INTRODUCTION
Endophytes are microorganisms that colonize the plant tissue without causing any adverse effect or producing symptoms on the plant [1]. Several fungi, bacteria, and actinomycetes have been reported as endophytes previously [2], and the fungal members are most common and frequently isolated from several plants [3-7]. Endophytes play several beneficial roles for their host plants like they can produce innumerable secondary metabolites [8] which shield the host from the pathogens, involve in the mineralization process, and add nutritive value. Endophytes can also produce many precursor molecules of plant metabolism and bioactive compounds which are generally found in the crude drugs like ‘Taxol’ [9]. Isolation of an endophyte with specific function thus definitely helps in the production of a desired compound or drug in a large scale and the practice is also a less time-consuming procedure. Till now, several endophytes are reported having excellent antimicrobial and antioxidant activities [10-12].

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negative pathogenic bacteria, namely, Escherichia coli MTCC1667, Salmonella typhimurium MTCC98, and Pseudomonas aeruginosa MTCC741 and three Gram-positive bacteria, namely, S. aureus MTCC96, Bacillus subtilis MTCC121, and Listeria monocytogenes MTCC657. The strains were procured from Microbial Type Culture Collection (MTCC), IMTech, Chandigarh, India. VBEF2 was cultured in ME broth at 28°C and after its proper growth, paper discs (6mm in diameter) of Whatman No. 1 filter paper soaked with the cell-free supernatants (CFS) of the VBEF2 culture were placed over the pathogenic bacterial lawn in the nutrient agar (NA) plates. In this assay, positive and negative controls were used ciprofloxacin (10 µg/ml) and sterilized dimethyl sulfoxide (DMSO), respectively. The plates were incubated at 35-37°C for 24 hrs.

**Minimum inhibitory concentration (MIC) of the ethyl acetate fraction of the endophyte**

As VBEF2 showed good antibacterial efficiency, its MIC values against one Gram-positive and one Gram-negative bacteria, i.e., S. aureus and E. coli, respectively, were determined. The CFS of VBEF2 was extracted with 40% ethyl acetate. After proper drying of the extract, different concentrations of it, namely, 25, 50, 100, 150, and 200 µg/ml were prepared in DMSO. These different concentrations were added to respective test tubes containing NB and fixed volume of bacterial culture. Next, the test tubes were incubated at 37°C for overnight. After proper incubation, 100 µl of each bacterial culture were spread on the NA plates with proper dilution. Finally, the plates were again incubated at 37°C for overnight and the MICs were calculated by CFU counting method.

**Mode of action of the endophyte**

The mode of action of the ethyl acetate extract of VBEF2 against S. aureus and E. coli was determined following time-kill study. Two simultaneous experimental sets for each bacterium were run as control and treated set. The ethyl acetate extract of VBEF2 was added in the actively growing cultures of S. aureus and E. coli at its MIC values in the treated sets. CFU for successive 10 hrs from both sets of each bacterium was recorded to determine the mode of actions of the extracts [16].

**Antifungal efficiency of the endophyte**

The antifungal activity of VBEF2 was checked by agar well diffusion method [17] against two plant pathogenic fungi, namely, Helminthosporium compactum MTCC351 and Alternaria alternata VBAW007 and two animal pathogenic fungi, namely, Candida albicans MTCC1644 and Aspergillus parasiticus MTCC2796. The fungal strains were taken either from the Mycology and Plant Pathology Laboratory, Visva Bharati, India or procured from MTCC, IMTech, Chandigarh, India. Pathogenic fungal cultures (100 µl) were spread on the respective MEA plates, in which wells were prepared by cork borer. 50 µl of CFS of fully grown VBEF2 was applied in the wells. Fluconazole (200 µg/ml) and sterilized DMSO were used as positive and negative controls. Experimental plates were incubated at 28°C for 3-5 days.

**Antioxidant activity**

The antioxidant assay of the ethyl acetate extract of VBEF2 was carried out using stable 2, 2-diphenyl-2-picrylhydrazyl (DPPH) [18]. A stock solution of DPPH (0.004%) was prepared for this assay. Another stock solution of ethyl acetate extract of VBEF2 was prepared by dissolving 0.01 g ethyl acetate in 1 ml of methanol. Next, different concentrations (5-150 µg/ml) were made by mixing specific volume of endophyte stock solutions and fixed volume of DPPH stock solution and incubated in dark condition for 30 minutes. Finally, after incubation, optical density of each set was measured using spectrophotometer at 517 nm. Methanol with DPPH solution (0.004%) was used as the blank set in this assay. Ascorbic acid was used as the control in this experiment. Next, percentage of inhibition (POI) of each concentration set was calculated, from which the IC50 value of the ethyl acetate extract of VBEF2 was determined. POI of each set was calculated using the formula given below:

\[ POI = \frac{[(a-b)/a] \times 100}{b} \]

POI of DPPH activity (%) = [(a-b)/a] \times 100; a = O.D. of blank set and b = O.D. of each set.

**RESULTS AND DISCUSSION**

**Antibacterial activity of the endophytic isolates**

Among the 17 endophytic fungal strains isolated from the leaves of S. wallichii (Table 1), only VBEF2 exhibited very good antibacterial potentials. Its CFS produced clear zone of inhibition ranging from 10 to 20 mm against the Gram-negative as well as Gram-positive pathogenic bacteria. It showed more antibacterial efficiencies against the Gram-positive bacteria compared to Gram-negative bacteria on the basis of the diameter of zone of inhibition it produced (Fig. 1). This was found may be due to the production of any antibacterial compounds that interfere with the peptidoglycan layers of cell wall of Gram-positive bacteria. The VBEF2 showed highest activity against S. aureus and lowest against P. aeruginosa. The test bacteria were found to be sensitive to ciprofloxacin and resistant to DMSO.

**MIC and the mode of action of VBEF2**

By counting the CFU of the test bacteria, the MIC values of ethyl acetate extract of VBEF2 were found to be 50 µg/ml and 150 µg/ml against S. aureus and E. coli, respectively. These results also attest the fact about the more antibacterial efficiency of the endophyte against Gram-positive bacteria (Table 2). The mode of action of the ethyl acetate extract of VBEF2 against both S. aureus and E. coli was also determined by counting CFU of the both control and treated sets at every hour. Based on the growth pattern of the bacteria observed in the treated set, the activity of the VBEF2 extract was found to be bactericidal against both the bacterial strains. In the treated sets, the growth of the bacteria declined sharply due to the bactericidal activity of the extract (Fig. 2a and b).

**Antifungal activity of VBEF2**

The CFS of the endophytic strain VBEF2 was found to show excellent antifungal range as it produced clear zones of inhibition against all the four pathogenic fungi used. The antifungal potential of VBEF2 was determined based on the diameter of zones of inhibition produced by it. The antifungal potential of the endophyte was much higher against the plant pathogenic fungi, i.e., H. compactum and A. alternata. Moderate antifungal activity of VBEF2 was also found against C. albicans and A. parasiticus (Fig. 3). All the test fungi were sensitive to fluconazole and resistant to DMSO. C. albicans is the causal organism for oral and vaginal candidiasis, and A. parasiticus is the pathogen responsible for aspergillosis. Hence, the ability of VBEF2 in controlling these pathogens was recorded to determine the mode of actions of the extracts [16].

| Leaf No. | Endophytic fungal isolate |
|---------|---------------------------|
| Leaf1   | VBEF1, VBEF2, VBEF3, VBEF4 |
| Leaf2   | VBEF5, VBEF6, VBEF7, VBEF8, VBEF9 |
| Leaf3   | VBEF10, VBEF11, VBEF12 |
| Leaf4   | VBEF13, VBEF14, VBEF15 |
| Leaf5   | VBEF16, VBEF17 |

**Table 2:** MICs of the ethyl acetate extract of VBEF2 CFS against S. aureus and E. coli

| Concentration (µg/ml) | CFU/ml |
|-----------------------|--------|
|                       | S. aureus | E. coli |
| Control               | 1.2×10³ | 2.1×10² |
| 25                    | 2.2×10³ | 1.7×10⁴ |
| 50                    | 1.8×10⁴ | 1.1×10⁸ |
| 100                   | 1.7×10⁴ | 2.1×10⁷ |
| 150                   | 3.1×10⁴ | 3.4×10⁶ |
| 200                   | 1.8×10⁵ | 2.1×10⁸ |

MIC: Minimum inhibitory concentration, VBEF: Visva-Bharati endophyte fungal, CFS: Cell-free supernatants, S. aureus: Staphylococcus aureus, E. coli: Escherichia coli
as well as the plant pathogenic fungi attests its applicability in the pharmaceutical as well as agriculture sector.

The ethyl acetate fraction of the CFS of VBEF2 showed very low IC_{50} value (19.01 µg/ml) in the DPPH scavenging antioxidant assay. This observation was considered as a good result as its IC_{50} value was found to be close with the IC_{50} value of the control ascorbic acid used (8.5µg/ml). DPPH scavenging pattern of the ethyl acetate fraction of VBEF2 is represented graphically (Fig. 4). Plant extracts contain several phenolic compounds such as flavonoids, phenolic acids, tannins, and phenolic diterpenes which are responsible for the antioxidant effects [19]. Previous reports revealed that the phytochemicals with antioxidant activity may reduce the risk of cancer and also improve health conditions. Till now, many reports have been published regarding the antioxidant potentials of several isolates of Aspergillus [20].

The colony of the isolate VBEF2 was observed with blackish mycelia with grayish border in MEA plates which are very common features of Aspergillus colony. Besides, the presence of unbranched conidiophores with terminal globose vesicles covered by chains of conidia and simple septation in the vegetative mycelia is observed under microscope also helped to identify the fungus as a species of Aspergillus (Fig. 5a and b). Previously, an endophytic species of Aspergillus isolated from the leaf of Justicia adhatoda showed antibacterial activities against S. aureus, P. Aeruginosa, and E. coli [21].

CONCLUSION

17 endophytic fungi were isolated from the leaves of S. wallichii, and among them, only VBEF2 exhibited its antibacterial potentials against all the six pathogenic bacteria used. Its low MIC values with bactericidal mode of action against both S. aureus and E. coli also indicate its antibacterial efficiencies against Gram-positive as well as Gram-negative bacteria. The endophyte was also found to show excellent antifungal potentials against both plant pathogenic as well as animal pathogenic fungi. In the DPPH scavenging antioxidant assay, VBEF2 also showed very good antioxidant activity by showing low IC_{50} value. Hence, the Aspergillus isolate can be a great prospect in the pharmaceutical sector for its excellent antimicrobial as well as antioxidation potentials.

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