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ORIGINAL ARTICLE

# Assessing the efficacy of phyllospheric growth-promoting and antagonistic bacteria for management of black rot disease of cauliflower incited by *Xanthomonas campestris* pv. *campestris*

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## Abstract

The study aimed to assess the potential of phyllospheric bacterial strains isolated from cauliflower plants as biocontrol agents against black rot disease caused by *Xanthomonas campestris* pv. *campestris*, through both in vitro and in vivo evaluations. A total of 46 bacterial strains were isolated from healthy and infected cauliflower leaves of both resistant and susceptible plants, and evaluated them for various traits, including plant growth-promoting activities and in vitro antagonistic activity against *Xanthomonas campestris* pv. *campestris*. Further, a pot experiment was conducted with the susceptible cauliflower genotype (Pusa Sharad) and 10 selected phyllospheric bacterial isolates to assess their biocontrol efficacy against the disease. The results showed that 82.60% of phyllospheric bacterial isolates were positive for phosphate solubilization, 63.04% for ammonia production, 58.69% for HCN production, 36.95% for siderophore production, and 78.26% had the capacity to produce IAA. Out of the 46 isolates, 23 exhibited in vitro antagonistic activity against *X. campestris* pv. *campestris* and 10 isolates were selected for a pot experiment under glasshouse conditions based on their good plant growth-promoting activities and antagonistic assay. The results revealed that bacterial isolate CFLB-27 exhibited the highest biocontrol efficiency (65.41%), followed by CFLB-24 (58.30%), CFLB-31 (47.11%), and CFLB-26 (46.03%). These four isolates were identified as *Pseudomonas fluorescens* CFLB-27, *Bacillus velezensis* CFLB-24, *Bacillus amyloliquefaciens* CFLB-31, and *Stenotrophomonas rhizophila* CFLB-26. This study provides valuable insights into the potential of phyllospheric bacteria as an effective tool for disease management in sustainable agriculture.

**Keywords** Black rot · Phyllospheric bacterial isolates · *Xanthomonas campestris* pv. *campestris* · Biocontrol efficiency · *Pseudomonas fluorescens* CFLB-27

## Introduction

Cauliflower (*Brassica oleracea* var. *botrytis*) is an important vegetable crop that is grown in many countries, including China, India, and Italy (Keck and Finley 2004; Abdelkhalik et al. 2019; He et al. 2020). Vitamins and minerals are abundant in cauliflower, making it a healthy food. However, bacterial, fungal, and viral infections frequently limit its production. Black rot disease, caused by *Xanthomonas campestris* pv. *campestris* (Xcc), is one of the most devastating diseases impacting crucifer crops around the world. Cauliflower producers have a lot to worry about because this disease spreads from seed to plant and may significantly decrease yields (Williams 1980; Singh et al. 2011). It is particularly prevalent in tropical and subtropical regions with warm and humid climates, where it can cause yield losses of up to 50%. The disease affects seed germination, seedling mortality, plant

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growth, curd formation, and curd quality, leading to low productivity and quality of cauliflower (Gupta et al. 2013).

Chemical disease management is undoubtedly an effective tool for managing plant diseases. However, it is not a long-term solution due to concerns over non-discriminatory use, exposure risks, health and environmental hazards, residue persistence, and resistance development. Additionally, the need for repeated applications increases the cost of plant protection (Christopher et al. 2010). To address these concerns, alternative control approaches have been adopted to manage plant diseases in a more environmentally friendly manner. One such approach is the use of phyllospheric microorganisms as antagonists. Phyllospheric microorganisms are microorganisms that reside on the surfaces of plant leaves (Stone et al. 2018). They can be mass multiplied at a commercial level and used as an alternative to chemical disease management for the management of black rot disease. The use of phyllospheric microorganisms as antagonists is a promising approach for managing plant diseases, as it is environmentally friendly, cost-effective, and sustainable (He et al. 2021). By utilizing these microorganisms, growers can reduce their reliance on chemical pesticides and promote a more sustainable approach to crop protection.

Although the use of biological control agents from the phyllosphere is less common than those from soil or roots, there have been successful treatments of some phyllosphere-associated diseases using biocontrol agents (Fernando et al. 2007). Recently, there has been increased interest in using native microorganisms as biocontrol agents due to their adaptation to local conditions and their ability to protect host plants against foreign pathogens (Kumar and Gopal 2015). The first step in biological control is identifying and deploying highly effective native strains. The phyllosphere is habitat to a wide variety of microorganisms; however, bacteria make up the vast majority of the population (Vorholt 2012; Wagi and Ahmed 2017; Koskella 2013). The phyllosphere contains a diverse population of bacteria, with *Pseudomonas* being the most common genus. The number of these bacteria changes based on the type of plant and other environmental factors (Aleklett et al. 2014; Kecskeméti et al. 2016; Steven et al. 2018; Andrews and Harris 2000; Hirano and Upper 2000). Phyllospheric bacteria are essential for maintaining plant health, but their complex interactions with biocontrol agents and the effects on plant health are still not fully understood. Phyllosphere communities can influence plant biogeography and ecosystem function by producing growth-promoting compounds and protecting hosts against pathogen infections. Despite their importance, little is known about phyllosphere bacteria (Jensen et al. 2013; Leff and Fierer 2013).

Plant growth-promoting bacteria (PGPB) can affect plant performance through direct methods such as phytohormone production, nitrogen fixation, nutrient solubilization, and indirect methods such as protection against plant pathogens (Glick 2014; Goswami et al. 2014; Álvarez and Biosca 2017; Girard et al. 2020; Shaikh and Sayyed 2015; Wang et al. 2019). Biocontrol agents employ various mechanisms to survive and compete, including the production of antagonistic compounds such as siderophores, antibiotics, hydrolytic enzymes, and volatile compounds (Khan 2005; Sahin et al. 2004). Biocontrol agents have been found to induce systemic resistance against a variety of plant diseases caused by fungal, bacterial, and viral pathogens (Radjacommare et al. 2002; Ramamoorthy et al. 2001). The employment of biocontrol agents to suppress disease is the result of interactions between the plant, pathogen, biocontrol agents, the microbial population on and around the plant, and the physical environment (Akhtar and Siddiqui 2010).

In light of the challenges posed by black rot disease in cauliflower cultivation and the limitations of chemical disease management, present study was aimed to explore the potential of phyllospheric microorganisms as a sustainable and environmentally friendly alternative for disease control. Phyllospheric bacteria associated with healthy cauliflower plants may exhibit plant growth-promoting traits and antagonistic activity against *Xanthomonas campestris* pv. *campestris*, the causative agent of black rot disease. We hypothesized that these beneficial bacteria could be harnessed to reduce the incidence and severity of black rot disease in cauliflower and enhance overall plant health and crop productivity. Specifically, we sought to identify and characterize bacterial species inhabiting the phyllosphere of both healthy and black rot-infected cauliflower plants. Our goal was to assess their ability to promote plant growth and act as antagonists against black rot disease. The overarching objective of this study was to provide valuable insights that contribute to the development of effective, eco-friendly strategies for managing black rot disease and enhancing the productivity and quality of cauliflower crops. By shedding light on the interactions between these phyllospheric microorganisms, the host plant, and the pathogen, we hoped to contribute valuable insights to the field of biological control for crucifer crops. Ultimately, our work strived to offer innovative solutions to enhance cauliflower yield, quality, and sustainability while reducing the reliance on chemical pesticides.

## Materials and methods

### Isolation of phyllospheric bacteria

Healthy and black rot-infected leaf samples of resistant (BR-161) and susceptible (Pusa Sharad) genotypes of cauliflower were collected from the field of Division of Vegetable Science, ICAR-IARI, New Delhi. To isolate the

phyllospheric bacteria, 30 disks each of 10-mm diameter were cut from each leaf sample using a 10-mm cork borer. These disks (1 g) were then mixed with 9 mL of sterile distilled water and shaken for 30 min. Serial dilutions were made up to $10^{-2}$. Further, 1-mL aliquots of the $10^{-1}$ and $10^{-2}$ dilutions were pour-plated on three different types of agar media, including nutrient agar (NA), tryptone soya agar (TSA), and King's B agar (KB). The plates were then incubated at 28 ± 2 °C for 72 h. After incubation, representative colonies were selected from each plate and subcultured. These subcultured colonies were stored as glycerol stocks at − 80 °C for further analysis.

### Morphological and biochemical characterization of phyllospheric bacterial isolates

All the isolates were studied for their morphological characteristics by colony characteristics, Gram staining, and KOH test and biochemical tests (catalase, peroxidase, starch hydrolysis test, gelatine liquefaction, arginine dihydrolase, citrate, and hydrogen sulfide production).

## Characterization of phyllospheric bacteria for plant growth-promoting traits

### Phosphorus solubilization

Bacterial isolates were screened for phosphate solubilization using Pikovskaya media (Pikovskaya 1948). After 48 h of growth, 1 mL of each culture was inoculated into Pikovskaya broth and incubated at 30° C for 3 to 5 days. After incubation, 1 mL of culture was mixed with 10 mL of ammonium molybdate solution, and the blue color intensity of the resulting solution was measured at 600 nm using a UV-VIS spectrophotometer (Mehta and Nautiyal 2001).

### Indole acetic acid production

Phyllospheric bacteria were tested for indole acetic acid (IAA) production by growing them in nutrient broth supplemented with tryptophan. After incubating the bacteria for 48–72 h, the amount of IAA produced was estimated using a method described by Hartmann et al. (1983), which involved measuring the intensity of the pink color at 530 nm. The quantity of IAA produced was then quantified using a calibration curve of standard IAA stock solution prepared in 50% ethanol. The results were expressed as µg/mL of IAA produced after 48 h of incubation.

### Siderophore production

The production of siderophores by 46 bacterial isolates was investigated using the method described by Schwyn and Neilands (1987). CAS-agar plates were prepared using CAS blue solution. After 48 h of growth, bacterial isolates were spotted onto the plates with micropipette and incubated at 28 °C in the dark for 5–7 days. Orange zones around the colonies indicated siderophore production. The CAS-agar control plates (uninoculated) were incubated under the same conditions and did not show any color change after 1–7 days of incubation.

### Ammonia production

The method used to estimate ammonia production by bacterial cultures was Dye's method (1962). To carry out the assay, bacterial isolates were inoculated into peptone water and incubated at 30 °C for 96 h. After incubation, 1 mL of Nessler's reagent was added to each tube. The development of a yellow color indicated ammonia production.

### HCN production

Bacterial isolates were screened for HCN production using Voisard et al. (1989) method. The isolates were streaked on nutrient agar amended with glycine (4.4 g/L) and a filter paper soaked in 0.5% picric acid in 2% sodium carbonate was placed on the lid of the petri dish. The plates were sealed with parafilm and incubated at 30 °C for 4 days. A change in color of the filter paper from yellow to red-brown indicated HCN production ability by the bacteria.

### Antagonistic activity of phyllosphere bacterial isolates against *Xcc* in vitro

The antagonistic potential of 46 phyllospheric bacterial isolates against *Xanthomonas campestris* pv. *campestris* (Xcc) was evaluated using the dual-culture method under in vitro conditions (Singh et al. 2011). Xcc culture was obtained from the plant bacteriology lab of ICAR-IARI, New Delhi, and was spread on nutrient agar medium. Three wells were made on each petri plate and 40-µL culture of phyllospheric bacterial isolates grown in nutrient broth medium (0.1 OD at 600 nm) was poured into each well. Control plates were prepared with sterile 0.001 M $MgSO_4$ solution. The inhibition zone formed by the bacterial isolates was measured after 48 h of incubation at 28 ± 2 °C. Isolates that formed an inhibition zone with a diameter of more than 0.8 cm were chosen for further study.

### Evaluation of efficacy of phyllospheric bacterial isolates for management of black rot disease under glasshouse conditions

Among 46 phyllospheric bacterial isolates, ten isolates were selected for further study based on their plant growth-promoting and antagonistic ability during in vitro conditions. A pot experiment was conducted in a glasshouse using Pusa Sharad, a black rot-susceptible genotype of cauliflower and ten selected phyllospheric bacterial isolates to assess their biocontrol potential against the black rot disease under glass house conditions. For preparing bacterial suspensions, all 10 cultures were grown on nutrient agar medium for 24 h at 28 °C, and single colonies were transferred to respective broths and incubated for 24 h at 28 °C with shaking at 150 rpm. Bacteria were pelleted with centrifugation for 5 min at 6000 rpm and resuspended in distilled water to give concentrations of $10^9$ CFU/mL.

Twenty-one-day-old cauliflower seedlings were transplanted in 15-cm-diameter earthen pots having autoclaved soil mixture of peat moss, vermiculite, and sand in the ratio 2:1:1 at 28 ± 2 °C. Forty-five-day-old plants were sprayed with 100 mL of a bacterial suspension ($10^9$ CFU/mL) of phyllobacterial strains. A control treatment was sprayed with 0.1 M SPB (pH 7.0). Thus, there were 11 treatments with three replications. The treatment details are provided in Table 5. Culture suspension of black rot causing pathogen (Xcc) was also prepared and inoculated to the cauliflower leaves after 72 h of phyllobacterial spray. Disease severity was recorded 14 and 21 days after inoculation by using disease rating scale 0–9 based on the relative lesion size (Vicente et al. 2002) and *DSI* was calculated using the formula:

$$DSI(\%) = \frac{\left[\Sigma(Rating\ no. \times No.\ of\ plants\ in\ rating)\right]}{(Total\ no.\ of\ plants \times Highest\ rating)} \times 100$$

Protection against Xcc was assessed by comparing the disease severity values.

Biological control efficacy (*BCE*) of antagonistic bacteria was determined as described formula by Guo et al. (2004):

$$BCE = [(DC - DT)/DC] \times 100$$

where *DC* is the disease of control and *DT* is the disease of the treatment group.

### Identification of the phyllospheric bacterial isolates

Four bacterial isolates (CFLB-27, CFLB-24, CFLB-31, and CFLB-26) were selected for further genetic identification. The bacterial DNA was extracted using a DNA isolation kit (ZYMO Research Corporation, USA). The 16S rRNA gene was amplified using universal primers (Edwards et al. 1989), and the resulting products were purified using Gene JET thermo scientific gel extraction kit and sequenced. The aligned sequences were analyzed for maximum homogeneity with available 16S rDNA gene sequences in NCBI database through BLASTn tools and the similarity index was used for their identification.

The sequences were aligned using Clustal W program and a phylogenetic tree (bootstrap method) was constructed using Mega-X software (Kumar et al. 2018). The Maximum Likelihood method (Tamura and Nei 1993) was used to infer ancestral states, and the Kimura-2 parameter model (Kimura 1980) was used to estimate rates among sites. These sequences have been submitted to NCBI and assigned accession numbers ON514075, ON514218, ON514187, and ON514222 for the four promising biocontrol bacterial isolates — CFLB-27, CFLB-24, CFLB-31, and CFLB-26, respectively.

## Results

### Isolation of phyllospheric bacteria

A total of 46 diverse bacterial isolates were isolated from the healthy and infected leaf samples of resistant and susceptible plants of cauliflower on different microbiological media, viz., Nutrient Agar, King's B, and Tryptone Soya Agar media, and purified them from different colonies. Out of forty six bacterial isolates, sixteen isolates were sourced from the healthy leaves of the resistant cauliflower genotype BR-161, while thirteen isolates were derived from slightly black rot infected leaves of the same resistant genotype. Additionally, eleven isolates were obtained from the healthy leaves of the susceptible cauliflower genotype Pusa Sharad, with the remaining seven isolates originating from leaves affected by disease in the susceptible genotype. Out of three media used for the isolation of phyllospheric bacteria, nutrient agar supported maximum population in log value followed by tryptone soya agar and King's B. Plate count of bacterial population cultured from different leaf samples ranged from 1.13 to 7.42 $Log_{10}$ CFU $g^{-1}$ (Table 1).

### Morphological and biochemical characterization of phyllospheric bacteria isolated from cauliflower leaves

The study involved screening 46 isolates of phyllospheric bacteria for various morphological characteristics such as color, size, margin, shape, texture, and pigmentation (Table 2). The isolates exhibited a smooth-rough texture and a color range from pure white to translucent, light brown, yellow, pinkish, and gray-white. The colonies varied in size from large to small (small ≤ 2 mm, medium 2–4 mm,

**Table 1** Abundance of phyllospheric bacterial population on black rot infected and healthy leaves of resistant and susceptible genotypes of cauliflower on different microbiological media

| Leaf samples | Phyllospheric bacterial population Log value (CFU/g of leaf tissue) | | |
|---|---|---|---|
| | Nutrient agar | Tryptone soya agar | King's B |
| **(a) BR-161 (resistant)** | | | |
| **Healthy leaf** | $7.42 \pm 0.10^{a}$ | $5.13 \pm 0.19^{a}$ | $3.45 \pm 0.23^{a}$ |
| **Black rot-infected leaf** | $7.12 \pm 0.16^{a}$ | $3.48 \pm 0.10^{b}$ | $2.44 \pm 0.05^{b}$ |
| **(b) Pusa Sharad (susceptible)** | | | |
| **Healthy leaf** | $6.45 \pm 0.12^{b}$ | $3.07 \pm 0.15^{c}$ | $1.37 \pm 0.13^{c}$ |
| **Black rot-infected leaf** | $6.39 \pm 0.14^{b}$ | $2.46 \pm 0.16^{d}$ | $1.13 \pm 0.09^{c}$ |

Data are the average of three replicates ± SD; grouping information between mean values of obtained data was carried out by Tukey's test and 95% confidence ($P \leq 0.05$); different letter points out significant differences in a column

and large > 4 mm) and had different shapes such as round, irregular, and uniform, with transparent, opaque, and semi-transparent appearances. Of the 46 isolates, 29 showed a negative reaction for the 3.0% KOH string test, indicating that 36.96% of the bacterial isolates belonged to Gram-negative bacteria, while the majority (63.04%) were Gram-positive. Most of the isolates were rod-shaped (37 isolates) and 9 were cocci-shaped. Further biochemical tests were conducted on all the isolates. Of the isolates, 95.65% showed a positive result in the catalase test, while 54.35% were positive in the peroxidase test. Arginine dehydrogenase production, citrate utilization, and $H_2S$ production were also recorded, and 63.04%, 76.08%, and 45.65% of phyllospheric isolates showed a positive reaction, respectively.

In addition, 58.69% of bacterial isolates hydrolyzed starch and 73.46% liquefied gelatine (Table 3). These results suggest that a diverse group of bacteria exists in the phyllosphere of cauliflower, and they exhibit various behaviors in biochemical tests.

## Plant growth-promoting activities of isolated phyllospheric bacteria

In this study, 46 bacterial isolates were screened for their ability to produce indole-3-acetic acid (IAA). Eight isolates, namely, CFLB-6, CFLB-9, CFLB-16, CFLB-17, CFLB-27, CFLB-31, CFLB-40, and CFLB-45, were strong producers of IAA, while eight other isolates, CFLB-12, CFLB-13, CFLB-15, CFLB-24, CFLB-25, CFLB-26, CFLB-39, and CFLB-41, were medium producers. The maximum IAA-producing isolate was CFLB-27 (65.93 µg/mL), followed by CFLB-45 (65.47 µg/mL) and CFLB-40 (64.05 µg/mL). Furthermore, 82.60% of the isolates exhibited phosphorus solubilization activity, with CFLB-27 showing the highest activity (50.42 µg/mL), followed by CFLB-45 (49.26 µg/mL) and CFLB-16 (47.94 µg/mL). About 63.04% of the isolates showed a positive reaction in ammonia production, while 58.69% of the phyllospheric bacteria produced hydrogen cyanide (HCN), albeit weakly. Additionally, 36.95% of bacterial isolates produced siderophores, and CFLB-27 was found to be a high producer of siderophores (Table 4).

## In vitro antagonistic activity of phyllospheric bacteria against cauliflower black rot causing pathogen *X. campestris pv. campestris*

In this study, it was found that 50% of the 46 phyllospheric bacterial isolates from cauliflower leaves exhibited antagonistic activity against *X. campestris* pv. *campestris*. The presence of these bacterial isolates resulted in a marked inhibition in the growth of *X. campestris* pv. *campestris* culture, as shown in Table 4. The isolate CFLB-27, obtained from the slightly black rot-infected leaf of the resistant genotype (BR-161) of cauliflower, showed the maximum inhibition zone of *X. campestris* pv. *campestris* (28.69-mm diameter), followed by CFLB-26 (18.58-mm diameter) and CFLB-24 (18.24-mm diameter). However, the isolate CFLB-30 exhibited the minimum inhibitory effect (1.21-mm diameter) after 48 h of incubation, as shown in Fig. 1.

## Biocontrol of black rot disease of cauliflower by phyllospheric bacteria under glass-house conditions

Based on the results of in vitro antagonistic and plant growth-promoting activities, the 10 most promising phyllospheric bacteria (CFLB-6, CFLB-12, CFLB-13, CFLB-16, CFLB-24, CFLB-26, CFLB-27, CFLB-31, CFLB-41, and CFLB-45) were selected for further screening for biocontrol of black rot disease of cauliflower (*X. campestris* pv. *campestris*) under glass-house conditions. The percent disease severity of the control group was 42.09% and 72.42% at 14 and 21 days, respectively, indicating a high level of disease pressure. All selected phyllospheric bacterial isolates significantly reduced disease severity of black rot disease of cauliflower as compared to control. Among the bacterial isolates, CFLB-27 showed the highest suppression of the black

**Table 2** Characterization of colonies of bacterial isolates, isolated from healthy and black rot-infected leaves of resistant (BR-161) and susceptible (Pusa Sharad) genotypes of cauliflower

| Serial no. | Isolate | Source | Size | Shape | Margin | Opacity | Elevation | Texture | Pigmentation |
|---|---|---|---|---|---|---|---|---|---|
| 1. | CFLB-1 | BR-161 (healthy leaf) | Medium | Circular | Entire round | Opaque | Convex | Smooth | Yellow-orange |
| 2. | CFLB-2 | BR-161 (healthy leaf) | Medium | Round | Round | Opaque | Convex | Smooth | White to slightly orange |
| 3. | CFLB-3 | BR-161 (healthy leaf) | Medium | Round with glistening | Round | Opaque | Convex | Smooth mucoid | Yellow |
| 4. | CFLB-4 | BR-161 (healthy leaf) | Medium | Round | Round | Opaque | Convex | Smooth | Yellow to slightly orange |
| 5. | CFLB-5 | BR-161 (healthy leaf) | Medium | Round | Entire margin | Opaque | Convex | Smooth | Yellow |
| 6. | CFLB-6 | BR-161 (healthy leaf) | Medium | Circular | Entire margin | Opaque | Convex undulated | Smooth | Beige |
| 7. | CFLB-7 | BR-161 (healthy leaf) | Small | Circular | Round | Opaque | Convex | Smooth | Off-white |
| 8. | CFLB-8 | BR-161 (healthy leaf) | Medium | Round | Entire margin | Opaque | Convex | Smooth | Off-white |
| 9. | CFLB-9 | BR-161 (healthy leaf) | Medium | Irregular | Undulate | Opaque | Flat | Irregular | White |
| 10. | CFLB-10 | BR-161 (healthy leaf) | Medium | Circular | Regular edges | Opaque | Convex with elevated center | Glistening smooth | Slightly orange |
| 11. | CFLB-11 | BR-161 (healthy leaf) | Medium | Round | Thick ridges | Opaque | Convex | Smooth moist | Gray white |
| 12. | CFLB-12 | BR-161 (healthy leaf) | Medium | Irregular | Undulate | Opaque | Flat | Irregular | Yellowish white |
| 13. | CFLB-13 | BR-161 (healthy leaf) | Small | Uniform | Entire round | Opaque | Convex | Smooth, shiny | Off-white with bluish tinge |
| 14. | CFL-B14 | BR-161 (black rot-infected leaf) | Small | Circular | Round | Opaque | Convex | Smooth, bright | Yellowish orange |
| 15. | CFLB-15 | BR-161 (black rot-infected leaf) | Medium | Uniform | Entire round | Semi-transparent | Flat | Smooth | Light brown |
| 16. | CFLB-16 | BR-161 (black rot-infected leaf) | Small | Uniform | Entire round | Opaque | Convex | Smooth, shiny | Yellowish with orange tinge |
| 17. | CFLB-17 | BR-161 (black rot-infected leaf) | Small | Uniform | Entire round | Opaque | Convex | Smooth | Yellowish off-white |

**Table 2** (continued)

| Serial no. | Isolate | Source | Size | Shape | Margin | Opacity | Elevation | Texture | Pigmentation |
|---|---|---|---|---|---|---|---|---|---|
| 18. | CFLB-18 | BR-161 (black rot-infected leaf) | Small | Round | Entire margin | Opaque | Convex | Smooth, moist surface | Yellow |
| 19. | CFLB-19 | BR-161 (black rot-infected leaf) | Small | Round | Entire margin | Opaque | Convex | Smooth | Yellow |
| 20. | CFLB-20 | BR-161 (black rot-infected leaf) | Small | Circular | Entire round | Opaque | Convex | Smooth, shiny | Yellowish |
| 21. | CFLB-21 | BR-161 (black rot-infected leaf) | Small | Uniform | Entire round | Opaque | Convex | Smooth, shiny | Off-white with bluish tinge |
| 22. | CFLB-22 | BR-161 (black rot-infected leaf) | Medium | Circular | Entire round | Opaque | Slightly convex | Smooth | Yellowish white |
| 23. | CFLB-23 | BR-161 (black rot-infected leaf) | Large | Circular | Entire margin | Opaque | Convex | Smooth | White |
| 24. | CFLB-24 | BR-161 (black rot-infected leaf) | Medium | Uniform | Entire round | Semi-transparent | Flat | Smooth | Light brown |
| 25. | CFLB-25 | BR-161 (black rot-infected leaf) | Medium | Irregular | Undulate | Opaque | Flat | Irregular | Red |
| 26. | CFLB-26 | BR-161 (black rot-infected leaf) | Small | Circular | Round | Semi-transparent | Convex | Smooth | Off-white |
| 27. | CFLB-27 | BR-161 (black rot-infected leaf) | Small | Uniform convex | Entire round | Opaque | Convex | Smooth, shiny | Off-white with bluish tinge |
| 28. | CFLB-28 | BR-161 (black rot-infected leaf) | Medium | Uniform | Round | Opaque | Convex | Smooth | Yellowish white |
| 29. | CFLB-29 | BR-161 (black rot-infected leaf) | Medium | Round with glistening | Round | Opaque | Convex | Smooth mucoid | Yellow |
| 30. | CFLB-30 | Pusa Sharad (healthy leaf) | Small | Round | Entire margin | Opaque | Convex | Smooth, moist surface | Yellow |
| 31. | CFLB-31 | Pusa Sharad (healthy leaf) | Medium | Uniform | Entire round | Semi-transparent | Flat | Smooth | Whitish brown |
| 32. | CFLB-32 | Pusa Sharad (healthy leaf) | Medium | Uniform | Round | Opaque | Convex | Smooth | Yellowish |

**Table 2** (continued)

| Serial no. | Isolate | Source | Size | Shape | Margin | Opacity | Elevation | Texture | Pigmentation |
|---|---|---|---|---|---|---|---|---|---|
| 33. | CFLB-33 | Pusa Sharad (healthy leaf) | Small | Uniform | Round | Opaque | Convex | Smooth | Yellowish orange |
| 34. | CFLB-34 | Pusa Sharad (healthy leaf) | Medium | Uniform | Round | Opaque | Convex | Smooth | Yellowish white |
| 35. | CFLB-35 | Pusa Sharad (healthy leaf) | Medium | Round with glistening | Round | Opaque | Convex | Smooth mucoid | Yellow |
| 36. | CLFB-36 | Pusa Sharad (healthy leaf) | Small | Round | Entire margin | Opaque | Convex | Smooth | Pinkish white |
| 37. | CFLB-37 | Pusa Sharad (healthy leaf) | Medium | Round with glistening | Round | Opaque | Convex | Smooth mucoid | Yellow |
| 38. | CFLB-38 | Pusa Sharad (healthy leaf) | Medium | Round with glistening | Round | Opaque | Convex | Smooth mucoid | Yellow |
| 39. | CFLB-39 | Pusa Sharad (healthy leaf) | Medium | Uniform | Entire round | Semi-transparent | Flat | Smooth | Yellowish brown |
| 40. | CFLB-40 | Pusa Sharad (black rot-infected leaf) | Medium | Uniform | Round | Opaque | Convex | Smooth, shiny | Whitish yellow |
| 41. | CFLB-41 | Pusa Sharad (black rot-infected leaf) | Medium | Round | Thick ridges | Opaque | Convex | Smooth moist | Gray white |
| 42. | CFLB-42 | Pusa Sharad (black rot-infected leaf) | Medium | Circular | Entire margin | Opaque | Convex | Smooth slimy | Whitish |
| 43. | CFLB-43 | Pusa Sharad (black rot-infected leaf) | Medium | Circular | Entire margin | Opaque | Convex | Smooth | Yellow |
| 44. | CFLB-44 | Pusa Sharad (black rot-infected leaf) | Small | Circular | Smooth margin | Opaque | Convex | Smooth | Cream to off-white |
| 45. | CFLB-45 | Pusa Sharad (black rot-infected leaf) | Small | Uniform | Entire round | Opaque | Convex | Smooth, shiny | Off-white |
| 46. | CFLB-46 | Pusa Sharad (black rot-infected leaf) | Medium | Round with glistening | Round | Opaque | Convex | Smooth, mucoid | Yellow |

rot disease of cauliflower at 14 and 21 days after inoculation, with percent disease severity of 14.28% and 25.05%, respectively, in cauliflower cultivar cv. Pusa Sharad (Table 5). The biocontrol efficacy (*BCE*) percentages ranged from 24.39 to 65.41%. Among the bacterial isolates, CFLB-27 had the highest *BCE* percentage (65.41%) followed by CFLB-24 (58.30%), CFLB-31 (47.11%), and CFLB-26 (46.03%). Isolate CFLB-6 showed minimum biocontrol efficacy (24.39%).

**Table 3** Morphological and biochemical characterization of bacterial isolates, isolated from healthy and black rot-infected leaves of resistant and susceptible genotypes of cauliflower

| Serial no. | Name of isolate | KOH test | Gram reaction | Shape | Peroxidase test | Catalase test | Starch hydrolysis | Gelatin liquefaction test | Arginine test | Citrate test | $H_2S$ test |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | CFLB-1 | − | + | Cocci | − | + | + | + | − | + | − |
| 2. | CFLB-2 | − | + | Rods | − | + | + | − | + | − | + |
| 3. | CFLB-3 | + | − | Rods | − | + | + | + | + | + | + |
| 4. | CFLB-4 | − | + | Rods | − | + | + | − | + | − | + |
| 5. | CFLB-5 | − | + | Rods | − | + | + | + | − | + | + |
| 6. | CFLB-6 | − | + | Rods | + | + | + | + | − | + | + |
| 7. | CFLB-7 | + | − | Rods | + | + | − | − | + | − | + |
| 8. | CFLB-8 | − | + | Rods | + | + | + | + | − | + | + |
| 9. | CFLB-9 | − | + | Rods | − | + | + | − | + | + | − |
| 10. | CFLB-10 | − | + | Cocci | − | + | + | + | − | + | − |
| 11. | CFLB-11 | − | + | Rods | − | + | − | + | + | + | − |
| 12. | CFLB-12 | + | − | Rods | − | + | + | − | + | + | − |
| 13. | CFLB-13 | + | − | Rods | + | + | − | + | + | + | − |
| 14. | CFLB-14 | − | + | Cocci | + | + | + | + | − | + | − |
| 15. | CFLB-15 | − | + | Rods | + | + | − | + | − | + | − |
| 16. | CFLB-16 | + | − | Rods | + | + | − | + | + | + | − |
| 17. | CFLB-17 | + | − | Rods | + | + | − | + | + | + | − |
| 18. | CFLB-18 | − | + | Rods | − | + | + | + | − | + | + |
| 19. | CFLB-19 | − | + | Cocci | + | − | − | + | − | + | − |
| 20. | CFLB-20 | − | + | Cocci | − | + | + | + | − | + | − |
| 21. | CFLB-21 | + | − | Rods | + | + | − | + | + | + | + |
| 22. | CFLB-22 | + | − | Rods | − | + | − | + | − | − | − |
| 23. | CFLB-23 | − | + | Cocci | − | + | + | + | − | + | − |
| 24. | CFLB-24 | − | + | Rods | + | + | − | + | − | + | − |
| 25. | CFLB-25 | − | + | Rods | − | + | + | − | + | + | − |
| 26. | CFLB-26 | − | + | Rods | − | + | − | + | + | + | − |
| 27. | CFLB-27 | + | − | Rods | + | + | − | + | + | + | + |
| 28. | CFLB-28 | − | + | Rods | + | + | + | − | + | − | − |
| 29. | CFLB-29 | + | − | Rods | − | + | + | + | + | + | + |
| 30. | CFLB-30 | − | + | Rods | + | + | + | + | − | + | + |
| 31. | CFLB-31 | − | + | Rods | + | + | − | + | + | + | − |
| 32. | CFLB-32 | − | + | Rods | − | + | + | − | − | − | + |
| 33. | CFLB-33 | + | − | Rods | + | + | − | − | + | − | + |
| 34. | CFLB-34 | − | + | Rods | + | + | + | − | + | − | − |
| 35. | CFLB-35 | + | − | Rods | − | + | + | + | + | + | + |

**Table 3** (continued)

| Serial no. | Name of isolate | KOH test | Gram reaction | Shape | Peroxidase test | Catalase test | Starch hydrolysis | Gelatin liquefaction test | Arginine test | Citrate test | $H_2S$ test |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 36. | CFLB-36 | − | + | Rods | + | + | + | + | − | + | + |
| 37. | CFLB-37 | + | − | Rods | − | + | + | + | + | + | + |
| 38. | CFLB-38 | + | − | Rods | − | + | + | + | + | + | + |
| 39. | CFLB-39 | − | + | Rods | + | + | − | + | + | + | − |
| 40. | CFLB-40 | + | − | Rods | + | + | − | + | + | + | + |
| 41. | CFLB-41 | − | + | Rods | + | + | − | + | + | + | − |
| 42. | CFLB-42 | − | + | Cocci | + | + | − | − | + | − | − |
| 43. | CFLB-43 | − | + | Cocci | + | + | + | − | + | − | − |
| 44. | CFLB-44 | + | − | Cocci | + | − | + | + | − | − | − |
| 45. | CFLB-45 | + | − | Rods | + | + | − | + | + | + | + |
| 46. | CFLB-46 | + | − | Rods | − | + | + | + | + | + | + |

Where '+' sign shows positive test and '−' sign shows negative test

## Identification of the phyllospheric bacterial isolates

Based on the 16S rDNA gene sequencing, bacterial isolates CFLB-27, CFLB-24, CFLB-31, and CFLB-26 showed 99.44%, 99.72%, 99.93%, and 99.79% similarity with *Pseudomonas fluorescens*, *Bacillus velezensis* strain FC37, *Bacillus amyloliquefaciens* isolate RHNK 22, and *Stenotrophomonas rhizophila* strain SBR05, respectively (Table 6 and Fig. 2). The 16S rDNA gene sequences of bacterial isolates CFLB-27, CFLB-24, CFLB-31, and CFLB-26 have been submitted to NCBI with the accession numbers ON514075, ON514218, ON514187, and ON514222, respectively.

## Discussion

Black rot disease is a serious problem for cauliflower (*Brassica oleracea* var. *botrytis*) cultivation in India. The disease is caused by *Xanthomonas campestris* pv. *campestris* and can affect the plant through various means, including infected seeds, transplants, soil, crop residues, and related weed species (Schaad and Alvarez 1993). Once the pathogen invades the host plant, it rapidly multiplies, producing high levels of extracellular polysaccharides and xanthan, which clog the plant's vascular system. This leads to the formation of characteristic "*V*"-shaped chlorotic lesions along the edges of the leaves, which then expand and darken, causing the veins to turn black. If left untreated, the entire plant may wither and die (Williams 1980; Kifuji et al. 2013; Tonu et al. 2013).

To prevent the spread of black rot disease, it is important to use disease-free seeds, transplants, and soil, as well as to practice good crop management, such as crop rotation and timely removal of crop residues. Additionally, early detection and treatment of infected plants can help limit the spread of the disease. Black rot disease of cauliflower is difficult to control using standard agronomic practices, and the use of chemical pesticides can have negative impacts on the environment and human health. The increasing demand for chemical-free agricultural products has made it necessary to develop more effective and environmentally friendly biocontrol agents. One promising approach is the use of bacterial antagonists that possess plant growth promotion and protection characteristics. Such bacteria can be utilized to produce novel, effective, and eco-friendly bioformulations that can replace synthetic fungicides (Hyder et al. 2020). The initial stage in developing an efficient bacterial strain for disease management is to isolate and identify potent antagonistic bacteria from natural habitats. This process involves screening different bacterial strains for their ability to effectively control plant pathogens while promoting plant growth. By identifying and characterizing strong antagonistic bacteria, we can develop sustainable and eco-friendly solutions for managing plant diseases. Plants harbor diverse microbial

**Table 4** In vitro plant growth-promoting and antagonistic activities of bacterial isolates, isolated from phyllosphere of black rot-infected and healthy leaves of resistant and susceptible genotypes of cauliflower

| Serial no. | Isolates | IAA production (µg/mL) | HCN production | Ammonia production | Phosphorus solubilization (µg/mL) | Siderophore production | Zone of inhibition (mm) |
|---|---|---|---|---|---|---|---|
| 1. | CFLB-1 | 0.00 | − | − | $5.39^{op}$ | − | 0.0 |
| 2. | CFLB-2 | $8.42^{op}$ | − | + | $12.35^{lmnop}$ | − | 0.0 |
| 3. | CFLB-3 | $28.44^{ijkl}$ | + | + | $31.08^{efgh}$ | − | 0.0 |
| 4. | CFLB-4 | $14.37^{nop}$ | + | − | 0.00 | − | 0.0 |
| 5. | CFLB-5 | $21.86^{lmno}$ | − | + | $19.53^{kl}$ | − | $4.23^{hi}$ |
| 6. | CFLB-6 | $49.08^{bcd}$ | + | ++ | $23.14^{cd}$ | − | $7.26^{f}$ |
| 7. | CFLB-7 | 0.00 | + | + | $19.49^{klm}$ | − | 0.0 |
| 8. | CFLB-8 | $28.02^{jklm}$ | − | + | $27.35^{fghij}$ | − | 0.0 |
| 9. | CFLB-9 | $60.09^{abcd}$ | + | ++ | $28.63^{fghij}$ | + | $4.55^{hi}$ |
| 10. | CFLB-10 | 0.00 | − | − | 0.00 | − | 0.0 |
| 11. | CFLB-11 | $41.73^{fghi}$ | + | + | $30.07^{efghi}$ | + | $7.46^{g}$ |
| 12. | CFLB-12 | $46.51^{efgh}$ | + | ++ | $42.17^{bcd}$ | + | $15.73^{cd}$ |
| 13. | CFLB-13 | $52.25^{cdef}$ | + | ++ | $42.91^{bcd}$ | ++ | $15.24^{de}$ |
| 14. | CFLB-14 | 0.00 | − | − | $12.05^{nop}$ | − | 0.0 |
| 15. | CFLB-15 | $50.15^{defg}$ | + | + | $36.15^{de}$ | + | $12.16^{f}$ |
| 16. | CFLB-16 | $63.27^{abc}$ | + | + | $47.94^{abc}$ | ++ | $16.57^{c}$ |
| 17. | CFLB-17 | $63.84^{abc}$ | + | + | $42.17^{bcd}$ | + | $4.64^{hi}$ |
| 18. | CFLB-18 | $25.15^{klmn}$ | − | + | 0.00 | − | 0.0 |
| 19. | CFLB-19 | $36.48^{hijk}$ | + | − | $33.15^{ef}$ | − | 0.0 |
| 20. | CFLB-20 | 0.00 | − | − | $8.95^{op}$ | − | 0.0 |
| 21. | CFLB-21 | $32.3^{ijkl}$ | + | + | $41.75^{cd}$ | − | $4.39^{hi}$ |
| 22. | CFLB-22 | $52.9^{cdef}$ | + | + | $31.9^{efg}$ | + | $3.54^{i}$ |
| 23. | CFLB-23 | 0.00 | − | − | $12.24^{lnop}$ | − | 0.0 |
| 24. | CFLB-24 | $54.49^{bcde}$ | + | +++ | $41.76^{cd}$ | ++ | $18.24^{b}$ |
| 25. | CFLB-25 | $54.44^{bcde}$ | + | + | $40.77^{cd}$ | + | $12.37^{f}$ |
| 26. | CFLB-26 | $53.12^{cdef}$ | + | ++ | $36.12^{de}$ | ++ | $15.35^{cde}$ |
| 27. | CFLB-27 | $65.93^{a}$ | − | +++ | $50.42^{a}$ | ++ | $28.69^{a}$ |
| 28. | CFLB-28 | $42.28^{fghi}$ | − | + | 0.00 | − | 0.0 |
| 29. | CFLB-29 | $32.79^{ijkl}$ | + | − | $28.79^{fghij}$ | − | 0.0 |
| 30. | CFLB-30 | $24.78^{klmn}$ | − | + | $22.11^{jk}$ | − | $1.21^{k}$ |
| 31. | CFLB-31 | $60.1^{abcde}$ | + | +++ | $42.43^{bcd}$ | ++ | $18.58^{b}$ |
| 32. | CFLB-32 | $40.61^{ghi}$ | + | − | $27.28^{fghij}$ | − | $5.23^{h}$ |
| 33. | CFLB-33 | 0.00 | − | − | $13.48^{lmno}$ | − | $2.31^{j}$ |
| 34. | CFLB-34 | 0.00 | − | + | 0.00 | − | 0.0 |
| 35. | CFLB-35 | $31.47^{ijkl}$ | + | − | $24.14^{hijk}$ | − | 0.0 |
| 36. | CFLB-36 | $32.2^{ijkl}$ | + | − | $18.87^{klmn}$ | − | 0.0 |
| 37. | CFLB-37 | $40.84^{ghi}$ | − | − | $28.17^{fghij}$ | − | 0.0 |
| 38. | CFLB-38 | $39.25^{ghij}$ | − | − | 0.00 | − | 0.0 |
| 39. | CFLB-39 | $52.54^{cdef}$ | + | + | $32.54^{ef}$ | + | $14.24^{e}$ |
| 40. | CFLB-40 | $64.05^{abc}$ | + | + | $19.05^{klmn}$ | + | $3.45^{i}$ |
| 41. | CFLB-41 | $56.76^{abcde}$ | + | ++ | $45.42^{abc}$ | + | $16.26^{cd}$ |
| 42. | CFLB-42 | 0.00 | − | − | $13.88^{lmno}$ | − | 0.0 |
| 43. | CFLB-43 | 0.00 | − | + | $11.15^{op}$ | − | 0.0 |
| 44. | CFLB-44 | $30.79^{ijkl}$ | − | − | 0.00 | − | 0.0 |
| 45. | CFLB-45 | $65.47^{ab}$ | + | ++ | $49.26^{ab}$ | + | $16.13^{cd}$ |
| 46. | CFLB-46 | $29.51^{ijkl}$ | + | − | 0.00 | − | 0.0 |

Where '−' sign shows negative test, '+' shows weak producer; '++' shows medium producer, and '+++' shows strong producer. Data are the average of three replicates; grouping information between mean values of obtained data was carried out by Tukey's test and 95% confidence ($P \leq 0.05$); different letter points out significant differences in a column

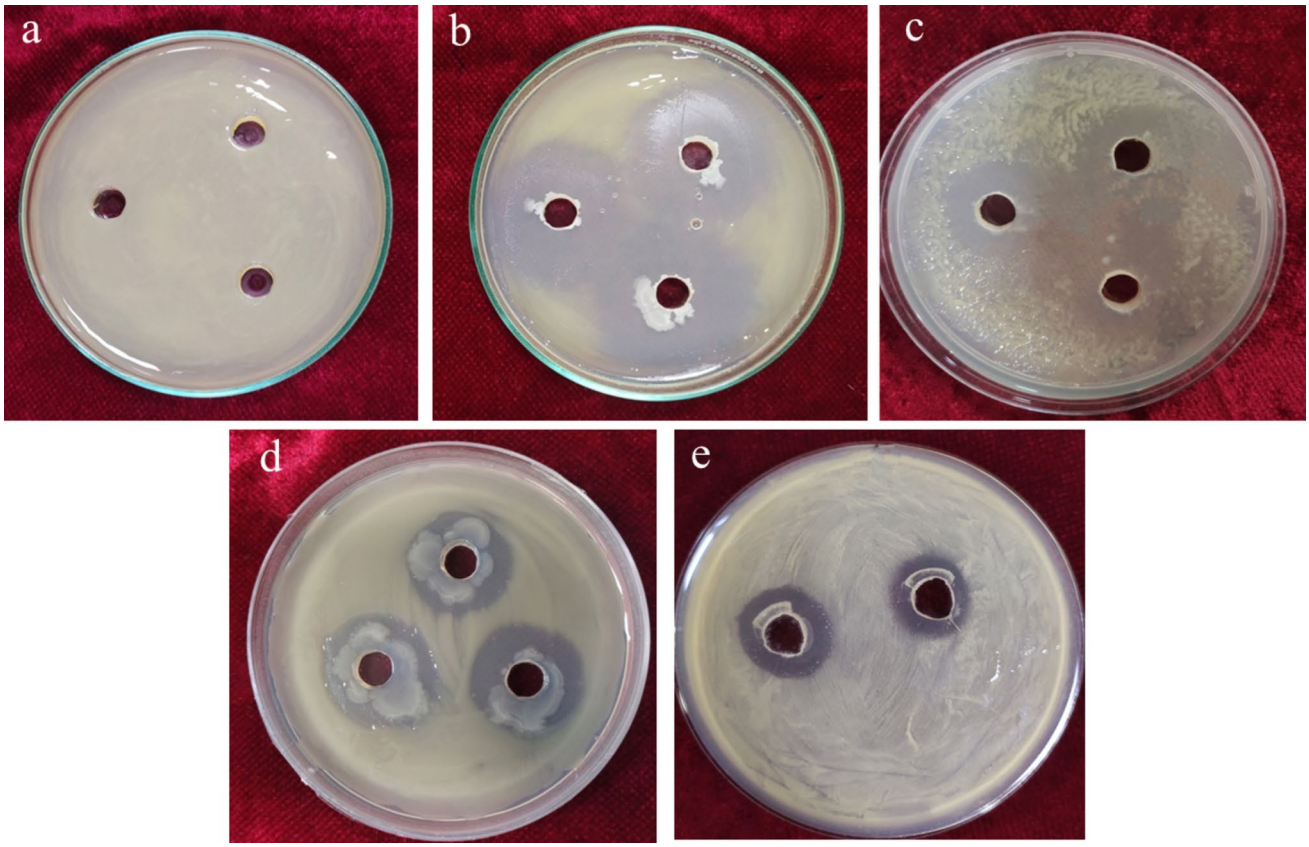


**Fig. 1** In vitro antagonistic activity of cauliflower phyllospheric bacterial isolate against *Xanthomonas campestris* pv. *campestris*. **a** Control, **b** CFLB-27, **c** CFLB-24, **d** CFLB-31, and **e** CFLB-26

communities, collectively known as the plant microbiome, on both endophytic and epiphytic habitats during their life cycle. The spatiotemporal succession of these microbial communities is influenced by a variety of biotic and abiotic factors (Jacobs et al. 2005).

Our present study reveals the diverse and abundant populations of phyllospheric bacteria in cauliflower plants and provides valuable insights for future research on potential biocontrol agents or beneficial plant growth-promoting bacteria. By understanding the diversity and function of these microbial communities, we can develop effective and eco-friendly strategies for managing black rot disease in cauliflower crops. In our present study, a total of 46 bacterial isolates were obtained from healthy and black rot-infected leaves of cauliflower, and were subjected to various tests to determine their potential as biocontrol agents or plant growth-promoting bacteria. The high percentage of gram-positive bacteria and their ability to produce beneficial enzymes suggest their importance in plant health. Further evaluation revealed that the isolates had a high capacity for phosphate solubilization, ammonia production, HCN production, siderophore production, and IAA production, indicating their potential as biocontrol agents or beneficial bacteria. In a previous study conducted by Adler et al. (2012), it was reported that pyochelin, a siderophore produced by *Pseudomonas aeruginosa*, demonstrated inhibitory activity in vitro against bacteria belonging to various genera, including *Xanthomonas citri* subsp. *citri*. Similarly, Abadi et al. (2020) studied the role of dominant phyllosphere bacteria with plant growth-promoting characteristics of maize (*Zea mays* L.). It was found that members of genera *Bacillus*, *Pseudomonas*, *Microbacterium*, *Stenotrophomonas*, *Enterobacter*, *Pseudarthrobacter*, and *Kocuria* were the most dominant PGPB in maize phyllosphere. Ghadamgahi et al. (2022) reported that *Pseudomonas aeruginosa* strain FG106 produces siderophores, ammonia, indole acetic acid (IAA), and hydrogen cyanide (HCN), and forms biofilms that promote plant growth and facilitate biocontrol of *Alternaria alternata*, *Botrytis cinerea*, *Clavibacter michiganensis* subsp. *michiganensis*, *Phytophthora colocasiae*, *P. infestans*, *Rhizoctonia solani*, and *Xanthomonas euvesicatoria* pv. *perforans*.

In our present study, phyllospheric bacteria from cauliflower leaves were found to have significant antagonistic activity against *X. campestris* pv. *campestris*, the pathogen responsible for cauliflower black rot. Fifty percent of the 46

**Table 5** Evaluation of phyllospheric bacterial isolates for suppression of *X. campestris* pv. *campestris* caused black rot disease of cauliflower

| Treatments | Percent disease severity | | Biological control efficacy (%) |
|---|---|---|---|
| | 14 days | 21 days | |
| **CFLB-6** | 26.94 (31.22)$^{b}$ | 54.75 (47.71)$^{b}$ | 24.39 |
| **CFLB-12** | 22.02 (27.94)$^{bcd}$ | 46.50 (42.97)$^{bc}$ | 35.79 |
| **CFLB-13** | 24.60 (29.63)$^{b}$ | 51.63 (45.92)$^{b}$ | 28.71 |
| **CFLB-16** | 23.95 (29.26)$^{bc}$ | 46.39 (42.91)$^{bc}$ | 35.95 |
| **CFLB-24** | 14.93 (22.64)$^{cd}$ | 30.20 (33.31)$^{de}$ | 58.30 |
| **CFLB-26** | 19.76 (26.35)$^{bcd}$ | 39.08 (38.67)$^{c}$ | 46.03 |
| **CFLB-27** | 14.28 (22.14)$^{d}$ | 25.05 (30.00)$^{e}$ | 65.41 |
| **CFLB-31** | 18.88 (25.71)$^{bcd}$ | 38.30 (38.21)$^{cd}$ | 47.11 |
| **CFLB-41** | 21.28 (27.44)$^{bcd}$ | 42.09 (40.43)$^{c}$ | 41.89 |
| **CFLB-45** | 26.25 (30.78)$^{b}$ | 52.24 (46.27)$^{b}$ | 27.86 |
| **Control** | 42.09 (40.43)$^{a}$ | 72.42 (58.32)$^{a}$ | 0.00 |
| **CD** | 3.969 | 3.024 | |
| **SE (m)** | 1.336 | 1.018 | |
| **SE (day)** | 1.890 | 1.440 | |
| **CV** | 8.119 | 4.173 | |

Data are the average of three replicates; figures in parenthesis indicate angular transformation values; grouping information between mean values of obtained data was carried out by Tukey's test and 95% confidence ($P \leq 0.05$); different letter points out significant differences in a column

bacterial isolates showed inhibition against the pathogen, with CFLB-27 from a slightly black rot-infected leaf of a resistant genotype exhibiting the highest inhibition zone of 28.69-mm diameter. *Pseudomonas fluorescens* CFLB-27 and *Bacillus velezensis* CFLB-24 were found to be the most effective bacterial isolates in reducing the severity of black rot disease, followed by *Bacillus amyloliquefaciens* CFLB-31 and *Stenotrophomonas rhizophila* CFLB-26. The strong biocontrol efficacy of *Pseudomonas fluorescens* CFLB-27 and *Bacillus velezensis* CFLB-24 was attributed to their potent antagonistic activity against the pathogen, which was observed in the in vitro study. The ability of these isolates to colonize the phyllosphere and establish beneficial interactions with the host plant could have also contributed to their biocontrol efficacy. The biocontrol efficacy of the phyllospheric bacterial isolates could also be attributed to their plant growth-promoting activity. The plant growth-promoting traits such as indole acetic acid (IAA) production, phosphate solubilization, HCN, ammonia, and siderophore production could have stimulated plant growth and induced systemic resistance against the pathogen. Petti et al. (2012) demonstrated that indole-3-acetic Acid (IAA) plays a crucial role in *Pseudomonas fluorescens*-mediated control of Fusarium head blight (FHB) disease of barley. They found that *P. fluorescens* inoculation resulted in increased levels of IAA in the plant, which primed the plant defense mechanisms and improved disease resistance. Ghazy and El-Nahrawy (2021) investigated that *Bacillus subtilis* MF497446 and *Pseudomonas koreensis* MG209738 were capable of producing siderophores that could chelate iron ions in the environment, thereby inhibiting the growth of *Cephalosporium maydis*, a fungal pathogen causing head smut disease in maize plants.

Munoz et al. (2022) conducted a similar study where they isolated 69 bacterial strains from the phyllosphere of tomato and lettuce, and evaluated their antimicrobial activity against bacterial pathogens such as *Pseudomonas syringae* pv. tomato and *Erwinia corotovora* subsp. *brasiliensis*. Among the isolated strains, *Bacillus subtilis* STRP31, *Bacillus velezensis* SPL51, and *Paenibacillus* sp. PL91 were identified as highly effective biocontrol agents against these pathogens. Several other studies have also reported the use of plant-associated microorganisms as biocontrol agents against crop diseases. For example, *Pseudomonas graminis*, isolated from the apple phyllosphere, showed control against fire blight caused by *Erwinia amylovora* (Mikicínski et al. 2016); *Pseudomonas protegens* CS1 from the lemon phyllosphere are used as a biocontrol against citrus canker (Michavila et al. 2017). Similar study was conducted by Romeiro et al. (2000) who isolated 129 bacterial residents from the phylloplane of healthy tomato plants and screened against *Pseudomonas syringae* pv. *tomato* among which *Pantoea agglomerans* and *Cedecea davisiae* were effective under greenhouse conditions. The study conducted by Macha et al. (2021) also supported that *B. velezensis* FZB42 acted as a potent antagonistic strain against Xcc. It is widely acknowledged that microorganisms associated with plants have significant roles to play in plant health, growth, and development, and also contribute to maintaining environmental balance (Ongena and Jacques 2008). The utilization of beneficial microorganisms presents

**Table 6** Results of 16S rDNA gene sequence similarities of bacterial isolates and GenBank accession numbers using BLASTn algorithm

| Isolate code | Sequence length (bp) | Closest related strain in NCBI database | Accession number | Similarity (%) | *E*-value |
|---|---|---|---|---|---|
| **CFLB-27** | 1425 | *Pseudomonas fluorescens* | KY490089 | 99.44 | 0.0 |
| **CFLB-24** | 1436 | *Bacillus velezensis* strain FC37 | OU487633 | 99.72 | 0.0 |
| **CFLB-31** | 1431 | *Bacillus amyloliquefaciens* isolate RHNK 22 | LM651914 | 99.93 | 0.0 |
| **CFLB-26** | 1441 | *Stenotrophomonas rhizophila* strain SBR05 | KX018309 | 99.79 | 0.0 |

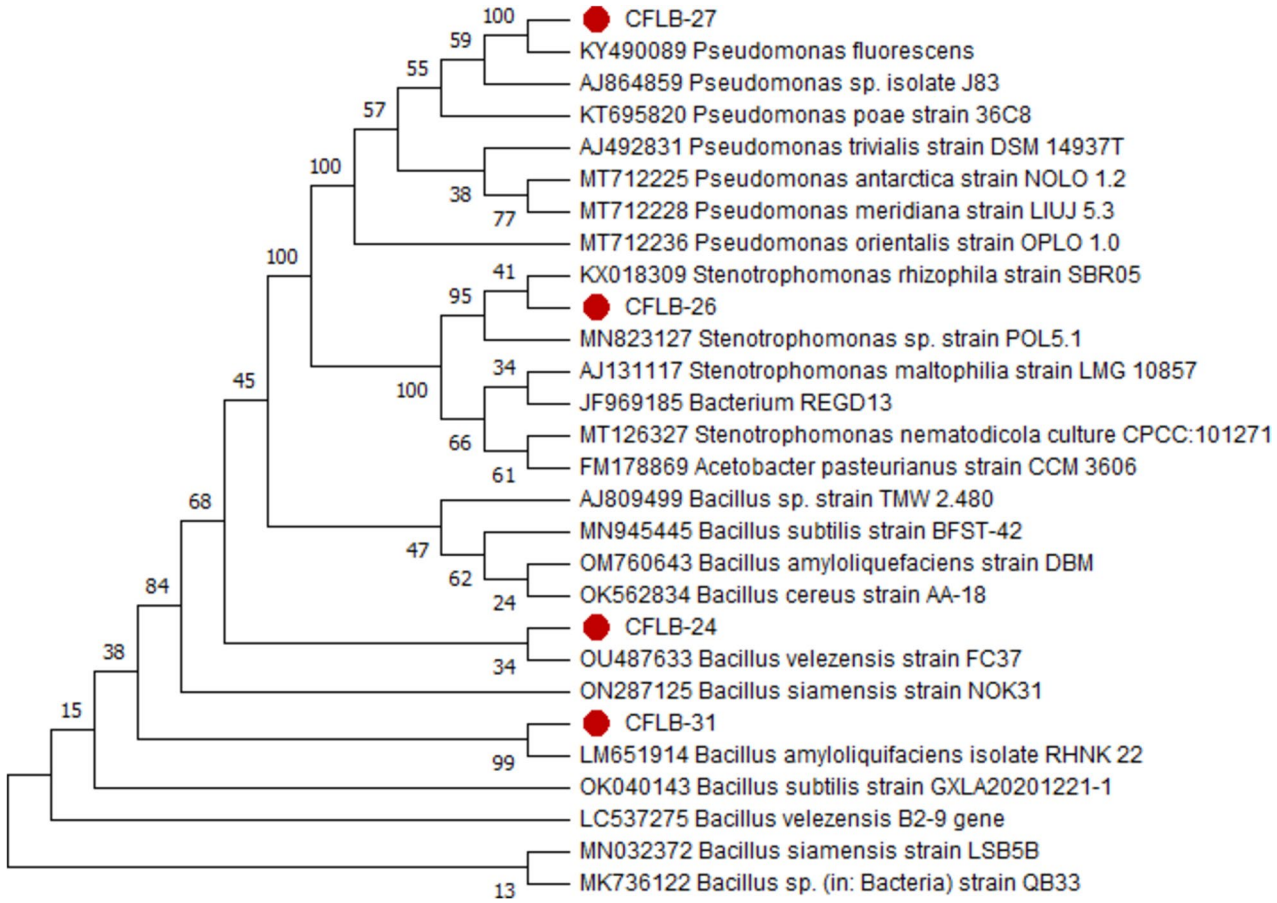


**Fig. 2** Phylogenetic tree constructed using Maximum Likelihood method based on Tamura-Nei model (1993) with 1000 bootstrap replications for phyllospheric bacterial isolates (CFLB-24, CFLB-26, CFLB-27, and CFLB-31) derived from Clustal W alignment of 16S rDNA partial sequences. Red circles indicate cauliflower phyllospheric bacteria

a promising approach to combat crop diseases and enhance yields, thereby ensuring adequate crop production (Heydari and Pessarakli 2010). By leveraging the positive effects of plant-associated microorganisms, we can promote sustainable agriculture and minimize the dependence on harmful chemicals. Therefore, exploring and utilizing beneficial microorganisms is crucial to enhance crop productivity and ensure food security in the long run.

## Conclusion

The use of chemical pesticides in agriculture has raised serious concerns about the safety of the environment and human health. As a result, there has been a growing interest in finding safer alternatives for disease management in crops. In this study, we have identified four novel potential bacterial strains isolated from the phyllosphere of cauliflower, namely, *Pseudomonas fluorescens* CFLB-27, *Bacillus velezensis* CFLB-24, *Bacillus amyloliquefaciens* CFLB-31, and *Stenotrophomonas rhizophila* CFLB-26, which have shown significant antagonistic activity against the pathogenic *Xanthomonas campestris* pv. *campestris*, the causative agent of black rot disease in cauliflower. Furthermore, our findings also indicate that these biocontrol strains have the ability to promote plant growth, which is an added benefit to the farmers. The results suggest that these phyllospheric bacteria could be used as safe and environment-friendly bioformulations at the field level for the management of black rot disease in cauliflower, and also for enhancing its quality and productivity. Overall, this study provides valuable insights into the use of beneficial microorganisms for sustainable agriculture and highlights the potential of phyllospheric bacteria as an effective tool for disease management and plant growth promotion.

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**Author contribution** Conceptualization: Dinesh Singh; data curation: Neelam Geat and Devendra Singh; formal analysis: Neelam Geat, Partha Saha, and Rajender Jatoth; funding acquisition: Dinesh Singh; investigation: Dinesh Singh and Neelam Geat; methodology: Neelam Geat, Devendra Singh, Pedapudi Lokesh Babu, Rajender Jatoth, and Partha Saha; project administration: Dinesh Singh; resources: Dinesh Singh; software: Devendra Singh; supervision: Dinesh Singh; validation: Devendra Singh; visualization: Dinesh Singh; roles/writing — original draft: Neelam Geat, Pedapudi Lokesh Babu, and Rajender Jatoth; writing — review and editing: Devendra Singh and Dinesh Singh.

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**Data availability** The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Declarations

**Competing interests** The authors declare no competing interests.

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