Title	Antagonistic Effect of Endophytic *Streptomyces* sp. Isolated from Root of *Centella umbellata* Schubert et Van Wyk on Pathogenic Fungi Causing Leaf Diseases in Cabbage
All Authors	Kay Thi Wai and Soe Myint Aye
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Antagonistic Effect of Endophytic *Streptomyces* sp. Isolated from Root of *Centella umbellata* Schubert et Van Wyk on Pathogenic Fungi Causing Leaf Diseases in Cabbage

Kay Thi Wai[1] and Soe Myint Aye[2]

Abstract

The endophytic bacterium was isolated from root of *Centella umbellata* Schubert et van Wyk and studied the antagonistic effect on pathogenic microorganisms that causing the leaf diseases of cabbage. This experiment was carried out at the Microbiology Laboratory of the Botany Department, University of Mandalay during the period of July-December 2015. The isolation of endophytic bacteria was carried out by using Starch Casein Agar (SCA) medium and International Streptomycetes Project-2 (ISP-2) medium. The identification of isolated bacteria was confirmed as *Streptomyces* species by the studies of morphological character and biochemical tests. Pathogenic fungi were isolated from the infected leaf of cabbage. The resulting strains were designated as KTW 01 (*Alternaria* sp.), KTW 02 (*Helminthosporium* sp.), KTW 03 (*Colletotrichum* sp.) and KTW 04 (*Aspergillus* sp.). The assay for antagonism was performed on Potato Dextrose Agar (PDA) medium by dual culture method. The pathogenic fungi *Alternaria* sp. and *Helminthosporium* sp. showed malformation of fungal hypha and deformation of spores. Although the isolated *Streptomyces* bacteria showed the positive effect on pathogenic fungi of leaf disease in cabbage, the *Streptomyces* showed the most antagonistic inhibition on *Helminthosporium* sp..

Key words: Streptomyces, antagonistic effect, leaf diseases in cabbage

Introduction

Endophytes are microorganisms that naturally occur in various parts of the plant parts like roots, stems and leaves. Endophytic microorganisms can be employed for their antibacterial, antifungal and antiviral activities. In controlling the plant diseases endophytes are relatively safe because it is non-toxic to its host plants and also non-toxic to the consumer if it can be used in the vegetables. Zucchi *et al*. (2008) stated that several efforts have been made to find less hazardous potions for controlling these plants pathogens among which the

[1] Kay Thi Wai, MRes.- Student, Department of Botany, University of Mandalay
[2] Dr Soe Myint Aye, Professor, Department of Botany, University of Mandalay

biological control using the microorganisms has been demonstrated to be a feasible alternative.

The application of biological control agent (BCAS) seems to be one of the promising approaches. Biocontrol involves the use of naturally occurring nonpathogenic microorganisms that are able to reduce the activity of plant pathogens and thereby supress diseases. Antagonistic microorganisms can complete with the pathogen for nutrients, inhibit pathogen multiplication by secreting antibiotics or toxins, or reduce pathogen population through hyper parasitisms (Svetlana *et al*. 2010).

Fungal phythopathogens pose serious problems worldwide and cause a number of plant diseases. Currently, there is an increasing Public concern regarding the continued use of agrichemicals to control the phytopathogenic Fungi. This awareness relies mainly in the noxious, effects of the pesticides on the environmental and human health (Costa 2013).

From the beginning of the 20^{th} century, the genus *Streptomyces* has become very important for the production of antibiotics in controlling human ailment as well as animal and plant diseases specially crop diseases. Almost all *Streptomyces* spp. have been proved to be antibiotic producers from economic and medical view points, extensive researcher have been carried out worldwide to screen antibiotic producer *Streptomyces* (Islam *et al*. 2014). Antibiotics produced by the filamentous bacteria, mainly *Streptomyces* spp., have been reported to be able to inhibit the development of a broad range of phytopathogenic fungi and bacteria (Berg *et al*. 2001). Also these compounds have often been related as one the most important tools to control the soil-borne diseases with low environmental impact and toxic effect for humans and animals, well-desired traits for new consumer's requirements (Cardoso *et al*. 2010).

White headed cabbage (*Brassica oleracea* L. var. *capitata* f. *alba* DC.) is a biennial herb in the Brassicaceae family which is commonly grown in temperate regions. Several insect pests and plant pathogens attack this plant worldwide (Rahimloo & Ghosta 2015). Kolte (2002) stated that one of the most common and important plant pathogens was caused by *Alternaria* species. It is reported that the yield loss caused by *Alternaria* blight disease on oilseed Brassicas is upto 60%. By studying on *Alternaria* leaf spots on *Brassica* species in Pernambuco, Michereff *et al*. (2012) reported that *A. brassicae* was found in all chinese cabbage fields, while *A. brassicicola* was found in all fields of cabbage, cauliflower and broccoli, an indication of host preferences.

Endophytic Actinomycetes was screened by Phuakjaiphaeo & Kunasakdakul in 2015 from *Centella asiatica* for their antifungal activities against *Alternaria brassicicola,* a plant pathogen causing *Alternaria* leaf spot of cabbage.

Dochhil et al. (2013) also reported that the endophytic Actinomycetes, *Streptomyces* was isolated from *Centella asiatica* (L.) Urban and can be used for producing indole acetic acid which enhanced seed germination and seedling growth of *Phaseolus vulgaris* L. However, no bodies have not used the *Centella umbellata* for isolation, characterization and studying the antagonistic effect on various pathogenic fungal microorganisms. Nowadays, *Centella umbellata* is one of the most widespread aquatic herbs and the plants invade most of the aquatic habitats like canals, ditches, ponds, and also in seaweed canals along the road-sides. The leaves are used as vegetables after preparing as (Myin-kwa) salads by some peoples. The most interested information to do a research is the presence of *Streptomyces* endophytes in roots of Genus *Centella* of family Apiaceae.

Therefore, the aims and objectives of the present research is to study the macroscopic and microscopic character of genus *Streptomyces* isolated from *Centella umbellata*, common name 'pennywort', to isolate and characterize the pathogenic fungus strains from cabbage and to investigate the antagonistic effect of isolated *Streptomyces* species on phyto-pathogenic fungi from cabbage.

Materials and Methods

The plant sample of aquatic plant 'penny wort' were collected from University of Mandalay in July 2015. The plant was studied, characterized and identified. Isolation of endophytes from root was done as soon as possible after the samples were brought to the laboratory of Botany Department University of Mandalay. The root samples were washed in running tap water to remove adhered epiphytes and soil debris. Effectiveness of surface sterilization was tested by the method of Schulz *et al*. (1993).

In the isolation of Streptomycetes, starch casein agar (SCA) medium were used as the basal culture media according to Williams *et al*. (1983). Morphological studies and colour determination of the selected isolate was studied in accordance with the International Streptomycetes Project (ISP) procedures according to Shirling & Gottlieb (1966). The staining procedures were carried out according to the methods described by Dubey and Maheshwari (2002).

The biochemical tests like gram staining, starch hydrolysis, Casein hydrolysis, sugar fermentation, nitrate reduction, motility, gelatin hydrolysis, Catalase and Oxidase were performed by following to Tittsler and Sandholzer (1936), Dickey & Kelman (1988), Atlas (1993) and Aneja (1996).

Isolation Method for Plant pathogenic fungi was followed to Phuakjaiphaeo & Kunasakdakul (2015). Potato Dextrose Agar (PDA) medium was used.

In vitro assay for antagonistic activity of endophytic Steptomycetes Isolate was performed on potato dextrose agar (PDA) by dual culture method as suggested by Yuan and Crawford (1995). The level of inhibition was defined as subtraction of radial mycelial growth γ_0 (in cm) of a control culture from the distance of the fungal growth in the direction of Streptomycetes (γ in cm) in the test cultures. The level of inhibition was calculated adapting following formula (Yuan & Crawford 1995).

$$\Delta\gamma = \gamma_0 - \gamma$$

where, $\Delta\gamma$ = The level of inhibition

γ_0 = Mycelial growth fungal pathogen (in cm) in control culture

γ = Mycelial growth fungal pathogen (in cm) in test inoculated

The antagonistic bioactivity of endopytic actinomycete was evaluated in term of ratings as described by Yuan and Crawford (1995).

Results

Morphological Character of Isolated *Streptomyces* sp. strain from root of *Centella umbellata* Schubert et van Wyk

The strain was red color, filamentous margin, mucoid after 24 hours incubation. It showed on International Streptomycetes Project -2 (ISP-2) medium. These strains also showed red color on Starch Casein Agar (SCA) medium. The strain of *Streptomyces* sp. grow erect aerial mycelium from substrate mycelium and finally aerial mycelium become chain spore formation after 3 days at 25°C incubation. Characteristics of spore chain type was straight to flexuous (Figure 1. F).

Biochemical Characteristics of *Streptomyces* sp.

Biochemical characteristics of *Streptomyces* sp. was analyzed (Table 1). It showed gram positive. It was found to be capable of hydrolyzing starch and casein. *Streptomyces* sp. was tested for their capability to ferment different types of carbohydrate such as sucrose, dextrose, glucose and manitol. The results showed that it was capable of fermenting sugar and producing gas. Nitrate reduction and motility test were found to be positive. Gelatin liquefaction test, catalase test and oxidase test showed the positive results for *Streptomyces* sp.

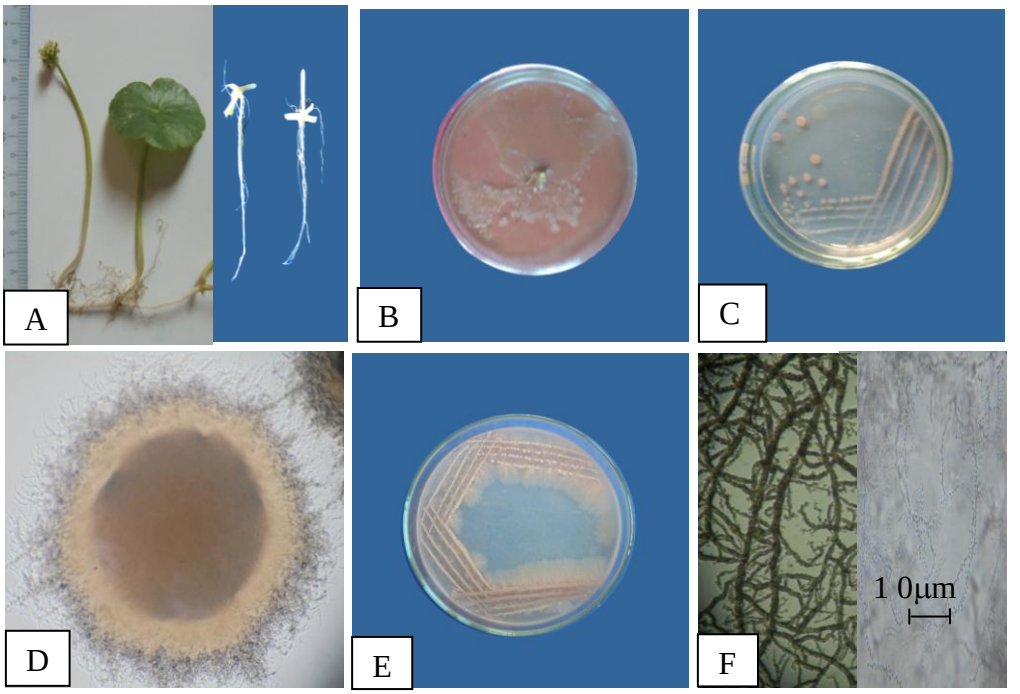

Figure 1.
A. The root sample of *Centella umbellata* Schubert et van Wyk
B. The bacteria strains growing in petri dish
C. *Streptomyces* sp. strain colony growing on ISP-2 medium
D. Single colony character of *Streptomyces* sp. on ISP-2 medium
E. *Streptomyces* sp. strain growing on SCA medium
F. Aerial mycelium and spore chain of *Streptomyces* sp. growing on SCA medium

Table 1 Biochemical Test for *Streptomyces* sp.

No.	Reaction	Response	Result
1	Gram staining	Purple colour	Positive
2	Starch hydrolysis	Clear zone is Formed around the growth zone	Positive
3	Casein hydrolysis	Clear zone is Formed around the growth zone	Positive
4	Fermentation test (Sucrose, Dextrose, Glucose, Manitol)	Yellow color change and gas production	Positive
5	Nitrate reduction	Colour change from clear to red	Positive
6	Motility test	Diffuse growth spreading from the line of inoculation	Positive
7	Gelatin hydrolysis	Liquifaction of gelatin	Positive
8	Catalase test	Production of free oxygen gas bubbles	Positive
9	Oxidase test	Purple color change	Positive

Observation of pathogenic fungi

Symptoms of leaf spot on cabbage may first develop on young plants in seedbeds where leaf spots, stunting, or damping off may occur. Dark brown to black leaf spots may appear on tissues of any age. The leaf spots enlarge in concentric circles and mature lesions have a bull's eye type appearance The symptoms of Black blotch leaves diseases were small irregular brown to black spots scattered randomly on the young leaves that can enlarge to form necrotic black patches are (Figure 2 A & B). Four strains of fungi were isolated from infected cabbage. These isolated strains were namely as KTW 01, KTW 02, KTW 03, KTW 04.

KTW 01 strain

Macroscopical characters of KTW 01 strain colonies was flat and olivaceous to dark brown on PDA medium after 3 - 7 days at 25°C and pH 6.5 - 7.0. Reverse colony was dark green with pale white color. In microscopical character, the hyphae are septate. Conidiophores are brown: bearing conidia. Conidia are borne on chains, ellipsoid, 8 - 13 μm width and 6 - 34 μm length (Figure 2 C, D &E). According to these macroscopical and microscopical characters, the fungus KTW 01 was *Alternaria* sp. (Table 2).

KTW 02 strain

The macroscopical characters of KTW 02 strain colony color was observed on potato dextrose agar at 25°C and pH 6.5 - 7.0 after 3 - 7 days. The colony was olive green with white age and become dark at mature. Reverse colony was pale green with greyish. In microscopical character, hyphae was septate, conidiophores was simple and arise as lateral branches. Conidia arise from conidiophore and yellow to brown containing 3-cells, cylindrical or ellipsoid, 6 – 13 μm width and 9 - 24 μm length. According to these macroscopical and microscopical characters, the fungus KTW 02 was *Helminthosporium* sp. (Table 2 and Figure 3. A, B &C).

KTW 03 Strain

Macroscopical character of KTW 03 strain was white velvet colony on PDA medium at 25°C and pH 6.5 - 7.0 after 3 - 7 days. Reverse colony was reddish brown color. In microscopical character, conidiophores are simple or branched. Conidia hyaline, one celled, cylindrical, 4 - 6 μm width and 8 – 19 μm length. According to these macroscopical and microscopical characters, the fungus KTW 03 was *Colletotrichum* sp. (Table 2 and Figure 3 D, E & F).

KTW 04 Strain

Macroscopical character was white colonies at firstly and turns to black with powdery and then mature black on potato dextrose agar medium at 25°C and pH 6.5 -7.0 after 3 - 7 days. Reverse colony was pale yellow color. Microscopical character of KTW 04 was showed that the hyphae was septate. Conidiophores were upright, simple and hyaline. Vesicle subglobose, sterigmata, biseriates-conidia arose from the tip of phialides and spherical and 5 μm in diameter (Figure 4.10 E). According to these macroscopical and microscopical characters, the fungus KTW 04 was *Aspergillus* sp. (Table 4.2 and Figure 4.10 B, C and D).

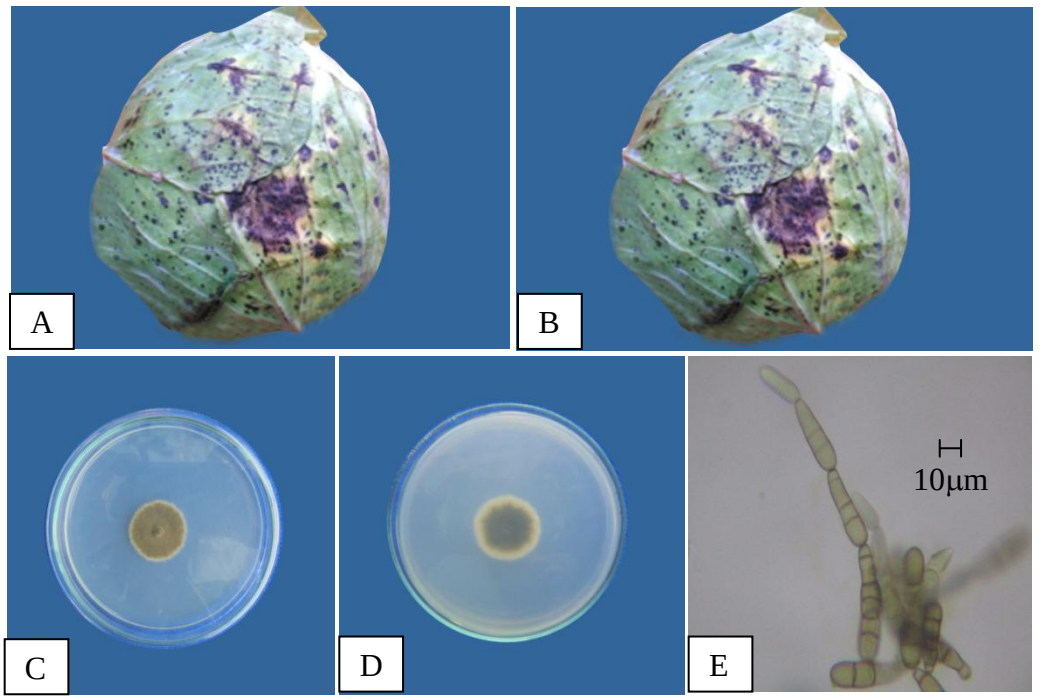

Figure 2. Disease Symptom and Characters of Isolated fungi from Cabbage

A. Disease symptoms of leaves spots (arrow)
B. Disease symptoms black blotch leaves (arrow)
B. Colony characters of *Alternaria* sp. (KTW-01) strain on PDA medium (4 days)
C. Reverse colony of *Alternaria* sp. (KTW-01) strain
D. Photomicrograph of *Alternaria* sp. (KTW-01) strain

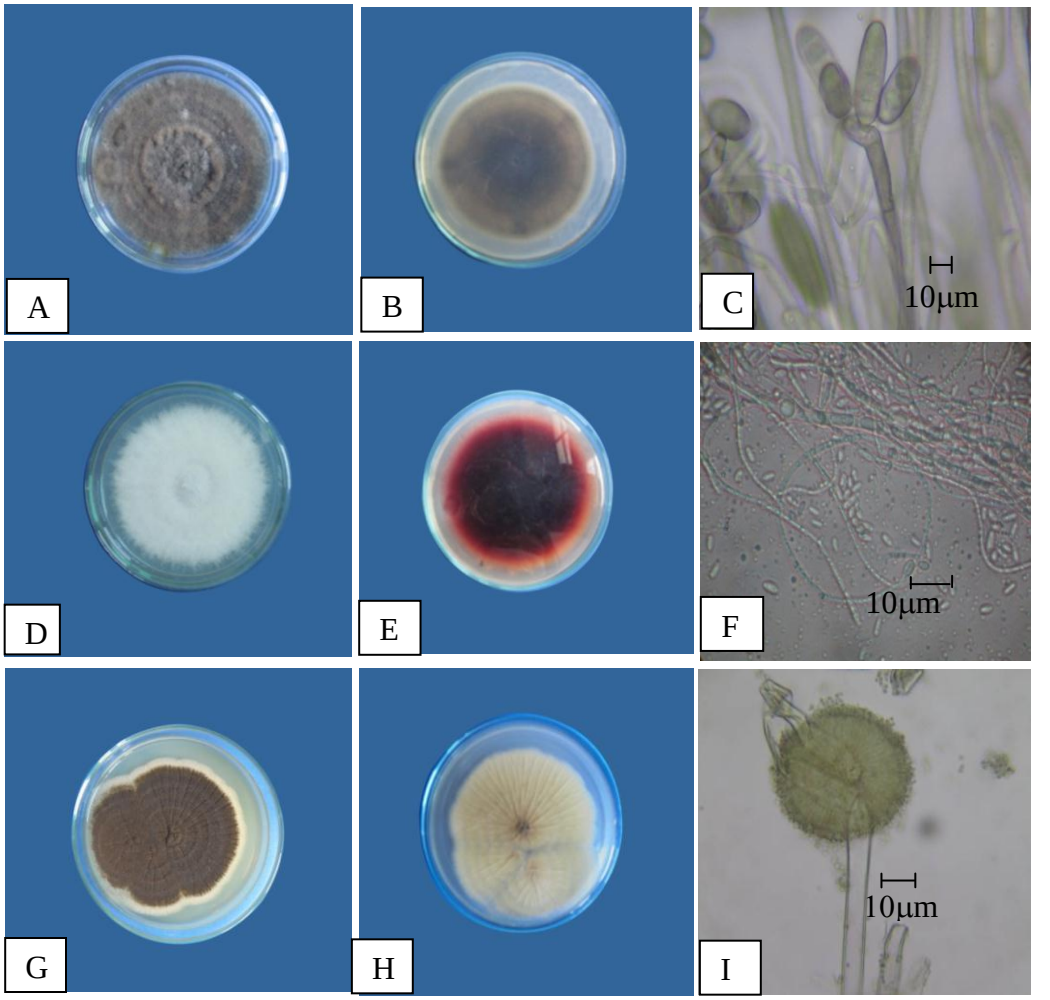

Figure 3. A. Colony characters on PDA medium (7 days), B. Reverse colony and C. Photomicrograph of *Helminthosporium* sp. (KTW-02) strain
D. Colony characters on PDA medium (7 days), E. Reverse colony and F. Photomicrograph of *Colletotrichum* sp. (KTW-03) strain
G. Colony characters on PDA medium (3 days), H. Reverse colony and I. Photomicrograph of *Aspergillus* sp. (KTW-04) strain

Table 2. Outstanding characters of fungal isolates from infected cabbage leaves

Isolated strain	Pathogenic fungi	Macroscopical characters	Microscopical characters	Diseases
KTW 01	*Alternaria* sp.	Flat, olivaceous to dark brow at mature	Hypae are septate. Conidiophores brown bearing conidia. Conidia are borne on chains, ellipsoid.	Leaves spots
KTW 02	*Helminthosporium* sp.	Olive green with white edge and become grayish at mature.	Hypae are septate, conidiophores was simple and arise as lateral branches. Conidia arose conidipohore: containing 3-cells, cylindrical or ellipsoid.	Leaves spots
KTW 03	*Colletotrichum* sp.	White velvet	Conidiophores are simple or branched. Conidia hyaline, one celled, cylindrical.	Black blotch leaves
KTW 04	*Aspergillus* sp.	White to black with powdery at mature.	Conidiophores were upright, simple and hyaline. Vesicle subgobose, strigmata biseriates-conidia arose from the tip of phialides and spherical.	Black blotch leaves

4.7. Antagonistic effect of *Streptomyces* sp. on four strains of pathogenic fungi

The strain of *Streptomyces* sp. showed inhibition of fungal pathogens with varying effectiveness (Figure 4). The assay for antagonism was performed on Potato Dextrose Agar (PDA) medium by dual culture method as suggested by Yuan and Crawford (1995).

Loopful of test antagonist *Streptomyces* sp. was streaked onto one side of each PDA plate. The plates were incubated at 30°C for 3 days. A 5 mm diameter agar plug of fungal mycelium of test fungal pathogen was transferred onto the center of the other side of each plate. Fungal plugs were also placed on *Streptomyces* sp. uninoculated PDA plates separately for all the test pathogens as uninhibited control. The plates were incubated at 30°C and examined for inhibition of growth after 6 days (Figure 4).

Streptomyces sp. was inhibited radial hyphae growth of *Alternaria* sp. in a dual culture test after 6 days (Figure 4.11 A and B). Under the microscope, hyphae and spores shape of *Alternaria* sp. were found in malformation (Figure 4. A – F). *Streptomyces* sp. also showed against the growth rate of *Helminthosporium* sp. (Figure 5 A and B). The antifungal effect of *Streptomyces* sp. has caused deformation of spores and hyphae of *Helminthosporium* sp. (Figure 5 C – F). Furthermore, *Streptomyces* sp. also slightly contaminated in growth rate of *Colletotrichum* sp. and *Aspergillus* sp. (Figure 6 A, B and Figure 7 A, B). Although microscopical structures of *Colletotrichum* sp. and *Aspergillus* sp. did not change apparently, the change of morphological characters were be found in some parts (Figure 6 C, D and Figure 7 C, D).

The strain of *Streptomyces* sp. exhibited good inhibition of *Alternaria* sp. (KTW 01), showed strong inhibition of *Helminthosporium* sp. (KTW 02), moderate degree of growth inhibition of *Colletotrichum* sp. (KTW 03) and weak inhibition of *Aspergillus* sp. (KTW 04) as shown in Table 3.

Table 3. The level inhibition of one strain *Streptomyces* sp. against pathogenic fungi of four strains after 6 days

Test Bacterial strain	Strain Fungus	Control Fungus (cm)	Test strain inoculated in Fungus (cm)	Level inhibition ($\Delta\gamma = \gamma_0 - \gamma$)	Rating
Streptomyces sp.	*Alternaria* sp.	5.2	1.6	3.6	+++
-	*Helminthosporium* sp.	8.6	3.3	5.3	++++
-	*Colletotrichum* sp.	8.5	5.5	3.0	++
-	*Aspergillus* sp.	7	5	2	+

++++ Strong inhibition ($\Delta\gamma >$ 5 cm)

+++ Good (4.0 cm > $\Delta\gamma$ > 3 cm)

++ Moderate (3cm > $\Delta\gamma$ > 2 cm)

+ Weak (2cm > $\Delta\gamma$ > 1 cm)

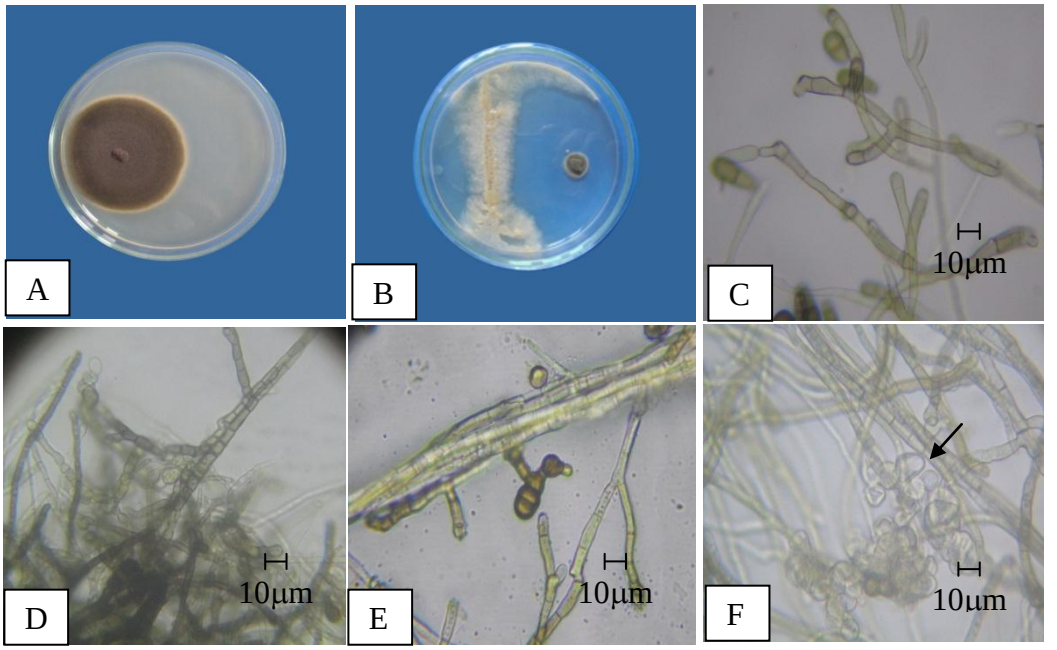

Figure 4. Inhibitory effects of *Streptomyces* sp. strain on *Alternaria* sp. after dual cultures for 6 days on PDA medium

A. Untreated control of fungus mycelium
B. Fungus mycelium in test inoculated
C. Healthy mycelia with regular normal growth in control Culture
D. Malformation growth (arrow) of fungal hyphae which culture with *Streptomyces* sp.
E. Regular normal growth of spores in control Culture
F. Spores showed deformation (arrow) which culture with *Streptomyces* sp.

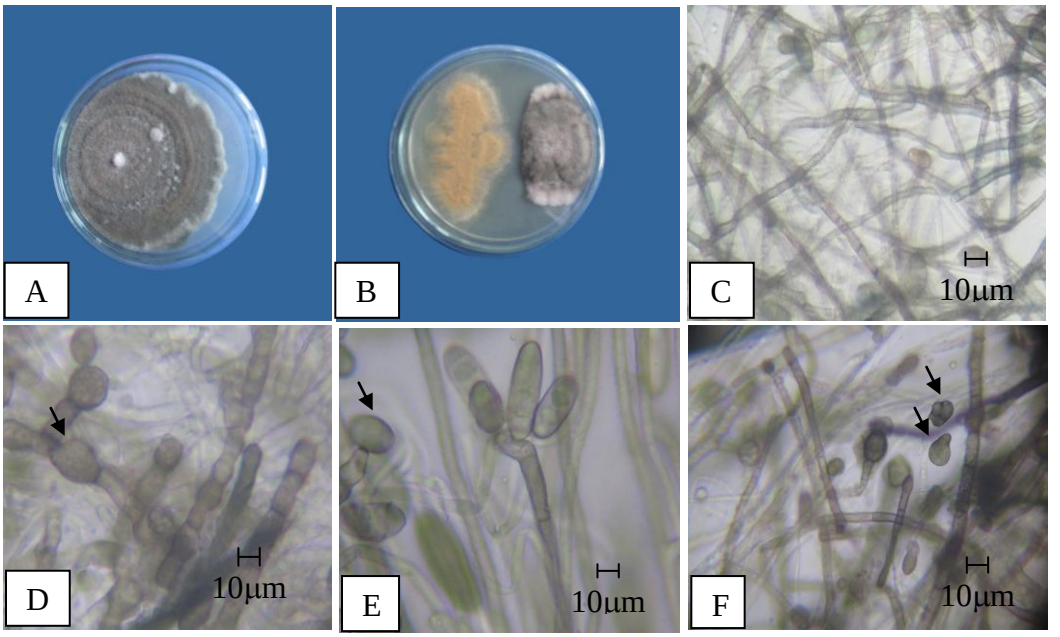

Figure 5. Inhibitory effects of *Streptomyces* sp. strain on *Helminthosporium* sp. after dual cultures for 6 days on PDA medium

A. Untreated control of fungus mycelium
B. Fungus mycelium in test inoculated
C. Healthy mycelia with regular normal growth in control treatment
D. Malformation growth (arrow) of fungal hyphae which culture with *Streptomyces* sp.
E. Regular normal growth of spores in control treatment
F. Spores showed deformation (arrow) which culture with *Streptomyces* sp.

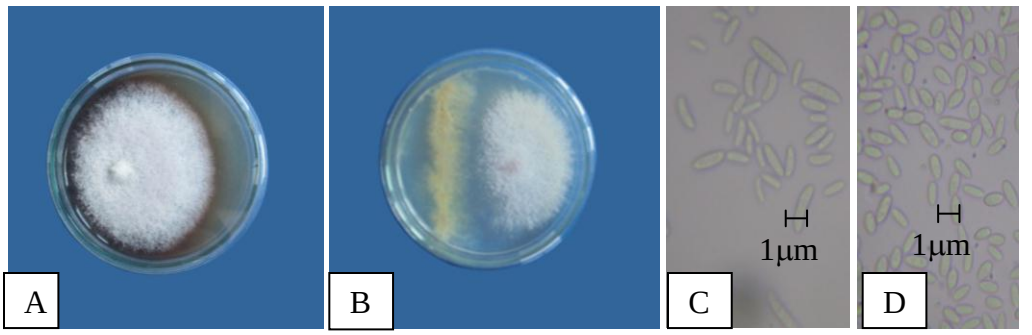

Figure 6. Effect of *Streptomyces* sp. strain on *Colletotrichum* sp. after dual cultures for 6 days on PDA medium

A. Untreated control of fungus mycelium
B. Fungus mycelium in test inoculated
C. Microscopic structure of conidia in untreated control
D. Microscopic structure of conidia in test inoculated

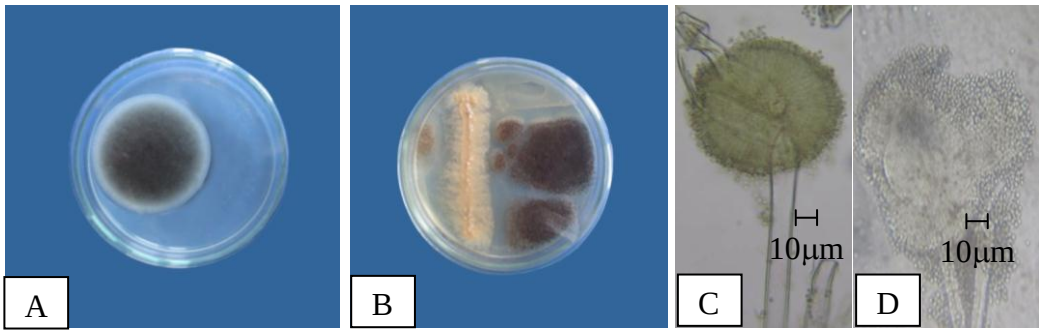

Figure 7. Effect of *Streptomyces* sp. strain on *Aspergillus* sp. after dual cultures for 6 days on PDA medium

A. Untreated control of fungus mycelium
B. Fungus mycelium in test inoculated
C. Microscopic structure of conidia in untreated control
D. Microscopic structure of conidia in test inoculated

Discussion and Conclusion

The present research work deals with the isolation, identification of microorganisms and studies of antagonistic effect. The endophytic bacterium was isolated from root of penny wort and studied the antagonistic effect on pathogenic fungi which caused a leaf disease on cabbage. According to the identification the host plant, it can be identified as *Centella umbellata* Schubert et van Wyk.

In the present investigation, bacteria strain were isolated from *Centella umbellata* root. Present research employed Starch Casein Agar (SCA) medium to isolate genus *Streptomyces* from *Centella umbellata* root. Fairbairn *et al.* (1986) described starch that is a suitable selective carbon source for Streptomycetes and Kim *et al*. (2003) also suggested that *Streptomyces* sp. can be readily recognized by their macroscopical and microscopical appearance on Starch Casein Agar medium. Further, International Streptomycetes Project -2 (ISP-2). medium employed for purified with point Shirling & Gottlieb (1966).

Identification was done according to the bacteria colony and microscopical character. *Streptomyces* sp. colonies was red color, mucoid and margin filamentous on International Streptomycetes Project -2 (ISP-2) and Starch Casein Agar (SCA) medium. Microscopical character of *Streptomyces* sp. grow aerial mycelium from substrate mycelium into chain spore. Characteristics of *Streptomyces* sp. was the chain types of spores were found to be Retiflexibiles. The colony characters and microscopic data is in accordance with the description

provided by Waksaman & Henrici (1943), Flardh & Buttner (2009) and Shirling & Gottlieb (1977).

Based on Gram Staining test, it showed violet crystal dye. Under microscope visualization, the color was violet. According to gram staining classification, the isolate belongs to the positive gram group.

Biochemical characteristics of the selected were analyzed. Nitrate reduction and catalase and oxidase were found to be positive. It was found to be capable of hydrolyzing starch, casein and liquefied gelatin. The strain was tested from their capability to ferment different types of carbohydrate such as sucrose, glucose, dextrose and manitol. The results showed that it was capable of fermenting sugar producing gas. According to the result, the one strain of bacteria character was confirmed as genus *Streptomyces* by referring to literature described by Waksaman & Henrici (1943).

Pathogenic fungi isolated from infected cabbage by using Potato Dextrose Agar (PDA) medium and employing Forcible Spore Discharge Methods and studies the fungi characters after designated as KTW 01, KTW 02, KTW 03, KTW 04 strains.

The macroscopical and microscopical character of KTW 01 strain was flat and olivaceous to brown colour colonies at mature. The hyphae are septate. Conidiophores are brown, bearing conidia. Conidia were born on chain. Conidia have 3 - 4 transverse septa, sometimes with 1 - 2 longitudinal septa at the widest part. Therefore, the present data are in agreement according to Wiltshire (1947). KTW 01 strain was confirmed as *Alternaria* sp.

The results have shown that distinguishing character of KTW 02 strain was septate hyphae. Conidiophore was simple or lateral branches. Conidia arose from the apical of conidiophores, 3-cells and ellipsoid. Morphology colony was olive green with white edge and then turn to greyish at maturity. These identifying character were in accordance with the report by Barnett (1955). Therefore, it was concluded that the fungus KTW 02 strain was *Helminthosporium* sp.

Bernett (1955) described that *Colletotrichum* is characterized by conidiophores simple, elongate and conidia hyaline, 1-celled, ovoid or oblong. These characters were observed in present results of KTW 03 strain. The characters of KTW 03 strain was confirmed as *Colletotrichum* sp.

The KTW 04 fungus was white colonies at firstly and turns to black with powdery and then mature black at room temperature 25°C and pH 6.5 - 7.0 after 3 - 7 days. The microscopical characters of KTW 04 were septate hyphae, upright conidiophore, subglobose vesicle, sterigmata, biseriates-conidia and spherical conidia. Among them, conidiophores position and conidia shape was key

character for the identification of KTW 04 strain. So, KTW 04 was confirmed as *Aspergillus* sp. according to report of Barnett (1955).

The level of antagonistic effects showed inhibition of fungal pathogens with varying effectiveness. It was based on the values of $^{\Delta\gamma}$ (cm) and further grouped in terms of rating. The strain of *Streptomyces* sp. showed inhibition of fungal pathogen with varying effectiveness that was *Alternaria* sp., *Helminthosporium* sp., *Colletotrichum* sp. and *Aspergillus* sp.

The strain of *Streptomyces* sp. (5.3 cm) exhibited a strong inhibition of *Helminthosporium* sp. and belong to ++++ rating, (3.6 cm) showed a good inhibition of *Alternaria* sp. belonged to +++ while, (3 cm) was a moderate degree of growth inhibition of *Colletotrichum* with rating ++ and (2 cm) was weak inhibition of *Aspergillus* sp. that had + rating. In the present data, the strain of *Streptomyces* sp. showed weak, moderate, good, strong inhibition level possess *Aspergillus* sp., *Colletotrichum, Alternaria* sp. and *Helminthosporium* sp. respectively.

Result of the observation on interaction between *Streptomyces* sp. and *Alternaria* sp. showed some changes in hyphae shape. These changes occured due to effect of antibiotic reaction produced by *Streptomyces* sp.

Besides swelling on the mycelium, the antifungal effect of *Streptomyces* has caused the spore deformation of *Alternaria* sp.

Further, the strain of *Helminthosporium* was againsted by the strain of *Streptomyces* sp. In the antagonistic test, the hyphae and spore of *Helminthosporium* showed malformation.

In the present research, *Colletotrichum* sp. and *Aspergillus* sp. were not distinctly found in change of spore and mycelium shape under examination by microscope due to strain of *Streptomyces* sp. showed moderate and weak inhibition on *Colletotrichum* sp. and *Aspergillus* sp.

The pathogenic fungi were slowly growth in a dual culture test. It is affected their structure into malformation. This event might be due to the production of antibiotic metabolites by the *Streptomyces*, which may penetrate the pathogen cell and inhibit its activity by chemical toxicity.

The previous researches reported that morphological change of mycelium related to antifungi activities resulted by *Streptomyces* sp. according to Mujoko *et al*. (2014), Phuakjaiphaeo & Kunasakdakul (2015) and Dalal & Kulkarni (2014). In the present work, the isolated strain of the *Streptomyces* species has antagonistic effect on phytopathogenic fungi *Alternaria*, *Helminthosporium, Colletotrichum*, and *Aspergillus*. The isolated bacteria strain showed antagonistic

effect especially to against *Alternaria* and *Helminthosporium* and slightly effected on *Colletotrichum* and *Aspergillus*.

In conclusion, the present work revealed that the characters of *Streptomyces* species, pathogenic fungi, and the level of inhibition rate on pathogenic fungi and deformation of pathogenic fungi structure by using endophytic bacteria *Streptomyces*. The research work also showed the antagonistic effect to against the disease of leaf diseases on the cabbage. This study will provide the information for further studies which are necessary to evaluate the effect of these potential biocontrol agents for the cultivation of cabbage in greenhouse and field conditions. Alternately, the effect of metabolites which are extracted from *Streptomyces* should also be studied in the future.

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