

Article

Composting of Vegetable Waste Using Microbial Consortium and Biocontrol Efficacy of *Streptomyces* Sp. Al-Dhabi 30 Isolated from the Saudi Arabian Environment for Sustainable Agriculture

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Abstract: Thirty-seven root-associated Actinomycetes were isolated from the tomato plant for plant growth promoting activity. Among these, ten were selected for phosphate solubilisation, the production of siderophores, and indole acetic acid. Out of ten, eight Actinomycetes solubilised phosphate, whereas, *Streptomyces* sp. Al-Dhabi 30 showed better activity (43.1 mg/dL). Actinomycetes produced siderophore and the concentration ranged between 1.6 and 42.1 µg/mL. *Streptomyces* sp. Al-Dhabi 30 showed the ability to produce a maximum amount of indole acetic acid (IAA) (43 µg/mL), chitinase (43.1 U/mL), cellulase (67 U/mL), and protease (121 U/mL) than other strains. Further, vegetable waste was used as the bulk material for composting using *Streptomyces* sp. Al-Dhabi 30 along with microbial consortium. Total nitrogen content was 3.8% in *Streptomyces* sp. Al-Dhabi 30 inoculated compost, whereas 2.7% organic nitrogen was detected in the control. In the compost vegetable waste, the C:N ratio was 10.07, whereas it was 17.51 in the control. The vegetable waste composted with *Streptomyces* sp. Al-Dhabi 30, *Lactobacillus plantarum* ATCC 33222, and *Candida utilis* ATCC 9950 showed antagonistic activity and the supplemented compost enhanced shoot, root height, and total weight in tomato plants. These findings clearly suggest the use of *Streptomyces* sp. Al-Dhabi 30 as a potential biocontrol agent.

Keywords: *Streptomyces* sp.; antagonistic activity; phytopathogens; tomato plant; growth promoters

1. Introduction

Biological waste is mainly found in municipal solid waste (MSW) and more than 75% MSW composed of biodegradable substances. However, the amount of this biodegradable waste in MSW depends on the country from where MSW is generated. For the past three decades, there has been increased population, urbanization and accelerating economy, stimulating a steep increase in MSW. According to Zhou et al. [1], China produces 96 million tonnes of kitchen waste/year, whereas India generates about 68.8 million tonnes/year. Many methods have been proposed to dispose of MSW and waste dumping in a disposal yard is a commonly used method. This method leads to soil pollution, the emergence of various diseases in cattle, human beings, and plants [2]. However, composting of biological waste by microbial consortia can add various advantages. An anaerobic digestion process has been effectively utilized in China to manage MSW and China aimed to treat about 30,000 tonnes of waste food/day [3]. Recently, Huiru et al. [4] analyzed the economic and technical feasibility of food waste digestion in anaerobic condition for electricity production in small plants. Agricultural fields are

mainly exposed to various types of organic waste, which raise the increased possibility of isolation and screening of various microbial consortia with novel applications. Organic waste degradation requires enzymatic action of microorganisms to hydrolyze highly complex polymers into simpler degradable molecules. Hence, inoculation of biodegradable waste with various beneficial microorganisms, such as bacteria, fungi, and actinomycetes for the synthesis of extracellular enzymes, such as lipase, pectinase, protease, amylase, and cellulase, enhances the organic waste degradation rate [5]. This can be achieved by co-culturing various microorganisms and each organism contributes either way by synthesizing unique enzymes. The biodegradation of waste by the application of a microbial consortium is a classical method, and the level of degradation of organic waste by microbial species is based on its structural and functional stability of the biomass. Analysis of an efficient biodegrading consortium can be performed by antagonistic property, synergistic activity, and concomitant production of various hydrolytic enzymes [6].

In recent times, the environment has been severely affected by continuous application of pesticides and various synthetic fertilizers. The unrestricted use of various pesticides and inorganic fertilizers affects soil fertility, resulting in productivity loss. Hence, an alternate approach should be implemented to reverse soil fertility. The eco-friendly approach is an alternate method to reverse soil fertility and plant productivity using various microbes. These microorganisms reduce C:N ratio and enhanced soil productivity [7]. Generally, the composting process involves many steps using diverse microbial consortia, such as fungi, bacteria, and Actinomycetes, collectively involved in humus formation. Composting is an eco-friendly method to reduce biodegradable organic waste into highly nutritious organic fertilizer. These organic fertilizers can be used as effective nutrients in vegetation [8].

Composted organic waste has generally been used to improve horticultural and agricultural soil to enhance crop growth and soil fertility. Also, composted nutrients effectively control soil-borne diseases. The applied compost suppressed soil-born plant pathogens and thus reduced the application of fungicides to the environment. Many mechanisms have been proposed to describe the control of soil-borne pathogens by compost, such as hyperparasitism, antibiotic production by various *Streptomyces* sp., and activation of disease-resistant genes of host plants [9]. Actinomycetes, mainly *Streptomyces* sp., have the potential to act against plant pathogens. These *Streptomyces* species are aerobic, Gram-positive bacterium and produce various antibiotics. It was estimated that more than 60% of the antimicrobial and antifungal agents developed for horticulture and agriculture have been extracted from *Streptomyces* spp. [10]. However, extensive hydrolytic enzyme screening on *Streptomyces* sp. and the composting process is limited. Until now, few chitinase-, cellulase- and antibiotics-producing organisms have been described from Actinobacteria. The genus *Streptomyces* is mainly considered as being able to produce many bioactive secondary metabolites that can effectively control various phytopathogens by improving endophytic or rhizosphere colonization [11]. Also, *Streptomyces* spp. enhances the bioavailability of various nutrients, alleviating abiotic stress and controlling phytopathogens. The antifungal secondary metabolites synthesized by *Streptomyces* showed activity against phytopathogens, such as *Rhizoctoniasolani* [12], due to the production of various enzymes and secondary metabolites. Actinomycetes can be used to enhance the availability of nutrients to the plants, which is an alternative to chemical fertilizers and pesticides.

2. Materials and Methods

2.1. Soil Samples

Tomato (*Solanum lycopersicum*) plant and rhizosphere-associated soil sample was collected from one plant pot using a sterile container. The surface soil was removed using a spatula and one sample (100 gm soil) was collected at approximately 10 cm depth. It was transported to the laboratory for analysis. Loosely adhered rhizosphere soil was removed and the tightly bound soil sample was subjected for the analysis of rhizosphere-associated Actinomycetes.

2.2. Isolation of Root-Associated Actinomycetes

Isolation of actinomycetes was performed by standard method using 1.0 g air-dried rhizosphere-associated soil sample collected from the tomato plant [13]. A serially diluted sample was plated over the actinomycetes isolation media (AIM). AIM was amended with Nalidixic acid (50 µg/mL), Nystatin (25 µg/mL), and Fluconazole (50 µg/mL) to inhibit the growth of fungi and bacteria. All plates were incubated for 7 to 21 days at 28 °C. Morphologically distinct actinomycete isolate was purified further using the same medium and maintained in 20% glycerol and stored at −80 °C for long-term use [14].

2.3. Phytopathogens

The phytopathogens, namely, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Bipolaris oryzae*, and *Fusarium solani*, were used. Inoculum of this spore suspension was prepared individually as suggested previously [15]. These fungal cultures were then stored on potato dextrose agar (PDA) slants.

2.4. Antimicrobial Compounds Extraction and Screening

The morphologically different ten actinomycetes isolates (Al-Dhabi 21, 22, 23, 24, 25, 26, 27, 28, 29, and 30) were cultivated into actinomycetes isolation broth and incubated for seven days at 28 °C and 150 rpm. After seven days, the culture was centrifuged at 10,000 rpm for 10 min and the supernatant used directly as the antimicrobial agents [16]. The extract was analyzed against plant pathogens (*Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus flavus*, *Bipolaris oryzae*, and *Fusarium solani*) using disc diffusion method as suggested by Kirby–Bauer with little modification. The antagonistic properties of the crude extract were compared with nystatin as the positive control. Antifungal property was evaluated by measuring the inhibitory zone of culture supernatant on the plates surface and the results reported were the mean of three independent repeats [17].

2.5. Characterization of Potent Actinomycete Strain

The strain Al-Dhabi 30 was selected for characterization studies based on its antagonistic potential. Morphological features, such as substrate and aerial mycelia, sporulation, and soluble pigments were analyzed after 7 days on ISP media. Assimilation of various sugars (arabinose, esculine, starch, mannitol, rhamnose, raffinose, sucrose, and xylose), lipid hydrolysis, and melanin production was analyzed. It was identified using phenotypic characters and 16S rDNA sequencing. Genomic DNA of the isolate Al-Dhabi 30 was extracted as suggested by Wilson [18] and sequenced using forward (27) and reverse (1525) primers, as described by Lane [19].

2.6. Screening and Quantification of Plant Growth-Promoting Activity of Actinomycetes

The phosphate solubilising property of the selected actinomycetes was analyzed using PKV agar media. After 5 days of incubation, a clear zone around the actinomycetes colonies indicated phosphate solubilising activity [20]. The positive strains were further cultured in National Botanical Research Institute Phosphate (NBRIP) broth medium and incubated for 8 days at 28 °C at 175 rpm using an orbital shaker incubator. The culture was centrifuged and the absorbance was checked using a UV-visible spectrophotometer at 880 nm [21]. Potassium dihydrogen phosphate (KH₂PO₄) was used as the standard and the results are expressed as mg phosphate/dL. The siderophore production by the selected Actinomycetes isolates was performed as described by Schwyn and Neilands [22], with minor modifications. Chrome azurol S agar medium was prepared and the culture was inoculated and incubated for 6 days at 28 °C. Siderophore production was confirmed by the development of halo zone of yellow–orange colour. Further, siderophore-producing strains were separately cultured in Gaus No. 1 medium with little modification [23]. The culture was incubated for 10–12 days at 28 °C in an orbital shaker at 175 rpm. The Csaky method [24] was used for the determination of siderophores. Indole acetic acid-producing ability of Actinomycetes was determined. Actinomycetes were grown

on ISP2 medium and incubated for 8 days at 28 °C. Then, a cork borer was used to cut the agar at 6 mm diameter. It was inoculated into ISP-2 liquid medium containing 0.2% L-tryptophan. The culture was incubated for 15 days at 28 °C using an orbital shaker at 175 rpm. Then, it was centrifuged at 10,000 rpm for 10 min and the clear supernatant was used for analysis. A 1 mL culture supernatant was mixed with 2 mL of Salkowski's reagent and kept in the dark for 30 min at 30 ± 2 °C. The developed red or pink colour was measured at 530 nm using a UV-visible spectrophotometer. The amount of IAA ($\mu\text{g/mL}$) from the culture supernatant was calculated using indole-3-acetic acid as the standard [25].

2.7. Hydrolytic Enzyme Screening and Assay from Actinomycetes

The morphologically different Actinomycetes species (Al-Dhabi 21, 22, 23, 24, 25, 26, 27, 28, 29, and 30) were analyzed for their ability to produce various hydrolytic enzymes. The isolates were grown on substrate agar plates (1%, *w/v*, casein, cellulose, pectin, colloidal chitin, and starch) for the determination of protease, cellulase, pectinase, chitinase, and amylase activity. The plates were incubated for 7 days and detected enzyme activity. Protease-positive Actinomycetes isolate shows halo zone around the isolated strain, whereas cellulase activity was determined after flooding with 0.1% Congo red (20 min incubation), and developing with 1 M NaCl (10 min). Gram's Iodine solution was flooded with starch agar plates for the determination of amylase activity. Pectinase-producing Actinomycetes were detected by adding iodine solution with pectin plates. Chitinase-producing ability of the strains was analyzed using colloidal chitin agar medium [26]. The selected ten actinomycetes isolates were inoculated in minimal medium and incubated for 7 days at 28 °C. To induce concomitant enzyme production, the substrates, such as casein, starch, pectin, chitin, and cellulose, were supplemented with the medium at 0.5% (*w/v*) level.

Pectinase activity of the sample was determined using pectin as a substrate. About 9.8 mL pectin (0.5%) was mixed with 0.2 mL culture supernatant. It was incubated for 10 min and 1 mL sodium carbonate (1 M), 5 mL iodine (0.1 N) were added and kept for 5 min. To this, 2 mL sulphuric acid (2 N) was added and kept in the dark for 15 min. The developed dark colour was further titrated against sodium thio sulphate (0.1 M) using soluble starch (1%) as an indicator. Protease activity of culture supernatant was assayed using casein (1%) as a substrate. The substrate was prepared in Tris buffer (pH 8.0, 50 mM). Then, 1.0 mL casein was mixed with 0.1 mL culture supernatant and incubated for 30 min. Then, 10% (*w/v*) trichloro acetic acid (5 mL) was added and incubated for 30 min. It was centrifuged at 5000 rpm for 5 min and the supernatant was measured at 275 nm against reagent blank using a UV-visible spectrophotometer. L-tyrosine was used as the standard for the determination of protease activity. Cellulase activity of the sample was assayed using 1% carboxy methyl cellulose (CMC) as a substrate. This substrate was prepared in 50 mM sodium phosphate buffer (pH 7.0); 1.0 mL substrate was mixed with 0.1 mL culture supernatant and incubated for 30 min; 1.0 mL dinitro salicylic acid (DNS) was added and kept on boiling water bath for 10 min. Brick red color was developed and diluted with 10 mL double-distilled water. The absorbance was read at 540 nm against reagent blank. Amylase was assayed from the culture supernatant using soluble starch as a substrate. Next, 1% soluble starch was prepared in sodium phosphate buffer (pH 7.0, 50 mM). To the substrate (1 mL), a 0.1 mL sample was added and incubated for 30 min. Further enzyme assay was continued following a similar procedure to the cellulase assay. Chitinase activity of the culture supernatant was determined using colloidal chitin as a substrate. This substrate was prepared at the 1% level using 50 mM acetate buffer (pH 5.5). A 1.0 mL substrate was mixed with a 0.1 mL sample and incubated for 30 min. After that, 1.0 mL DNS was added and further assay was continued following a similar procedure to the cellulase assay.

2.8. Vegetable Waste Composting and Physio-Chemical and Biological Properties

In composting, the nature of the raw material directly influences the quality of the mature compost. The vegetable waste, such as cabbage, cucumber, cauliflower leaves, and brinjal waste were collected, dried, mechanical blended, and water was sprayed for microbial composting. Then,

5.0 kg of vegetable waste was used for the composting process. The microbial consortium, including rhizosphere-associated *Streptomyces* sp. Al-Dhabi 30, *Candida utilis* ATCC 9950, and *Lactobacillus plantarum* ATCC 33222, were added with vegetable waste, whereas *Streptomyces* sp. Al-Dhabi 30 was not inoculated in the control. However, the control vegetable waste was amended with *C. utilis* ATCC 9950 and *L. plantarum* ATCC 33222. The selected microorganisms have a known producer of various enzymes and have the ability to degrade vegetable waste during composting, whereas *Streptomyces* sp. Al-Dhabi 30 has the potential to synthesize plant growth promoting substances, including antibiotics. Microbial composting vegetable waste was performed for 42 days. Finally, the mature compost was subjected to physical, chemical properties, antifungal activities, followed by plant growth promoting activities [4]. Moisture content of the compost was tested by standard method. Compost was taken in a container and dried in an oven at 110 °C until constant weight was achieved and percentage moisture level was calculated. The nutrient factors, such as organic carbon (%), nitrogen (%), carbon and nitrogen ratio, pH and electrical conductivity (EC), were determined. To the mature compost, double-distilled water was added at 1:10 (*w/v*) ratio and kept on rotary shaker at 200 rpm for 30 min. This sample was used for nutrient and antagonistic activity analysis. Nitrogen content was estimated by the Kjeldahl method, as suggested by Bremner [27]. Organic carbon content in the compost was tested by the method of Walkey and Black [28]. Antagonistic activity of the mature compost was determined using the well diffusion method [15].

2.9. Analysis of Plant Growth Promoting Activity of Compost

The experimental compost amended with *Streptomyces* sp. Al-Dhabi 30 and control compost was used for analysis. The selected tomato plant seed ($n = 20$) was sterilized and prepared according to the method of Indananda et al. 2010 [29]. The seeds were supplied with required water for germination and allowed for 4–5 days under dark condition. After 15 days of seed germination, mature compost was supplied into the experimental pot, whereas control compost was added with the control pot. Treatment was continued for three weeks and growth performance (shoot length, root length, and weight) were analyzed. Results were analyzed using one-way analysis of variance (ANOVA) with posthoc Tukey HSD test.

3. Results

3.1. Actinomycetes and Their Antagonistic Properties Against the Selected Phytopathogens

The antagonistic property of the Actinomycetes was tested against five plant pathogens. In total, 37 Actinomycetes were successfully recovered from the rhizosphere-associated soil. Among the 37 Actinomycetes isolates, only ten Actinomycetes showed activity against at least one phytopathogen. About 65% isolates showed potent antifungal activity against *F. xysporum*, *F. solani*, and *A. niger*, five strains showed activity against *F. oxysporum*, *A. niger*, *A. flavus*, and *Bipolaris oryzae*. In the present investigation, one Actinomycete (*Streptomyces* sp. Al-Dhabi 30) showed potent activity against all tested plant pathogens. Antifungal activity and the corresponding zone of inhibition (mm) were presented in Table 1. The molecular level characterization of the strain by 16S rRNA gene sequence amplification and sequencing confirmed that the strain belonged to the *Streptomyces* species and the obtained sequence was publicly available in PubMed, with the accession number 16S rDNA sequence deposited in GenBank under the accession number MN709219.

Table 1. Antagonistic activity of Actinomycetes (Zone of inhibition in mm) isolated from root-associated soil of tomato plant against phytopathogens.

| Actinomycetes | Antagonistic Activity (mm) | | | | |
|-------------------------------------|----------------------------|-----------------|------------------|------------------|------------------|
| | <i>F. oxysporum</i> | <i>A. niger</i> | <i>A. flavus</i> | <i>B. oryzae</i> | <i>F. solani</i> |
| <i>Streptomyces</i> sp. Al-Dhabi 21 | 22 | 11 | 0 | 0 | 17 |
| <i>Streptomyces</i> sp. Al-Dhabi 22 | 0 | 0 | 18 | 17 | 0 |
| <i>Streptomyces</i> sp. Al-Dhabi 23 | 19 | 16 | 12 | 0 | 0 |
| Actinomycete Al-Dhabi 24 | 0 | 16 | 22 | 19 | 0 |
| Actinomycete Al-Dhabi 25 | 0 | 0 | 20 | 20 | 17 |
| <i>Streptomyces</i> sp. Al-Dhabi 26 | 19 | 21 | 17 | 0 | 0 |
| <i>Streptomyces</i> sp. Al-Dhabi 27 | 12 | 0 | 0 | 0 | 18 |
| <i>Streptomyces</i> sp. Al-Dhabi 28 | 0 | 0 | 17 | 18 | 0 |
| <i>Streptomyces</i> sp. Al-Dhabi 29 | 18 | 19 | 21 | 0 | 0 |
| <i>Streptomyces</i> sp. Al-Dhabi 30 | 22 | 17 | 16 | 21 | 20 |

3.2. Production of Plant Growth Promoting Factors by Actinomycetes

Antagonistic Actinomycetes (Al-Dhabi 21, 22, 23, 24, 25, 26, 27, 28, 29, and 30) were subjected for the synthesis of IAA in the minimal medium. Among these 10 Actinomycetes isolates, 8 actinomycetes were able to produce IAA. IAA production ranged between 5 and 43 $\mu\text{g/mL}$ (Table 2). The strain *Streptomyces* sp. Al-Dhabi 30 showed the ability to produce a maximum amount of IAA (43 $\mu\text{g/mL}$) among the tested actinomycetes species. The selected ten actinomycetes were subjected to screen the ability to solubilise inorganic phosphate from the medium. All selected Actinomycetes showed the ability to solubilise phosphate. Phosphate degrading ability was maximum in *Streptomyces* sp. Al-Dhabi 30 (43.1 mg/dL) (Table 2). Siderophore production was detected from 80% of the selected isolates. These isolates formed a clear orange zone around the Actinomycetes colony. Eight Actinomycetes produced siderophore and the concentration ranged between 1.6 and 42.1 $\mu\text{g/mL}$. Among the *Streptomyces* species, *Streptomyces* sp. Al-Dhabi 30 produced the maximum amount (42.1 $\mu\text{g/mL}$) of siderophore (Table 2).

Table 2. Plant growth promoting activity of Actinomycetes isolated from the root-associated soil from tomato plant.

| Actinomycetes | Plant Growth Promoting Activity | | |
|-------------------------------------|---------------------------------|--------------------------------|----------------------------------|
| | IAA ($\mu\text{g/mL}$) | Phosphate Solubilizing (mg/dL) | Siderophore ($\mu\text{g/mL}$) |
| <i>Streptomyces</i> sp. Al-Dhabi 21 | 12 | 10 | 1.6 |
| <i>Streptomyces</i> sp. Al-Dhabi 22 | 0 | 21 | 0 |
| <i>Streptomyces</i> sp. Al-Dhabi 23 | 9 | 9 | 18.2 |
| Actinomycete Al-Dhabi 24 | 32 | 14.5 | 40.2 |
| Actinomycete Al-Dhabi 25 | 3 | 39 | 2.42 |
| <i>Streptomyces</i> sp. Al-Dhabi 26 | 19 | 27 | 2.3 |
| <i>Streptomyces</i> sp. Al-Dhabi 27 | 21 | 12.1 | 5.67 |
| <i>Streptomyces</i> sp. Al-Dhabi 28 | 5 | 4.2 | 0 |
| <i>Streptomyces</i> sp. Al-Dhabi 29 | 0 | 9.2 | 1.9 |
| <i>Streptomyces</i> sp. Al-Dhabi 30 | 43 | 43.1 | 42.1 |

3.3. Enzyme Production by Actinomycetes

The selected all ten actinomycetes showed protease production. Of them all, strain Al-Dhabi 30 showed 19 mm zone in casein agar plates. Also, enzyme production was found to be maximum (121 U/mL) in *Streptomyces* sp. Al-Dhabi 30. Cellulase production ranged between 18 U/mL and 67 U/mL. Only about 60% of the selected actinomycetes strains were able to hydrolyze cellulose material. Pectinase production was maximum in *Streptomyces* sp. Al-Dhabi 22 (2.1 U/mL) compared to other isolates. Also, three Actinomycetes did not show any pectinase activity in the culture supernatant. All selected Actinomycetes species showed amylase activity and ranged between 0.3 and 19.2 U/mL (Table 3).

Table 3. Production of extracellular enzymes by Actinomycetes species isolated from the root-associated soil from tomato plant.

| Actinomycetes | Enzyme Activity (U/mL) | | | | |
|-------------------------------------|------------------------|-----------|-----------|---------|-----------|
| | Protease | Cellulase | Pectinase | Amylase | Chitinase |
| <i>Streptomyces</i> sp. Al-Dhabi 21 | 42 | 0 | 1.8 | 0.3 | 29.4 |
| <i>Streptomyces</i> sp. Al-Dhabi 22 | 110 | 0 | 2.1 | 19.2 | 0 |
| <i>Streptomyces</i> sp. Al-Dhabi 23 | 48 | 52 | 0 | 13 | 40.2 |
| Actinomycete Al-Dhabi 24 | 19 | 0 | 0.43 | 12 | 0 |
| Actinomycete Al-Dhabi 25 | 56 | 42 | 2 | 10.5 | 29.2 |
| <i>Streptomyces</i> sp. Al-Dhabi 26 | 43 | 18 | 1.1 | 9.5 | 35.9 |
| <i>Streptomyces</i> sp. Al-Dhabi 27 | 10 | 0 | 0 | 5 | 0 |
| <i>Streptomyces</i> sp. Al-Dhabi 28 | 2.1 | 38 | 0.2 | 10.2 | 25.5 |
| <i>Streptomyces</i> sp. Al-Dhabi 29 | 92.5 | 22 | 0 | 4.2 | 19.4 |
| <i>Streptomyces</i> sp. Al-Dhabi 30 | 121 | 67 | 1.3 | 13.4 | 43.7 |

3.4. Characterization of Actinomycetes

The suitable plant growth promoting strain Al-Dhabi 30 was characterized by morphological, biochemical, and 16S rDNA sequencing and identified as *Streptomyces* sp. Al-Dhabi 30. It produced aerial mycelium and was ash colour. It was a non-acid fast organism, Gram-positive. Sporulation was observed on solid media; this organism produced filamentous and highly branched mycelium. It was catalase-positive and reduced nitrate effectively. It hydrolyzed arabinose, mannitol, rhamnose, raffinose, sucrose, xylose, esquinine, and starch and failed to hydrolyze lipid substrate. The strain Al-Dhabi 30 could hydrolyze maltose, lactose, glucose, mannitol, and salicin.

3.5. Composting of Vegetable Waste

The vegetable waste decomposition started after inoculation of vegetable waste with microbial consortium. Biodegradation of waste was observed after the 7th day of incubation due to fungal and Actinomycetes growth. In the experimental compost, degradation was rapid and a sharp decrease in decolouration, changes in physical texture and volume also decreased considerably. These rapid changes inferred the use of *Streptomyces* sp. in waste composting. The vegetable waste was completely decomposed after 42 days of incubation and about $\frac{3}{4}$ volume decreased during this period. Total nitrogen content (%) was 2.7% in the control, whereas 3.8% organic carbon was detected in the experiment. In the compost vegetable waste, the C:N ratio was 10.07%, whereas it was 17.51% in the control after 42 days. The compost was extracted with water at a 1:10 ratio (compost and water) and it showed antagonistic activity against the selected fungal pathogens (*F. xyloporum*, *A. niger*, *A. flavus*, *B. oryzae*, and *F. solani*). These phenomena clearly suggested the antibiotic potential of *Streptomyces* sp. Al-Dhabi 30. The physiochemical properties and antifungal potential of compost is described in Table 4.

Table 4. Physical, chemical, and biological properties of mature compost.

| Parameters | Control | Experiment |
|-------------------------|----------------|------------|
| Physical properties | | |
| Colour | Brownish black | Black |
| Moisture | 17.42 | 34.49 |
| Chemical properties | | |
| pH | 7.4 | 7.48 |
| Electrical conductivity | | |
| (ds/m) | 4.1 | 4.5 |
| Nitrogen (as N%) | 2.7 | 3.8 |
| Organic carbon (%) | 47.28 | 38.29 |
| C:N ratio | 17.51 | 10.07 |
| Antifungal activity | | |
| <i>F. oxysporum</i> | – | +++ |
| <i>A. niger</i> | – | +++ |
| <i>A. flavus</i> | + | +++ |
| <i>B. oryzae</i> | – | +++ |
| <i>F. solani</i> | – | +++ |

– indicates negative, +indicates low activity and +++indicates high activity.

3.6. Experimental Trial of Plant Growth Analysis Treated with Compost

The efficacy of *Streptomyces* sp. Al-Dhabi 30 promoting plant growth was determined at 42 days after planting. Tomato plant treated with experimental compost showed better effect than control pots. The vegetable waste together composed by *Streptomyces* sp. Al-Dhabi 30 enhanced shoot and root length compared to control seedlings after 21 days of sprouting. The root length of the control tomato plant was 1.92 ± 0.26 cm and the length was increased in the *Streptomyces* sp. Al-Dhabi 30 inoculated compost (2.87 ± 0.32 cm) ($p < 0.0001$). Likewise, the shoot length was 13.4 ± 0.42 cm in the control tomato plant, whereas 17.1 ± 0.22 cm in the tomato plant treated with *Streptomyces* sp. Al-Dhabi 30 compost after 42 days ($p < 0.001$). Likewise, the total weight of the plant also increased about 15% in the *Streptomyces* sp. Al-Dhabi 30 compost vegetable waste. Collectively, *Streptomyces* sp. Al-Dhabi 30-assisted compost mediated plant growth promotion and the results were statistically significant ($p < 0.01$). Growth promoting activity of compost on the growth of tomato plant is described in Table 5.

Table 5. Effect of *Streptomyces* sp. Al-Dhabi 30 on growth promoting activity in tomato plant.

| Experimental Procedure | Root Length (cm) | Shoot Length (cm) | Total Weight of Plant (gm) |
|------------------------|----------------------|-----------------------|----------------------------|
| 21 days treatment | | | |
| Control | 1.92 ± 0.26 | 6.9 ± 0.33 | 1.92 ± 0.53 |
| Treatment | 2.87 ± 0.32 **** | 9.42 ± 0.28 **** | 3.1 ± 0.41 *** |
| 28 days treatment | | | |
| Control | 2.4 ± 0.32 | 7.42 ± 0.29 | 2.53 ± 0.38 |
| Treatment | 3.82 ± 0.28 **** | 11.32 ± 0.18 **** | 4.6 ± 0.26 * |
| 35 days treatment | | | |
| Control | 3.6 ± 0.39 | 11.2 ± 0.11 | 3.1 ± 0.23 |
| Treatment | 4.9 ± 0.41 *** | 15.2 ± 0.13 ** | 5.3 ± 0.47 ** |
| 42 days treatment | | | |
| Control | 5.1 ± 0.33 | 13.4 ± 0.42 | 4.76 ± 0.31 |
| Treatment | 6.8 ± 0.27 **** | 17.1 ± 0.22 ** | 6.21 ± 0.29 * |

* $p < 0.01$, ** $p < 0.001$, *** $p < 0.0001$ and **** $p < 0.00001$.

4. Discussion

A total of 37 Actinomycetes were isolated from the root-associated soil of tomato plant. These relatively large numbers of Actinomycetes suggested that rhizosphere is an important source of Actinomycetes. These plant-associated actinomycetes have the potential activity against various phytopathogens. Of these, five Actinomycetes strains have potent activity against *F. oxysporum*, *A. niger*, *A. flavus*, and *B. oryzae*. Among all tested isolates, *Streptomyces* sp. Al-Dhabi 30 showed potent activity against *F. oxysporum*, *A. niger*, *A. flavus*, *B. oryzae*, and *F. solani*. *Streptomyces* species produce various metabolites and are highly active against *R. solani* [12]. In a study, Verma et al. 2009, [30] isolated endophytic Actinomycetes from *A. indica*, A. Juss, and the isolated *Streptomyces* sp. showed antimicrobial activity. Recent studies indicated that the *Streptomyces* strains revealed protective bioactivity against various microbial pathogens also [31,32]. The present finding and earlier studies [33,34] showed that *Streptomyces* is the predominant organism in endophytic form or associated with root.

In recent years' application of chemical fertilizers, herbicides and pesticides increased rapidly and are a serious concern in crop production and environmental management. Hence, industrialists and researchers seek an alternate approach using natural resources for the sustainable production of agricultural products in horticulture and agriculture [33]. It was found that plant-associated actinomycetes have various responses, including plant growth promoting activity. In a study, Verma et al. [34] reported on the growth promoting property of actinomycetes species. The selected ten antagonistic Actinomycetes were synthesized using IAA, effectively solubilised inorganic phosphates and producing siderophore. These growth promoters enhanced the growth performance of plants. The selected actinomycetes showed potential antifungal activity and plant growth promoting potential. Among 10 actinomycetes, eight organisms effectively produced plant growth promoting biomolecules. Of these, seven isolates were identified as *Streptomyces* sp.; especially, root associated with *Streptomyces* sp. Al-Dhabi 30 has the potential to produce plant growth promoting molecules, such as IAA, siderophore, and phosphate solubilising property. These findings are in good agreement with previous studies by Hamdali et al. [35], who detected 39, 58.9, 83.3 mg/dL phosphate solubilisation by *Micromonospora aurantiaca*, *Streptomyces griseus*, and *Streptomyces cavourensis*, respectively. In a study, Chen et al. [36] reported phosphate solubilising property of bacteria from the subtropical soil and analyzed their tricalcium phosphate solubilising abilities. Also, they reported the influence of phosphate solubilising organisms in acidification of the medium, and stated that the soluble phosphate concentration and pH of the medium were inversely proportional. This leads to the production of gluconic acid, a very low molecular weight organic acid. Hence, the plant-associated Actinomycetes play a very important role in the growth of plants.

The screened Actinomycetes, mainly *Streptomyces* species, produced a considerable amount of siderophores. These siderophores have the potential to inhibit the growth of phytopathogens. The growth of phytopathogen inhibiting property by *Streptomyces* species due to the production of *siderophores* has been described previously [31]. Tan et al. [37] reported that synthesis of siderophore for the growth of the plant and phytopathogen antagonism *Streptomyces* sp. Al-Dhabi 30 produced a maximum level of siderophore, and these results are in good agreement with previous reports. Nimnoi et al. [38] reported that *Pseudonocardia halophobica* showed a high amount of siderophore producing ability (39.30 µg/mL). However, the amount of siderophores production in the present study was found to be higher than previous reports, suggesting the growth promoting potential of the *Streptomyces* sp. Al-Dhabi 30. In a study, Khamna et al. [39] reported hydroxamate type and catechol types siderophore from *Streptomyces* CMU-SK 126 isolated from *Curcuma mangga* rhizospheric soil.

The phosphate solubilisation property was found to be high in the *Streptomyces* sp. Al-Dhabi 30 compared to other actinomycetes isolates. The production of IAA varied between 3 and 43 µg/mL and this range is in accordance with observations made previously by various research groups. Nimnoi et al. [40] reported IAA-producing bacteria associated with rhizosphere, root tissue and rhizoplane of *Leucaena leucocephala*, *Ficu sreligiosa* L., and *Piper sarmentosum* Roxb. Khamna et al. [39]

isolated Actinobacteria from rhizosphere soil and showed the ability to produce siderophore, IAA, and various antifungal compounds. Recently, Verma et al. [34] isolated plant growth promoting *Streptomyces* from *Azadirachtaindica* A. Juss and reported the ability to inhibit the growth of phytopathogens. Actinomycetes, mainly the genus *Streptomyces*, has a lot of potential to produce various phytohormones, secondary metabolites against phytopathogens and this lead shows a lot of advantages to colonize to rhizosphere [11].

The selected substrates were added with the minimal medium for the production of various enzymes because most of the enzymes are inducible. About 70% of the actinomycetes were synthesized chitinase enzyme. This enzyme is very important for the hydrolysis and recycling of nitrogen and carbon trapped in an insoluble form of chitin and it is also widely used for making biopesticides and mosquito control [41]. Protease production ranged between 2.1 and 121 U/mL, and all the selected actinomycetes were synthesized protease. Of these, only six isolates produced cellulases and *Streptomyces* sp. Al-Dhabi 30 produced the maximum amount (67 U/mL). Pectinase production ranged between 0.43 U/mL and 2.1 U/mL. *Streptomyces* sp. Al-Dhabi 22 produced the maximum amount of amylase compared to strain Al-Dhabi 30. However, protease, cellulase, and chitinase activity was maximum in *Streptomyces* sp. Al-Dhabi 30. Considering this fact, strain Al-Dhabi 30 has the potential to degrade organic biomass from the environment. In a study, Gopinath et al. [42] analyzed the biodegrading ability of organic waste, tested by assaying various hydrolytic enzymes. These hydrolytic enzymes are useful in the composting process and help to breakdown complex molecules to simpler ones.

The vegetable waste compost was made after inoculation with antifungal compound-producing *Streptomyces* sp. Al-Dhabi 30. Biodegradation of waste was visible, the colour changed to brown and volume also decreased from two weeks of composting. The carbon and nitrogen ratio decreased in the experimental compost from 17.51 to 10.07%. It shows the potential composting properties of *Streptomyces* sp. Al-Dhabi 30 on vegetable waste. Kitchen waste, vegetable waste have been used previously by various research groups for composting. In a study, Karnchanawong and Nissakla [43] used organic waste for the preparation of compost using microbial consortia and improved composting was achieved using mature compost as a starting material, because these mature types of compost consist of various microbial consortia, including Actinomycetes. In a study, Actinomycetes consortium alone was used to compost domestic agro-residues and the mature compost has been used to treat pulp-paper mill effluent [44]. These previous studies reported composting of agro-waste without bacteria and fungi. Previously, Tanaka et al. [45] reported that *Micromonospora* sp. and *Streptomyces* sp. completely degraded yeast debris. These previous literatures and present finding show the efficiency of *Streptomyces* sp. Al-Dhabi 30 on composting. Kitchen waste contains mainly agro-industrial residues. These residues are composed of large proportions of lignin (10–35%), hemicelluloses (15–35%) and cellulose (35–50%) [46]. In general, naturally associated microbial consortia accelerated composting process. Considering this fact, tomato root-associated soil sample has been used for the isolation of Actinomycetes. Generally, bacteria, fungi, and Actinomycetes are involved in the composting process, because all these three exist in the natural environment. In this work, an attempt was made to use enzyme-producing thermophilic actinomycete along with microbial consortium to compost vegetable waste to obtain antifungal and growth-promoting compost. During the composting process, high heat is generated during the middle phase and the population of mesophilic organisms was found to be less at this stage. However, inoculation of thermophilic Actinomycete could improve the degradation potential of the compost. Actinomycete strain Al-Dhabi 30 was selected for composting based on its rapid growth compared to other species. Also, this organism was able to synthesize various hydrolytic enzymes influencing composting. The group of enzymes, including cellulases, amylases, proteases, chitinases, and pectinases, involved breakdown of all lignocellulosic materials from the vegetable waste.

In this observation, *Streptomyces* sp. Al-Dhabi 30 composed nutrients showed the ability to produce significant plant growth promoting activity under experimental conditions. This actinomycete

species showed the maximum root and shoot length of experimental plant compared to the control plant after 21, 28, 35, and 42 d of sprouting. These findings indicated the plant growth promoting activity of *Streptomyces* sp. Al-Dhabi 30 compost vegetable waste. Root and shoot length was found to be less in the control experiment. These kinds of plant growth promoting Actinomycetes could improve the bioavailability of nutrients and be alternates to fertilizers and chemical pesticides and has been reported previously [47]. In this experiment, the actinomycetes colonized tomato plant and promoted plant growth through biosynthesis of various growth promoters, such as IAA and siderophores, and also have potential activity against various plant pathogens. These synthesized compounds enhanced the growth of shoot and root. In the experimental tomato plant, root length increased 33%, whereas shoot length was enhanced 27% after six weeks. Many previous reports stated that Actinobacteria can enhance shoot and root length in various plants, and such an increase may show advantages to the plants to promote growth and biocontrol efficacy. In a study, Lamsal et al. [48] reported influential growth in tomato using rhizobacterial isolates, where root and shoot length was 26% and 18% higher, respectively, than the control. This kind of phytopathogen control property can manifest as space and nutrient competition, predation, induced systemic resistance, hypovirulence, and parasitism [49]. All these mechanisms indirectly enhance the growth of plants. Biocontrol property is very important and it has been reported as the first and foremost important to colonize the root tip of plants. Taken together, the findings of these studies show that *Streptomyces* sp. Al-Dhabi 30 is highly suited as a potent plant growth promoting and biocontrol inoculum for sustainable agriculture.

5. Conclusions

From this study, we conclude that various *Streptomyces* species, including *Streptomyces* sp. Al-Dhabi 30 associated with root of tomato plant have tremendous potential as an antagonistic agent against *F. oxysporum* (22 mm) and *B. oryzae* (21 mm). The *Streptomyces* sp. Al-Dhabi 30 effectively produced indoleacetic acid (43 µg/mL) and siderophores (42.1 µg/mL). The total weight of tomato increased over 15% compared to the control compost and was statistically significant. The selected microbial consortium has the potential to degrade vegetable waste and enrich the compost with phytohormones and antagonistic secondary metabolites. This compost is useful as potential plant growth promoting activity.

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