

Bacterial endophytes of aloe vera and their potential applications

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Abstract

The study was aimed to assess culturable bacterial endophytes from the medicinal plant Aloe vera, their antimicrobial spectra against pathogens, and the potential of bacterial endophytes in textile and paper dyeing. Culturable seventeen bacterial endophytes were isolated from the Aloe vera plant out of which 16 showed varied antimicrobial activity against both human pathogens i.e., bacteria and fungi *E. coli*, *S. pyogenes*, acne bacterial isolate (ABI), *A. niger*, and *F. oxysporum*. Simultaneously, the bacterial endophyte ENDB3 is producing extracellular green-brown color pigment under submerged (SmF) condition and the extracted pigment has shown promising results in textile and paper dyeing at lab scale without using mordant. All the bacterial endophytes showed resistance against standard antibiotics (penicillin G P(10 units), Oxacillin (1 mcg), Cephalathin (30 mcg), Clindamycin (2 mcg), Erythromycin (15 mcg), and Amoxycylav (30 mcg)) at the specific concentration used. Concludingly, bacterial endophyte ENDB3 is found capable to produce bioactive molecules with pharmaceutical and dyeing industries which may provide a new path in the pursuit of new biological sources of drug and natural dyeing candidates. Hence, we suggest further evaluation and characterization of their bioactive molecules for pharmacological and dyeing potential.

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1. Introduction

Aloe vera is an evergreen medicinal plant of *Xanthorrhoeaceae* family. It is native to the Saudi Arabian Peninsula, sub-Saharan Africa, and Western Indian Ocean islands. It is found in a different type of climatic conditions including coastal areas, grassland, and desert [1]. The Aloe plant has therapeutic and curative values and is becoming the source for many potential uses. The exudates of fleshy leaves are widely used in cosmetics (hair conditioner, moisturizers, etc.) and for medicinal purposes [2] such as wound healing, burns, anticancer activity [3], diabetes, and antimicrobial activity [4, 5].

Pathogenic microorganisms developed resistance to many commercially available medicines [6] which opens the door to find new bioactive compounds from natural sources. Recently, there is an emerging focus and interest in the study of endophytes of medicinal plants. They are the alternative sources for bio- bioactive compounds. Endophytes are the microbial species, colonizing endosymbionts in inter and intracellular parts of plants [7, 8]. It is hypothesized that because of the host-microbe relationship endophytes may become the source of many pharmacological bioactive compounds [7]. Hence endophytes and their bioactive compounds are known to be potent sources of novo natural products with possible exploitation and exploration in pharmacy and other industries. *Aloe vera* is a medicinal plant that provides a range of endophytic bacteria this becomes a motivating factor to explore their bioactive compounds for different industrial and medicinal applications. On the other hand, acne is also one of the serious issues of concern with many socio-health impacts which has to be solved, till today no product is suggested to control acne effectively. The bioactive molecules of the bacterial endophyte

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of *Aloe vera* can be used as a source of natural drugs and in other potent industrial applications. Bioactive compounds can be used as natural colorants like food colorants, additives [9], natural dyes [10], cosmetic products, [11] and, functional foods [12] which represent an opportunity for the use of natural pigments in different industries. For the production of natural pigment, the possibility to exploit microorganisms has been recommended [13]. In the present work, endophytic bacteria from *Aloe vera* were isolated, their bioactive molecules were screened for antimicrobial assay and extracellular pigment was explored for its industrial importance. This attempt is novel as the application of extracellular pigments of endophytic bacteria from *Aloe vera* has not been reported, although antimicrobial activities of endophytic fungi and actinomycetes have been reported. In the present study, we have focused to isolate the endophytic bacterial flora of *Aloe vera*, and attempts were made to find out the antimicrobial activity of endophytes against human pathogens and to explore screening of textile and paper dyeing potential of pigment produced by ENDB3.

2. Materials and methods

The *Aloe vera* plant sample was collected from the medicinal garden of College of Life Sciences, Cancer Hospital and Research institute, Gwalior (M.P.), India (geographically located in 26.2183° N and 78.18° E). Samples were immediately processed for analysis. Collected samples were washed with double distilled water and the samples were left for 10-15 minutes then the surface sterilization of the sample was conducted by using 3% H₂O₂ washing for 3 min. followed with three times washing with sterile distilled water, excess water was allowed to drain. The plant sample was cut into small pieces of size 2-3 cm soon after the single piece of collected plant sample was inoculated on a semisolid nutrient agar plate, incubated at 35±2°C for 48 hrs. BTB (Bromo thymol blue) was used as a pH indicator.

Pus sample from acne was collected with a sterile swab then spread, inoculated on a nutrient agar plate. The plate was incubated at 35±2 °C for 24 hr. The pure isolate was obtained by following the method described above.

The pure colonies were obtained by subsequent rounds of quadrant streaking method on agar plates (1.5% W/V agar) was followed to obtain pure colonies of bacterial endophytes, incubation was held at 35±2 °C. The pure colonies were used for further studies. Similar was followed to obtain a pure culture of acne pathogen.

Morphological parameters of each isolate such as colony size, shape, polysaccharide production, elevation, consistency, growth, and color were observed and recorded. The shape was determined by following simple and Gram staining methods.

The antimicrobial potential of bacterial endophytes was evaluated against pathogenic bacterial and fungal strains included *Escherichia coli* MTCC-1302, *Streptococcus pyogenes* MTCC-1925, *Aspergillus niger* MTCC-281, *Fusarium oxysporum* MTCC-284, and acne bacterial isolate (ABI) pathogen. Kirby–Bauer disc diffusion method described by Bauer [14] was followed to perform the test. The pathogens were pre-cultured in Mueller Hinton (overnight for bacteria), potato dextrose broth (5 days for fungi), 0.5 ml of each broth culture was spread over agar plates then the sterile discs were impregnated with pre cultured bacterial endophytes, placed on pathogen seeded plates. The incubation was held at 35 ± 2°C for 48 h (bacteria) and 25°± 2°C C for 5 days (fungi), the zone of inhibition was determined. Antibiotic disc of tetracycline and nystatin were used as a positive control for bacteria and fungi respectively. The experiment was performed in triplicates.

Antibiotic sensitivity pattern of bacterial endophytes was studied against different antibiotics where hexa disc of antibiotics (HiMedia) included penicillin G P (10 units), Oxacillin (1 mcg), Cephalathin (30 mcg), Clindamycin (2 mcg), Erythromycin (15 mcg) and Amoxyclav (30 mcg) was used. The pre-incubated bacterial endophyte culture was spread plated on MH agar medium then the antibiotic disc was placed and incubated at 35±2°C for 48 hr. The sensitivity of each isolate was recorded in terms of zone of inhibition produced [15].

The endophytic bacterial isolate ENDB3 is capable to produce extracellular green-brown pigment diffused in the agar medium therefore attempts were made to assess its pigment production capabilities and their potential exploration in dyeing industries. 1 ml of a pre-incubated overnight culture of selected bacterial strain (ENDB 3) was inoculated in 50 ml nutrient broth in an Erlenmeyer flask incubated at 35 ± 2°C for 72 hr under static and unoptimized conditions. Optical density was recorded at 550nm after an interval of 12 hr. The produced dark green-brown color pigment was extracted by filtration followed by centrifugation at 10,000 rpm, performed in triplicates. For assessment of dyeing potential, the extracted pigment was applied to paper, cotton, and cotton cloth pieces. The plates were left for 24hr under the soaking conditions without mordant after soaking the dyed objects were washed with water and allowed to air dry at room temperature.

Data were analyzed by one-way analysis of variance (ANOVA) to determine statistical significance. A p -value of ≤ 0.05 was considered to be statistically significant.

3. Results

The results showed that the *Aloe vera* plant enriched with bacterial endophytes, total 17 different isolates namely, ENDB1 to ENDB17 were isolated from *Aloe vera* plant sample and identified on the basis of macro and microscopic attributes (see Table 1). All the isolated culturable bacterial endophytes are gram-positive in nature and rod in shape.

Table 1. Morphological characteristics of bacterial endophytes

| Strain No- | Shape | Size | Elevation | Polysaccharide production | Colour | Consistency | Growth | Gram Staining |
|------------|-------|-----------|----------------|---------------------------|-----------------|-------------|--------|---------------|
| ENDB1 | Rod | Pin point | Convex | No | Creamish | Translucent | Slow | positive |
| ENDB2 | „ | „ | „ | Low | Creamish | „ | „ | „ |
| ENDB3 | „ | Small | „ | No | Green-Brown | „ | Fast | „ |
| ENDB4 | „ | Pin point | Flat | Low | Yellowish-white | „ | „ | „ |
| ENDB5 | „ | Small | Flat and rough | High | White | „ | „ | „ |
| ENDB6 | „ | Small | Flat | Low | Creamish | „ | „ | „ |
| ENDB7 | „ | Pin point | Flat | Low | White | „ | „ | „ |
| ENDB8 | „ | Small | Convex | High | Creamish | „ | „ | „ |
| ENDB9 | „ | Pin point | Flat | Low | Creamish | „ | „ | „ |
| ENDB10 | „ | Pinpoint | Convex | Low | White | „ | „ | „ |
| ENDB11 | „ | Moderate | Convex | High | Creamish | „ | „ | „ |
| ENDB12 | „ | Pin point | Raised | High | Creamish | „ | „ | „ |
| ENDB13 | „ | Pin point | Convex | Low | Creamish | „ | „ | „ |
| ENDB 14 | „ | Moderate | Raised | No | Creamish | „ | „ | „ |
| ENDB15 | „ | Pin point | Convex | Low | White | „ | „ | „ |
| ENDB16 | „ | Pin point | „ | Low | Creamish | „ | „ | „ |
| ENDB17 | „ | Pin point | „ | Low | Creamish | „ | „ | „ |

3.1. Antimicrobial activity of endophytic bacterial isolates

A total of seventeen bacterial endophytes were isolated from the *Aloe vera* plant, all the isolates were subjected to antimicrobial screening. A total of sixteen isolates have shown antimicrobial activity against tested bacteria, 94.11% of the endophytic bacteria were active and shows prominent antimicrobial activity. Among all the bacterial endophytes isolates ENDB12 showed maximum antimicrobial activity against *E. coli* and the lowest was found in ENDB 1 whereas ENDB4, 5, 8, 14, 16, and 17 have no inhibition activity against *E. coli*. Similarly, isolate ENDB8 has the highest antimicrobial activity against *S. pyogenes*, lowest was exhibited by ENDB11 and ENDB 16 and 17 has shown no antimicrobial activity against *S. pyogenes* and except ENDB14 (no zone of inhibition) rest all the 16 bacterial endophytes have shown good antimicrobial activity against acne bacterial isolate (ABI). ENDB3 showed maximum activity against ABI and the lowest was displayed by ENDB13.

Although the bacterial endophytes have shown promising results against fungal pathogens, isolate ENDB10 has shown the highest activity against *A. niger*, lowest was exhibited by ENDB5 and ENDB14,15,16 and 17 have exhibited no activity. Similarly, ENDB10 showed maximum antimicrobial activity against *Fusarium oxysporum*, ENDB 14, 15, 16, and 17 have displayed no activity whereas the ENDB12 showed the lowest activity against *Fusarium oxysporum*. Therefore, it could be concluded that among all the tested microorganisms only, 64.7% (11/17) bacterial endophytes were found to be active against *E. coli*, Similarly, 88.23% (15/17), 94.11% (16/17), 76.4% (13/17), and 76.47% (13/17) bacterial endophytes were shown to be active against *S. pyogenes*, acne bacterial isolate (ABI), *A. niger* and *Fusarium oxysporum* respectively (Fig. 1, 2, 3, 4, and 5). Statistical significance ($p \leq 0.05$) of the data was obtained by Analysis of variance (One way ANOVA) for differences in means.

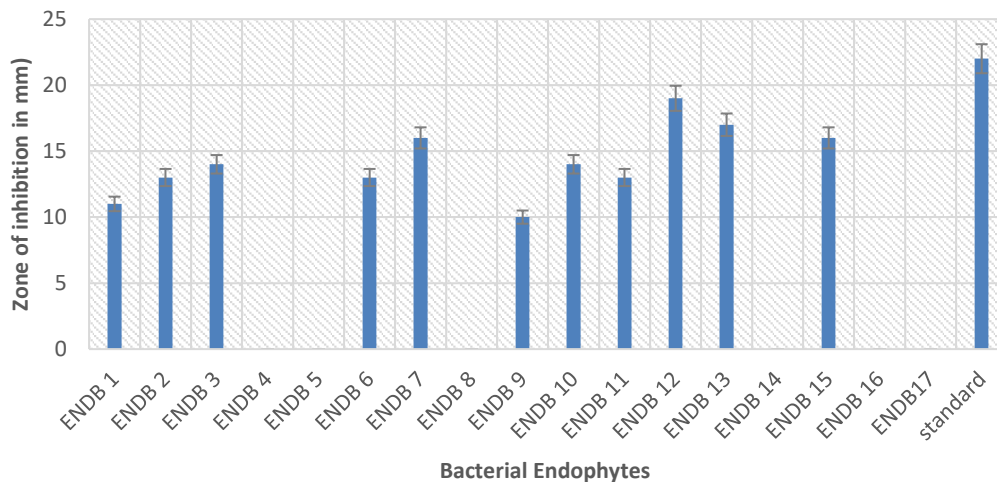


Figure 1. Antimicrobial potentiality spectra of bacterial endophytes against *E. coli*

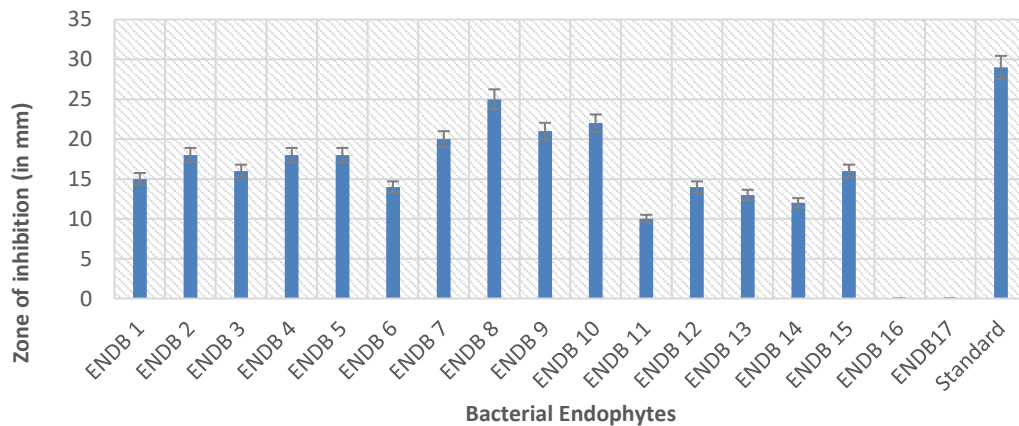


Figure 2. Antimicrobial potentiality spectra of bacterial endophytes against *S. pyogenes*

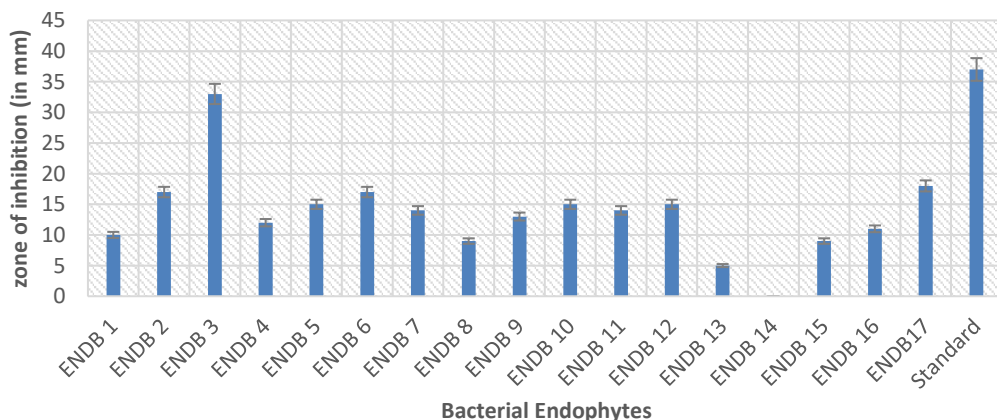
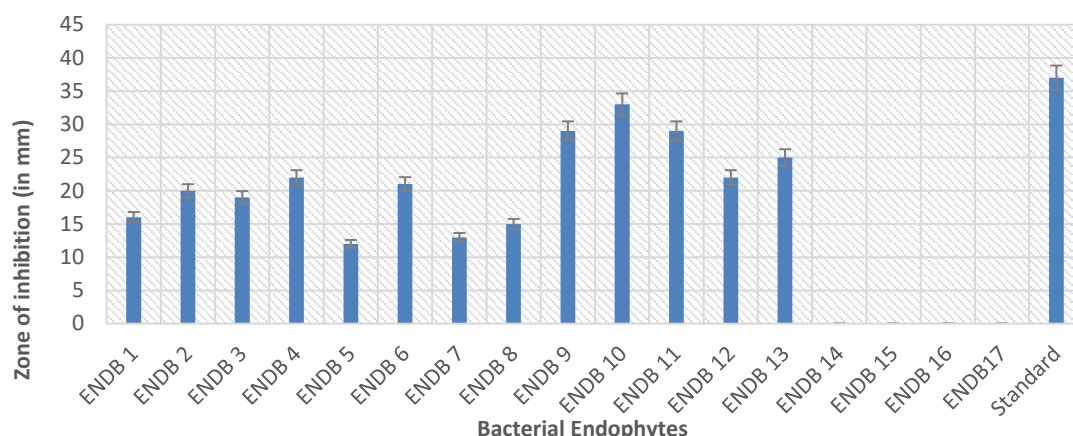
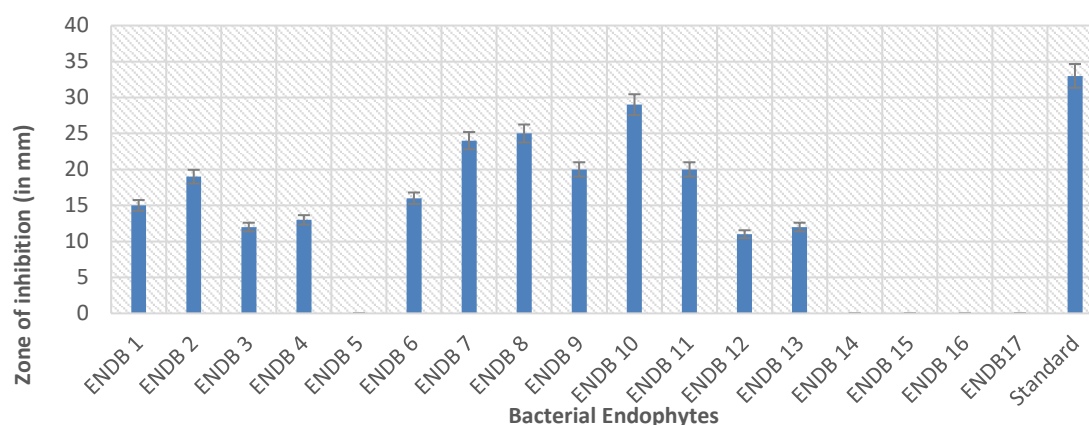


Figure 3. Antimicrobial potentiality spectra of bacterial endophytes against ABI

Figure 4. Antimicrobial potentiality spectra of bacterial endophytes against *A. niger*Figure 5. Antimicrobial potentiality spectra of bacterial endophytes against *Fusarium oxysporum*

In the present investigation results of antibiotic susceptibility of obtained bacterial endophytes against standard penicillin G P(10 units), Oxacillin (1 mcg), Cephalathin (30 mcg), Clindamycin (2 mcg), Erythromycin (15 mcg), and Amoxyclav (30 mcg) were evaluated and the tested antibiotics displayed no inhibitory effect on the growth of bacterial endophytes, therefore, it could be concluded that at the given concentration all the bacterial endophytes were resistant to all the used antibiotics (see Table 2).

Table 2. Antibigram of bacterial endophytes against six standard antibiotics

| Bacterial Endophyte | Penicillin G P (10 units) | Oxacillin (1 mcg) | Cephalathin (30 mcg) | Clindamycin (2 mcg) | Erythromycin (15 mcg) | Amoxyclav (30 mcg) |
|---------------------|---------------------------|-------------------|----------------------|---------------------|-----------------------|--------------------|
| ENDB 1 | - | - | - | - | - | - |
| ENDB 2 | - | - | - | - | - | - |
| ENDB 3 | - | - | - | - | - | - |
| ENDB 4 | - | - | - | - | - | - |
| ENDB 5 | - | - | - | - | - | - |
| ENDB 6 | - | - | - | - | - | - |
| ENDB 7 | - | - | - | - | - | - |
| ENDB 8 | - | - | - | - | - | - |
| ENDB 9 | - | - | - | - | - | - |
| ENDB 10 | - | - | - | - | - | - |
| ENDB 11 | - | - | - | - | - | - |
| ENDB 12 | - | - | - | - | - | - |
| ENDB 13 | - | - | - | - | - | - |
| ENDB 14 | - | - | - | - | - | - |
| ENDB 15 | - | - | - | - | - | - |
| ENDB 16 | - | - | - | - | - | - |
| ENDB 17 | - | - | - | - | - | - |

- = Resistant strains against used antibiotics.

3.2. Application in paper and textile dyeing

In the present investigation, ENDB3 produced an extracellular brilliant green-brown color pigment under submerged conditions (Fig. 6). The obtained pigment showed good results in the dyeing of paper, cotton, and cotton fabric without using mordant, visibly mark difference observed after 24 hr of dyeing with extracted pigment (Fig. 7).



Figure 6. Pigment produced under submerged condition

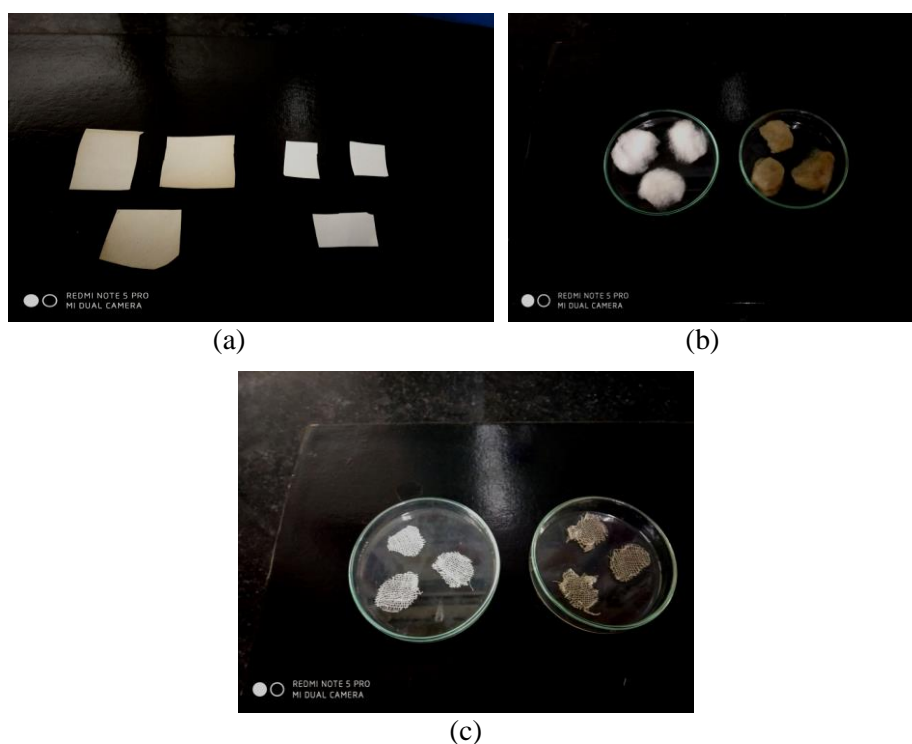


Figure 7. Dyed objects: (a) Paper: Pre (L) and After dyeing (R) (b) cotton: Pre (R) and After dyeing (L) and (c) Cloth: Pre (R) and After dyeing (L)

4. Discussion

The recent attraction has been focused to study the endophytic microbial flora of medicinal plants because they have a wide range of applications and are found as symbionts with the host plant, various have been studied the endophytic microbial flora of many medicinal plants. It is found that there is a huge diversity of endophyte microbes found in various parts of plant tissues. Similarly, the findings suggested by Strobel et al. [16] and Lilley et al. [17] reported 114 endophytic isolates of sugar beet of 23 different genera supported the presence of endophytes in plant tissues. In the present study, we have obtained 17 different strains of bacterial endophytes from the medicinal plant Aloe vera (see Table 1) and their antimicrobial potential was evaluated (Fig. 1, 2, 3, 4, and 5). Similar, studies have been reported by Akinsanya et al. [18] they studied the endophytic bacterial flora of the Aloe vera plant and reported 29 different species from the plant simultaneously they also studied the antimicrobial spectra of the isolates and focused on the antimicrobial activities of bioactive molecules of

bacterial endophytes of Aloe vera plant. Santos et al. [19] also studied the fungal endophytic flora of the Aloe vera plant and their antimicrobial potentials against pathogens were reported. Endophytic microorganisms have many important properties because they are connected to their metabolic capabilities and can provide protection to the plant by producing a variety of bioactive molecules [20, 21, and 22].

Assessment of antimicrobial potentials of bacterial endophytes of Aloe vera plant has been performed against bacterial and fungal pathogens. There seems to be antimicrobial effects on the growth of pathogens in terms of inhibition were tested (Fig. 1,2,3,4, and 5). Similarly, Huang et al. [23] Li and Strobel [24], and Sinha et al. [25] also studied the antimicrobial activities of plant endophytes. In this work isolated, total of 17 bacterial endophytes from the Aloe vera plant and their antimicrobial potential was assessed out of these 16 bacterial endophytes (94.11% of isolated bacterial endophytes) were shown to be active against *E. coli*, *S. pyogenes*, acne bacterial isolate, *A. niger* and *F. oxysporum* and inhibited the growth of tested microorganisms. Various have been suggested a similar result, for example, 27.6% isolated microbial strains of *Camptotheca acuminata* showed antimicrobial activity against pathogens [26], similarly, 8.3% isolated microbial strains from *Aquilaria sinensis* and *Dracaena cambodiana* displayed antimicrobial activity against pathogens [27] whereas Akinsanya et al. [18] reported that endophytic bacterial flora of Aloe vera plant displayed about 48.3% antimicrobial activity against human pathogens and Jalgaonwala [28] suggested <10% antimicrobial activity was exhibited by bacterial endophytes against pathogenic microbes. Endophytic microorganisms ever been the largest source for bioactive secondary metabolites, for example, the endophytic strains of fungal genus *Nigrospora* reported as a source of bioactive molecules [29, 30, and 31]. In this work, the best antifungal activity was found in ENDB10 against *F. oxysporum* and the best antibacterial activity was displayed by ENDB3 against acne bacterial isolate. The present study suggested that the bacterial endophyte ENDB10 has shown good antifungal activity against both tested pathogenic fungi (*A. niger* and *F. oxysporum*) and ENDB14, 15, 16, and 17 have shown no activity against tested fungi (*A. niger* and *F. oxysporum*) whereas the bacterial endophyte ENDB3 has shown the maximum and wide spectrum of antimicrobial activity against the bacterial pathogen used.

Antibiogram of isolated bacterial endophytes against six standard antibiotics exhibited resistance against the specific concentration of all the antibiotics (Penicillin, Oxacillin, Cephalathin, Clindamycin, Erythromycin, and Amoxyclav) used (see Table 2). Burman et al. [32] reported similar findings where bacterial isolates showed resistance to standard antibiotics like ampicillin (10 µg/disc), penicillin G (10 µg/disc) and amoxicillin (30 µg/disc).

Natural pigments are highly recommended to overcome the adverse effects of artificial and synthetic colorants [33, 34] used worldwide. The synthetic colorants have toxic impacts on humans as well as many adverse impacts on the environment [35]. The production of bacterial pigment is an emerging field with potential in different industrial sectors. Extracellular pigments are easy, cheap to obtain, eco-friendly and have the potential to be used in textile dyeing [36]. In this study, the extracellular pigment was produced by bacterial endophyte ENDB3 under static conditions since few studies of pigment production from bacterial endophytes have been reported yet. Khanam and Chandra [37] also reported dye yielding endophytic bacteria from the plant *Beta Vulgaris* L. Many authors have suggested that bacterial pigment and their exploration of dyeing potential in different industries [13, 38, and 39], they have also reported bacterial pigment production and their application in textile and other dyeing industries. The extracted pigment can be potentially used in various sectors of industries like textile [40], paper dyeing [40, 41, and 42], soap coloring, candles [42], food colorant [42], etc. In this work, the bacterial pigment is used to dye fabric, cotton and papers, although Ahmad [42] also reported the similar use of bacterial pigment for dyeing fabric, soaps, candles, and papers. The finding contributes to increasing the industrial and pharmacological potential value of bioactive molecules of bacterial endophytes from the Aloe vera plant.

Furthermore, observations indicate that the bacterial endophytes of the Aloe vera plant have industrial potential as they produce bioactive molecules that could be used as antimicrobial agents and natural colorants.

It could be concluded that the endophytic microbes of the Aloe vera plant have medicinal and other industrial potentials. Hence, the study recommends further investigation is required of these bioactive molecules of bacterial endophytes from the Aloe vera for dyeing and pharmacological industries.

5. Conclusion

The present investigation revealed that the bacterial endophytes of the Aloe vera plant have a wide variety of bioactive molecules that inhibit the growth of pathogenic micro-organisms both bacteria and fungi. ENDB3

displayed the best antibacterial activity against acne bacterial isolate whereas ENDB10 showed maximum antifungal activity against *F. oxysporum*. Bacterial endophytes reflected resistant antibiogram towards different standard antibiotics (at specific concentrations) used. The isolate ENDB3 has shown very good antibacterial activity against acne bacterial isolate and the strain has also shown promising results in paper and textile dyeing. Therefore, we found that bacterial endophytes of Aloe vera have great industrial potential to be explored especially, in pharmacological and dyeing industries. This study has established bacterial endophyte ENDB3 as a cost-effective source of antimicrobial bioactive molecules and bacterial pigment that may have diverse industrial applications (in Pharma and Dyeing industries). Further studies are recommended to identify the bioactive molecules produced by ENDB3 and ENDB10 in order to discover new drugs with antibacterial, antifungal activity respectively. Hence, optimization studies are needed to explore the potential of ENDB3 in textile and paper dyeing effectively, large-scale production investigation of the bioactive molecules is recommended to be used as novel antibiotic and dyeing pigment which can open new opportunities for commercialization of the antibiotic and dyeing pigment bioactive molecules.

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Declaration of competing interest

The authors declare that they have no any known financial or non-financial competing interests in any material discussed in this paper.

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