidhyalaya, Bhopal, Madhya Pradesh, India*

[2]*Centre for Scientific Research & Development, People's University, Bhopal, Madhya Pradesh, India*

**Corresponding author: farazkhan020987@gmail.com*

ABSTRACT

The emergence of new diseases and development of drug resistance has increased in recent years. The prime focus is in need for novel and safe bioactive compounds to support all aspects of mankind. Ever increasing diseases throttled search for bioactive compounds and drug development for the public health. The present study was conducted to evaluate the effects of culture filtrate of isolated endophytes; *Melanospora fusispora*, *Alternaria alternata, Fusarium moniliforme* and *Cladosporium Cladosporium* from *Ocimum sanctum* which were able to inhibit the growth of *Salmonella typhii* and *Salmonella typhimurium*. It is concluded that endophytes isolated from *Ocimum sanctum* leaf possess certain compounds with therapeutic value against *Salmonella typhii* and *Salmonella typhimurium*.

Keywords: Typhoid, Antibacterial, Salmonella, Bioactive metabolites, Endohytic Fungi.

1. INTRODUCTION

Foodborne diseases are an important cause of morbidity and mortality worldwide. WHO identifies foodborne disease outbreaks and incidents, including those arising from natural, accidental and deliberate contamination of food, as major global public health threats in the 21st century. World Health Organization (WHO) Global Food borne Infections Network (GFN) indicate that *Salmonella typhimurium* and *Salmonella* ser. *Enteritidis* account for nearly 80% of all human isolates reported globally [1]. *Salmonella typhii* and *Salmonella typhimurium* are rarely encountered outside endemic countries or in returned travelers and have a worldwide distribution. While the true incidence is unknown, there are an estimated 93.8 million episodes and 155,000 deaths each year [2]. Salmonella are facultative intracellular pathogens that can survive within host macrophages, unlike typhoidal Salmonella, which have the ability to evade the immune system, NTS tend to induce a localised inflammatory response in immuno-competent individuals, provoking a large influx of polymer-phonuclear leukocytes to the intestinal lumen. They can also colonize small and large intestinal mucosa thus facilitating prolonged periods of shedding [3]. Transmission is predominantly foodborne, although other modes include consumption of contaminated water, contact with infected animals and nosocomial exposure [4]. The incubation for NTS gastroenteritis depends on the host and the inoculum. It is usually 12-36 hrs, although incubation periods of up to nearly 2 weeks have been reported with certain strains [5].

Since this salmonella induced diseases requires the drugs that are more effective and less toxic, requiring treatment to be administered specifically increasing the probability of success. This situation possesses a serious problem for low income countries, especially those with a high prevalence of malnutrition and hygienic food accessibility. Therefore, salmonellosis, typhoid and diarrheal diseases need to be tackled with high priority in global public health and biomedical research [6]. Trend in the occurrence of Salmonella induced diseases along with the development of resistant strains intensify the search for more efficient drugs [7, 8]. There are widespread claims by traditional Ayurveda that Salmonella diseases can be treated using medicinal herbs [9].

Various endophytes had been recovered from medicinal herbs and produced secondary metabolites. However, the claims have least scientific justification as India has published limited literature on plants flora and its symbionts screened against *S. typhii* and *S. typhimurium*. This situation is auxiliary complicated with an increase in loss of biodiversity of the country [10].The results of the study will provide a base to claim and support

endophytic fungi enrichness to produce anti-salmonella bioactive metabolites which can be used as futuristic drugs.

2. MATERIAL AND METHODS

2.1. Test pathogens

Test pathogens for the present study; *Salmonella typhi* (MTCC 733) and *Salmonella typhimurium* (MTCC 3214) were obtained from Microbial Type Culture Collection, Institute of Microbial Technology (IMTECH), Chandigarh, India for testing the antimicrobial activity of isolated endophytes.

2.2. Screening of strains

The isolated endophytic fungi from *Ocimum sanctum* [11] may have capability to produce antibacterial metabolites. Screening was done to pick potential endophytic fungi strains i.e., *Melanospora fusispora*, *Alternaria alternata, Fusarium moniliforme* and *Cladosporium Cladosporium* from *Ocimum sanctum* leaves. Stock culture slants for inoculation were prepared for primary screening to initiate fermentation by subculturing strains on Potato dextrose agar media, pH 6.5±0.2 at 27±2°C for 7 days [12-14].

2.3. Preparation of cell free culture filtrate

Two-stage fermentation procedure was used for the production of anti-bacterial metabolites of recovered endophytic fungi from *Ocimum sanctum* leaves. The endophytic fungi grown in submerged culture (shaking) in the first stage and the second stage as stationary culture. For small scale fermentation, a pure fungal disc (normally in the log phase of growth) from Potato dextrose agar media (Yeast extract 3.0 gms, Malt extract 3.0 gms, Peptone 5.0 gms and Glucose 20.0 gms) pH 7.4 were inoculated in 500 ml Erlenmeyer flask containing 100 ml Wickerham medium and were incubated at 25±2°C on a rotary shaker (240rpm) for 5days. These cultures were used as seed cultures (First stage) [14, 15].

For production of anti-bacterial metabolites, 10ml of seed cultures were transferred to flasks containing 250 ml of Potato dextrose broth medium (Second stage). The flasks were incubated at 27±2°C for 21days as stationary culture (Second stage) [16,17]. After completion of incubation period, culture broths were centrifuged at 3,000 rpm for 15 min at 4°C. Supernatant was collected and filtered through Whatman no. 1 filter paper (HIMEDIA) and sterilized with Millipore membrane (0.22µm pore diameter). The filtrate thus obtained was used to determine antibacterial activity. Each experiment was conducted in triplicate set.

2.4. Antibacterial activity

Well diffusion method for antibacterial susceptibility testing was carried out according to the standard method [18], to assess the presence of antibacterial activities of the samples. For sensitivity testing pour plate technique was used, *Salmonella typhii* and *Salmonella typhimurium* (1ml) was taken in sterile petriplate and molten nutrient agar was added under aseptic conditions, mixed well and allowed to solidify for 1hr. Then, well was formed in the seeded medium with sterile cork borer of 8mm inner diameter. Crude extracts of endophytes and antibiotics (100µl) each was dispensed into the wells and allow the diffusion of the extract at 4°C for 30 min. Standard antibiotics as positive and DMSO was kept as negative control respectively. The plates were then incubated at 37°C for 24hr. After the incubation, the plates were examined for zone of inhibition (ZOI) using antibiotic zone reader scale.

3. RESULTS AND DISCUSSION

Typhoidal and non-typhoidal infection by Salmonella is a serious threat to human health. Ciprofloxacin is the last drug of choice to clear the infection. Ciprofloxacin, a gyrase inhibitor, kills bacteria by inducing chromosome fragmentation, SOS response and reactive oxygen species (ROS) in the bacterial cell [19]. Naturally occurring compounds found in plant species has shown to possess antimicrobial activity and serve as a significant antimicrobial agent against pathogenic microorganisms. The occurrence and spread of antibiotic resistant bacteria are pressing public health problems worldwide and many bacteria have become and continue to be resistant to nearly all antimicrobial agents. The resistance rates are higher in developing countries.

Natural products led to provide new and important discoveries in the drug development process [20]. Natural biodiversity products and their semi synthetic derivatives have indeed provided novel drug leads for treatment of salmonella borne diseases [21].

There are numerous studies on endophytic fungi isolated from healthy plant tissues [22]. Plant internal tissue provide suitable conditions to endophytic fungi

which indirectly promote plant growth, moreover a symbiotic relation creates an environment with resistance to stress condition by producing secondary metabolites. However, the interaction between the host plant and endophytic fungi is still widely researched for understanding the co-benefits. Endophytes relation with medicinal plants is fascinating area of research as they produce bioactive substances which may act as futuristic medicines [23].

In amazing review the up to date and comprehensive information on compounds from endophytic fungi from 1995 to 2011 was summarized [24], together with the botany, phytochemistry, pharmacology and toxicology, and discussed the possible trends and the scope for future research on endophytes. This has led to greater emphasis on the exploration of fungi associated with higher plants as possible sources of novel biologically active compounds as anti-microbial (*Salmonella* sp.). In the present study culture filtrate of *Melanospora fusispora*, *Alternaria alternata, Fusarium moniliforme* and *Cladosporium cladosporium* were found to inhibit the growth of *Salmonella typhii* and *Salmonella typhimurium* and exhibit zone of inhibition. The results showed that crude extract of *Alternaria alternate* possess bioactive compound which was able to produce maximum inhibition zone of 22mm and 23mm against *Salmonella typhii* and *S. typhimurium* respectively. It was significant to observe the crude extracts, which displayed bioactivity similar to ciprofloxacin (Positive control). The results of the experiment conducted to test antagonistic activity of endophytes is shown in table 1.

Table 1: Antagonistic activity of entophytes

Endophytes/Antibiotics	Zone of Inhibition (mm)	
	Salmonella typhii	*Salmonella typhimurium*
Crude extract of *A. alternata*	22	23
Crude extract of *F. moniliforme*	18	21
Crude extract of *C. cladosporium*	21	22
Crude extract of *M. fusispora*	17	14
Ciprofloxacin	25	26

Antibacterial activity of endophytes are well supported in findings [25], they reported the antibacterial activities of endophytic fungi from mangrove plants *Rhizophora apiculata* L. and *Bruguiera gymnorrhiza* (L.) Lamk. on *Salmonella typhii*. It was found significant inhibitory activity against *Salmonella typhii*. Similar findings had been reported [26] and studied the antibacterial activities of endophytic bacteria isolated from *Taxus brevifolia* which exhibited significant anti-bacterial activity against foodborne pathogenic bacteria.

4. CONCLUSION

The results indicate that endophytic fungi isolated from *Ocimum sanctum* leaves serve as a novel source of natural antibacterial agent against foodborne pathogenic bacteria, with potential applications in the pharmaceutical industry.

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