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ORIGINAL PAPER

Mode of antagonism of a biocontrol bacterium *Lysobacter* sp. SB-K88 toward a damping-off pathogen *Aphanomyces cochlioides*

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Abstract The biocontrol bacterium *Lysobacter* sp. SB-K88 suppresses damping-off disease in sugar beet and spinach caused by *Aphanomyces cochlioides* and *Pythium* sp. through characteristic plant colonization and antibiosis against the pathogens. This study aimed to unravel further details on mode of antagonism of SB-K88 against a damping-off pathogen *A. cochlioides* AC-5. The SB-K88 substantially inhibited growth and decomposed AC-5 mycelia and suppressed the release of zoospores from the hyphae. The excised root tips of sugar beet seedlings from seeds previously inoculated with SB-K88 were less attractive to AC-5 zoospores. Although aerial growth was not affected, however, root hairs of SB-K88 inoculated sugar beet seedlings were remarkably shorter and thicker than those of uninoculated control. When exposed to zoospores, the SB-K88 inhibited motility of zoospores and/or caused lysis, and then aggregated around the dead cystospores or lysed residues within 3–6 h likely to be micro-predatory behavior to a eukaryotic organism. Confocal laser scanning microscopic analysis revealed that number of lipid bodies and activities of mitochondria were markedly increased in the affected hyphae compared with control hyphae as visualized by established vital stains. Taken together, these results suggest that *Lysobacter* sp. SB-K88 suppresses damping-off diseases through exerting multifaceted antagonistic effects against the peronosporomycetes.

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Introduction

Members of the genus *Lysobacter* are typically found in soil and water habitats and are characterized by gliding motility and the ability to lyse other microorganisms, including fungi and nematodes (Christensen and Cook 1978). Some strains of *Lysobacter* spp. have been reported as potential candidates for biocontrol of fungal and Peronosporomycete phytopathogens (Nakayama et al. 1999; Zhang and Yuen 1999; Zhang and Yuen 2000; Yuen and Zhang 2001; Zhang et al. 2001; Islam et al. 2005a, b; Kobayashi et al. 2005; Yu et al. 2007). Several new species of *Lysobacter* have recently been reported with high promise for biocontrol activity against fungal and peronosporomycete phytopathogens (Park et al. 2008; Ji et al. 2008; Postma et al. 2008, 2009). However, the mode of action of antagonisms of these potential biocontrol agents against phytopathogens is poorly understood.

Antagonistic bacteria vary greatly in their modes of action, root colonization ability and degrees of host specificity, and may use mechanisms such as competition, antibiosis (Thomashow and Weller 1988; Nakayama et al. 1999), production of hydrolytic enzymes (Zhang and Yuen 2000; Ahmed et al. 2003), or a combination of all these processes to suppress the pathogen (Bais et al. 2004; Islam et al. 2005b). The full potential for biological control of peronosporomycete pathogens has not been explored but several recent studies have described progress in the control of those pathogens by mycoparasitism (Whipps 2001; West et al. 2003; Walker et al. 2002), increasing defense systems in plants (Kilic-Ekici and Yuen 2003) and antibiosis (Nakayama et al. 1999; Islam et al. 2005b; Yu et al. 2007).

One of the strains of *Lysobacter* species, the SB-K88 which was isolated from the rhizoplane of sugar beet has shown high potential to suppress damping-off diseases in sugar beet and spinach caused by *Aphanomyces cochlioides* and *Pythium* sp. (Nakayama et al. 1999; Islam et al. 2004a, 2005a, b). Control of damping-off diseases in sugar beet and spinach by SB-K88 has been found to be associated with dense root colonization through characteristic perpendicular attachment and production of lytic antibiotics (xanthobaccin A, B, C; Hashidoko et al. 1999; Nakayama et al. 1999; Islam et al. 2004a, 2005a, b). Furthermore, it has recently been demonstrated that the inhibitory effect of *Lysobacter* sp. SB-K88 on *A. coclioides* is linked to disruption of filamentous actin (F-actin) and ultrastructural alterations in the zoospores and hyphal cells (Islam 2008). Few attempts have been made to understand the modes of action of newly identified biocontrol bacteria *Lysobacter* spp. at cellular and molecular levels of the pathogens, host plants and bacteria itself. Hence, little information is available concerning how disease pathogens are controlled by *Lysobacter* species. Nothing is known about the effect of SB-K88 on roots of seedling produced from seeds previously inoculated with bacteria.

Although it has been focused on antibiosis and characteristic plant colonization behavior of *Lysobacter* sp. SB-K88 in relation to damping-off disease suppression in sugar beet and spinach (Nakayama et al. 1999; Islam et al. 2004a, 2005b), understanding detail mechanism of host plant, SB-K88 and peronosporomycete pathogen interactions is needed for considering the practical use of this biocontrol agent. Thus, the objectives of the present work were to (i) evaluate the strain SB-K88 to inhibit mycelial growth and zoosporogenesis of *A. cochlioides*; (ii) investigate chemotactic behavior of zoospores toward SB-K88 inoculated roots of spinach and sugar beet seedlings; (iii) effect of SB-K88 inoculation on morphology of sugar beet roots; and (iv) behavior (tactic) of SB-K88 toward killed cystospores and residues of lysed *A. cochlioides* cells; and (v) visualize nuclei, lipid bodies and mitochondrial activities in the affected hyphae by using vital stains. Experimental results suggest that the SB-K88 inhibited hyphal growth and developmental transition of zoospores by affecting metabolic activities in the cells of the pathogen. This paper describes new information on the mode of antagonism of biocontrol bacterium *Lysobacter* sp. SB-K88 against a soilborne phytopathogen *A. cochlioides* that coexists in the same environment.

Materials and methods

Microorganisms

The bacterial strain SB-K88 was isolated from the fibrous roots of sugar beet (Homma et al. 1993) and identified as *Lysobacter* sp. based on 16S rRNA gene sequencing data (accession no. AB190258) and other traits (Islam et al. 2005b). The SB-K88 was cultured in a 500-ml flask containing 200 ml of a nutrient solution at 25 °C with shaking at 100 rpm (Islam et al. 2005b). The culture fluid was centrifuged at 8,000 *g* for 15 min at 5 °C, and then the bacterial pellets were washed three times with sterilized phosphate buffer (8 mM, pH 7.2) and used for seed coating and bioassay. The test pathogen *A. cochlioides* AC-5 was kindly donated by Professor R. Yokosawa, Health Science University of Hokkaido, Japan.

Mycelial dry weight

To study antagonistic effect of *Lysobacter* SB-K88, mycelial growth of *A. cochlioides* was measured in presence of different populations of bacteria. The zoospores of AC-5 were produced exactly following protocols described previously (Islam and Tahara 2001; Islam et al. 2007). For mycelial growth, zoospores of *A. cochlioides* at the rate of 6×10^4/ml were inoculated to 100 ml of potato dextrose broth (PDB) in an Erlenmeyer flask and allowed to grow for 7 days at 23 °C, and then a series of bacterial (SB-K88) population was added to the flask and mixed thoroughly. Mycelial dry weight of control samples was measured before the inoculation of bacteria, to observe whether bacteria can decompose the grown mycelia. After 10 days of mycelia-bacteria interactions, mycelia were harvested by vacuum filtration (5 μm), washed and dried in an oven at 70 °C overnight. Dry weight was determined using a Mettler scale. Each treatment was replicated three times.

Zoospore release and chemotaxis assay

To study the effect of SB-K88 on zoosporogenesis, AC-5 was cultured on corn meal agar medium (CMA, Difco Laboratories, Sparks, MD, USA) for 6 days at 20 °C. *Lysobacter* sp. strain SB-K88 was cultured in a 500-ml flask containing 200 ml of a nutrient solution at 25 °C for 15 days with shaking at 100 rpm. The bacterial cells were washed and harvested as described earlier (Islam et al. 2005b). The preparation of sample for induction of zoosporogenesis was carried out on plastic Petri dishes as described (Islam and Tahara 2001, Islam et al. 2003) and varying populations of SB-K88 cells were added to the aquatic medium before incubation of washed mycelial blocks of AC-5 into sterilized distilled water to release zoospores. After 16 h of incubation, the number of zoospores released per milliliter of water was counted (Islam et al. 2007). A control treatment (without bacterial inoculation) was also included. Each treatment was replicated thrice.

Chemotaxis of zoospores toward root tips of spinach and sugar beet previously inoculated with SB-K88 was

performed as described earlier (Islam et al. 2004b). A set of control (uninoculated) root tips was also tested.

Seed coating, plant culture and SEM

Surface sterilized seeds of sugar beet cultivar Abendrot were coated with SB-K88 (ca. 10^8 CFU/seed) and grown in 0.3% gellan gum containing 0.2× Hoagland's S medium in test tubes (Islam et al. 2005b). To study morphology of roots and colonization of bacteria on plant surfaces, 2-week-old seedlings were carefully removed from a soft gel composed of gellan gum and gently washed six times with sterilized phosphate buffer (8 mM pH 7.2). The roots of seedling were separated using a sharp blade and fixed with 2% glutaraldehyde overnight. The remaining preparations and procedures for scanning electron microscopic (SEM) study were carried out as described previously (Islam et al. 2001, 2002).

Dual culture tests and aggregation assay

Interactions between *A. cochlioides* AC-5 and *Lysobacter* sp. strain SB-K88 were studied exactly following protocols described earlier (Islam 2008). Briefly, agar discs (6 mm in diameter) were collected from the growing edges of AC-5 hyphae on CMA (17 g/l) and placed 3 cm apart from the colonies of SB-K88. Plates were allowed to grow at 27 °C in the dark. In dual culture assay, SB-K88 inhibited growth of approaching AC-5 hyphae by inducing excessive branching and curling in the hyphal tips (Islam 2008). For confocal laser scanning microscopic (CLSM) observation, AC-5 hyphal samples were harvested with a sterile cork borer (6 mm i.d.) from the colony edge growing toward the SB-K88 colonies after 5 days.

Various dilutions of washed SB-K88 cells were added to the suspension of motile zoospores of *A. cochlioides* AC-5 taken into a small Petri dish (3 cm i.d.). The zoospores became halted and then encysted or lysed in presence of SB-K88 at 1×10^9 (CFU/ml) or higher. The interacting organisms in the Petri dish were kept undisturbed for 3–6 h at room temperature (23 °C) and then observed under a light microscope connected to digital camera (Deora et al. 2006). Each treatment was replicated thrice and representative images are shown.

CLSM specimen preparation and observation

The following stains were selected in order to observe the physiological changes evoked in the hyphae: Nile red (Sigma N-3013, Steinheim, Germany) for lipid bodies; 3,3¢-diheptyloxacarbocyanine iodide ($DIOC_7$; Molecular Probes D-378, Eugene, OR, USA) for mitochondria and Hoechst 33258 (bisbenzimide; Sigma–Aldrich H 6024, St. Louis, MO, USA) for visualization of nuclei. Stock solutions of the stains were made at 1 mg/ml in dimethylsulfoxide (DMSO) except for Hoechst 33258. The final working concentrations and staining times were as follows: Nile red at 2 µl/ml for 20 min and $DIOC_7$ at 0.02 µl/ml for 60 min (Deora et al. 2006). Hoechst 33258 was used at 20 µg/ml ultrapure MilliQ (Millipore, Molsheim, France) water for 5 min. The detail procedures of specimen preparation and staining were described elsewhere (Deora et al. 2006). Five mycelial disks from each of five replicated plates of the treated and negative control were sampled. An average of five hyphae was examined in each specimen.

The sections with stained hyphae were observed with a Zeiss confocal laser scanning microscope LSM410 (Carl Zeiss, Oberkochen, Germany) in order to detect the various fluorescence signals (Deora et al. 2006). The instrument was equipped with an Axiovert 135 M microscope with objective lenses containing magnifications/numerical apertures of 20/0.5 and 40/0.75. For Nile red and $DIOC_7$, an excitation wavelength of Ar488 and LP515 nm emission filter were used, and for Hoechst 33258, an excitation of UV-Ar364 nm and emission filter BP395-440 nm. Ar488 was used in order to mediate observations with differential interference contrast (DIC) microscopy. For fluorescence microscopy, images were sequentially collected from single optical sections along the z-axis at intervals of 1–1.5 µm. Approximately 10–30 optical sections were obtained from a total depth of optical sectioning of 1 µm. Randomly selected regions of specimens were scanned to compile images using the z-series program for confocal laser scanning microscopy (CLSM). The optical sections were reconstructed into stereoscopic images with the CLSM reconstruction program. Images were processed by adding the following pseudocolors with Adobe Photoshop v.6.0.1: green for Nile red and $DIOC_7$, and blue for Hoechst 33258 to detect lipid bodies, mitochondrial localization and activity, and behavior of nuclei, respectively. However, only a single optical section was obtained for DIC images.

Results

Antagonism of SB-K88 against mycelial dry weight and zoosporogenesis of *Aphanomyces cochlioides*

To see whether SB-K88 affect mycelial growth of *A. cochlioides* in PDB medium, the various doses of SB-K88 cells (50 to 1×10^9 CFU/ml) were added to the Erlenmeyer flasks containing AC-5 cultivation at day 7. The dry weight of mycelia of AC-5 at day 7 was (68.2 ± 2.7) mg. The effects of SB-K88 on growing mycelia of *A. cochlioides* (AC-5) in PDB medium was evaluated and presented in Table 1. The

Table 1 Effect of *Lysobacter* SB-K88 on mycelial growth of *Aphanomyces cochlioides*

Bacterial population (CFU/ml)[a]	Dry weight of mycelium (mg)[b]
5×10^4	51.8 ± 6.2
5×10^3	56.6 ± 1.6
5×10^2	73.9 ± 11.6
5×10^1	198.2 ± 7.2
Control (1)	226.1 ± 24.8
Control (2)[c]	68.2 ± 2.7

[a] CFU/ml of PDB inoculated after 7 days of mycelial growth

[b] The values are means ± SE based on three replicates after 10 days of bacterial inoculation

[c] Dry weight of mycelia calculated at time of bacterial inoculation to check the decomposition effect

mycelial dry weight (56.6 ± 1.6) mg of AC-5 remarkably decreased in presence of SB-K88 at 5×10^3 CFU/ml or higher density than that of initial dry weight (68.2 ± 2.7) mg of mycelia before addition of bacteria indicating decomposition of mycelia by SB-K88. The mycelial dry weight of uninoculated control was (226.1 ± 24.8) mg, which was much higher than those of dry weights of mycelia in SB-K88 inoculated flasks. Addition of SB-K88 as low as 50 CFU/ml, also decreased mycelial dry weight by 13% from uninoculated control indicating that SB-K88 suppresses AC-5 mycelial dry weight in liquid medium.

Production of zoospores from the *A. cochlioides* mycelia was also significantly reduced (>25%) in the presence of SB-K88 at a dose of 5×10^5 CFU/ml (Fig. 1). The relative percentage of released zoospores was severely affected (less than 15% of control) at a dose of 1×10^8 CFU/ml. However, no significant differences in relative zoospore release were observed at a short range between 5×10^6 CFU/ml and 5×10^7 CFU/ml. The SB-K88 at a dose of 1×10^{10} CFU/ml or above completely blocked the process of zoosporogenesis in *A. cochlioides* (data not shown). However, presence of bacterial population less than 1×10^2 CFU/ml did not show any effect on zoosporogenesis in sterilized distilled water.

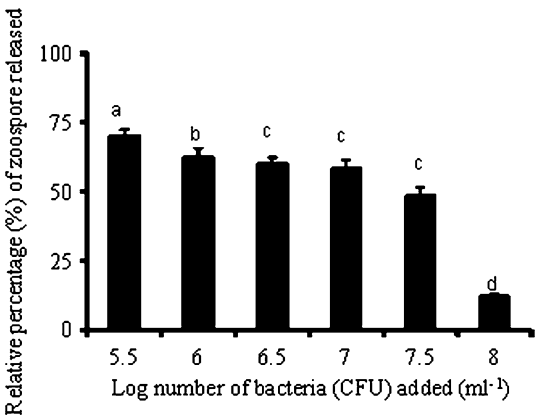

Fig. 1 Relative percentage of zoospores released from the mycelia of *Aphanomyces cochlioides* in the presence of various density of SB-K88. Averaged number of zoospores produced (released) in the control (without bacteria) Petri dish was ca. 2.1×10^5/ml. The error bars represent the standard errors of the mean. Each treatment was replicated four times

Lysobacter sp. SB-K88 aggregates to dead or lysed spores of *Aphanomyces cochlioides*

Lysobacter sp. SB-K88 is a rod-shaped gliding bacteria having characteristic polar fimbriae (Fig. 2a, b). When living bacterial suspension of SB-K88 (1×10^9 CFU/ml or more) added to the suspension of motile AC-5 zoospores, the motility of zoospores ceased. The sessile spores immobilized or became round cystospores and then germinated or killed. Interestingly, the SB-K88 cells aggregated to the immobilized residues (Fig. 3c) or killed cystospores (Fig. 3b) or hyphal germling (Fig. 3d) and formed a mass of cells within 3–6 h incubation at room temperature. Figure 3a shows an ungerminated cystospores from dish having no bacterial inoculation (control). This phenomenon is likely to micro-predation popularly known as wolf-pack feast reported earlier (Martin 2002).

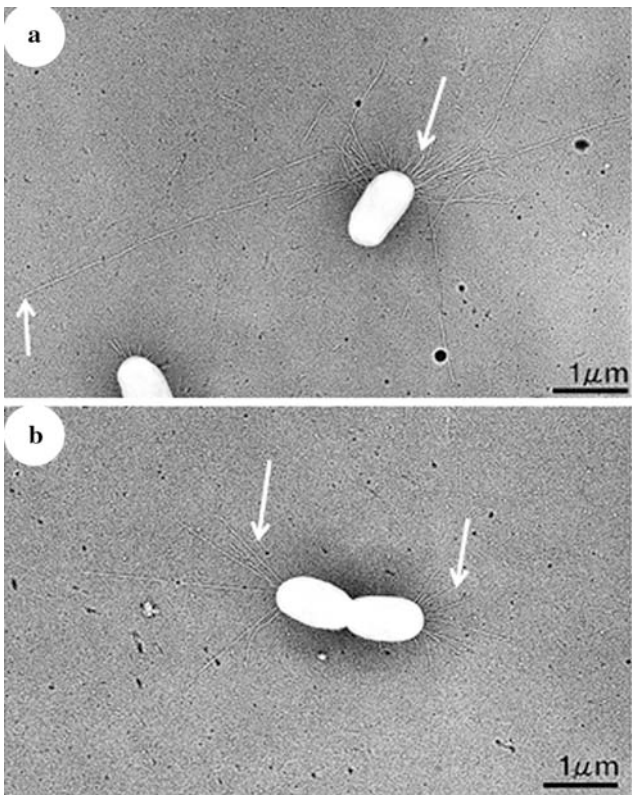

Fig. 2 Transmission electron micrographs showing morphological features of *Lysobacter* sp. SB-K88. **a** An SB-K88 cell with polar fimbriae (*arrows*); and **b** an SB-K88 cell dividing into two cells having polar fimbriae

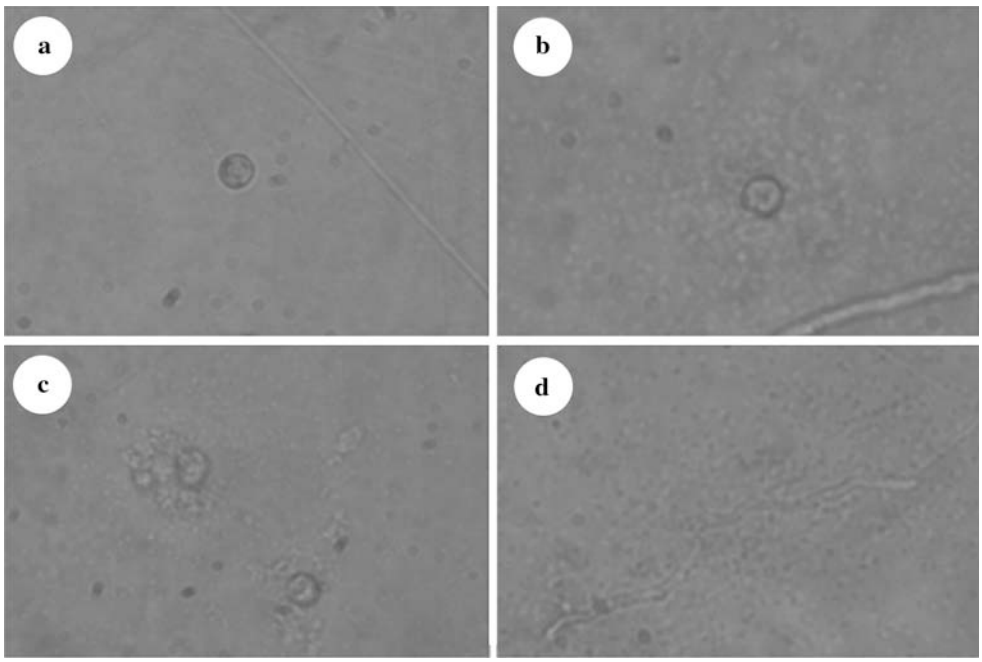

Fig. 3 Light micrographs showing aggregation behavior of *Lysobacter* sp. SB-K88 to dead cystospores or residue of lysed spores or hyphal germling of *Aphanomyces cochlioides* (AC-5). **a** A cystospore free from bacterial colonization (control); **b** SB-K88 aggregated on and around a partially lysed cystospore; **c** aggregation of SB-K88 cells to residues and partially immobilized two cystospores; **d** aggregation of SB-K88 cells on and around a newly formed hypha (the trace of cystospore is not seen)

Interruption of homing responses of zoospores by SB-K88

When excised roots tips of 7-days-old seedlings of spinach and sugar beet from seeds previously inoculated with SB-K88 were immerged into zoospore suspension, zoospores were very less attracted to and aggregated at the root tips compared to control seedlings (without bacterial inoculation; data not shown). In control root tips, zoospores rapidly aggregated and formed a mass of cystospores on and around the tips. All aggregated cystospores germinated to form germ tubes within 60 min (Islam et al. 2002). Some zoospores those approached to the SB-K88 inoculated roots were halted and immobilized indicating that *Lysobacter* sp. SB-K88 interrupts homing responses of pathogenic *A. cochlioides* zoospores.

Lysobacter sp. SB-K88 affects nuclei, mitochondria and lipid bodies in *A. cochlioides* hyphae

By using Hoechst 33258 to visualize behavior of nuclei, specifically division and localization, it became evident that the number of nuclei per unit length was not significantly increased in growth stunted deformed hyphae (Fig. 4b). However, the shape of nuclei in negative control was oval-shaped (Fig. 4a) while in the affected hyphae they became in spindle-shaped (Fig. 4b). Nile red staining confirmed remarkable increase in the number and size of lipid bodies with characteristic globular shapes and variable sizes throughout the bacteria-exposed hyphae during interaction at day 5 (Fig. 4d) as compared to the negative control (Fig. 4c). The lipid bodies in the affected hyphae appeared to be sunk to the vacuoles while in control they remained in the cytoplasm (Islam 2008). Different patterns of the fluorescence intensities were observed in negative controls and the affected hyphae that were stained with $DIOC_7$; these variations showed the difference of mitochondrial distribution and activity at different stages of interaction. In the affected hyphae, the mitochondria were significantly more abundant especially in swollen or deformed part of the hyphae (Fig. 4f). These results were in contrast to the negative control that exhibited less density of mitochondria (Fig. 4e).

SB-K88 alters root morphology of inoculated sugar beet seedlings

To see whether SB-K88 has any effects on root growth of inoculated plants, roots of 2-week-old sugar beet seedlings from seeds previously inoculated with SB-K88 were examined by scanning electron microscopy (SEM; Fig. 5). SEM analysis revealed dense colonization on the root surface mostly in characteristic perpendicular fashion (Fig. 5c–f). The SB-K88 also colonized on cotyledons and leaves of sugar beet and spinach in similar pattern (Fig. 5e, f). Interestingly, although root hairs were free from bacterial colonization, the root hairs of SB-K88 inoculated sugar beet seedlings (Fig. 5a) were remarkably shorter and thicker than those of uninoculated control seedlings (Fig. 5b). The SB-K88 displayed higher tendency to colonize at the bases of root hairs (Fig. 5c, d) as well as ridges of leaf surface except the stomata (Fig. 5e).

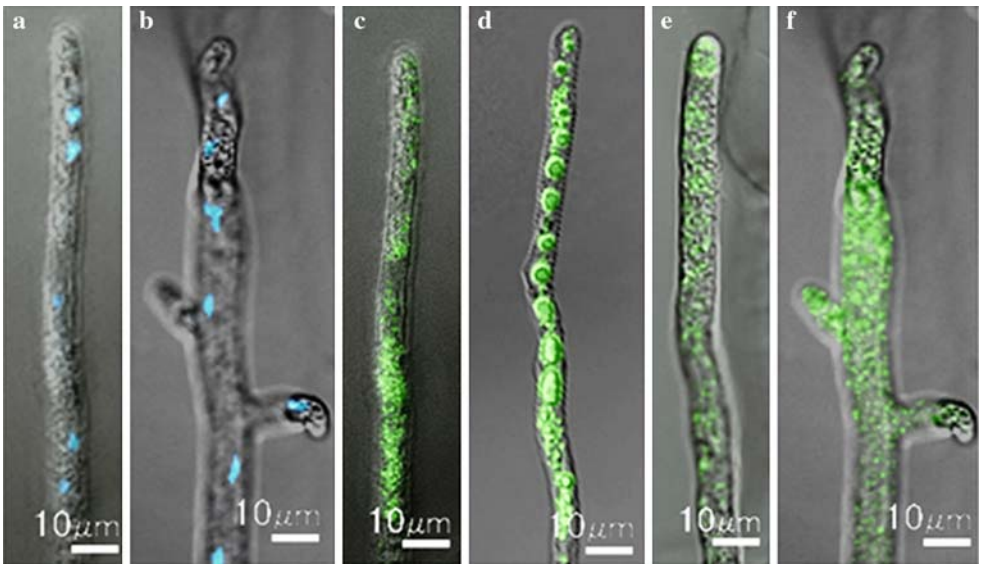

Fig. 4 Confocal laser scanning micrographs showing intracellular changes of nuclei, lipid bodies and mitochondrial activity in *Apahnomyces cochlioides* hyphae challenged with *Lysobacter* sp. SB-K88 on PDA medium. **a** Control hypha, nuclei oval-shaped. **b** Affected hypha with nuclei spindle-shaped. **c** Control hypha with lipid bodies organized into the cytoplasm. **d** Affected hypha with lipid bodies sank into the vacuoles. **e** Control hypha, mitochondrial density lower. **f** Affected hypha with higher mitochondrial density

Discussion

Zoospores are the means of infecting plants by most of the phytopathogenic peronosporomycetes (Islam and Tahara 2001). In present study, in vitro bioassay revealed that the SB-K88 remarkably inhibited zoosporogenesis (Fig. 1) as well as caused necrosis of the growing hyphae (Table 1). These results suggest that inhibition of both mycelial growth and production of zoospores by the SB-K88 might be linked to suppression of damping-off disease upon seed inoculation with bacteria (Nakayama et al. 1999; Islam et al. 2004a, 2005a, b). Inhibition of hyphal growth of fungi and peronosporomycetes by different strains of *Lysobacter* spp. on dual culture assay in agar medium has been reported by many earlier investigators (Nakayama et al. 1999; Folman et al. 2003; Islam et al. 2005a, b; Park et al. 2008), however, this is the first report on suppression of zoospore release by *Lysobacter* sp. SB-K88 from the mycelia of a soilborne peronosporomycete phytopathogen *A. cochlioides*.

Chemotaxis of zoospores is critical for locating host and overall success in infection by peronosporomycete phytopathogens. Disruption of chemotaxis of zoospores is thought to eliminate the possibility of infection of plants by zoosporic phytopathogens (Islam et al. 2004b). Bioassay revealed that roots of sugar beet and spinach seedlings from seeds previously inoculated with SB-K88, were very less attractive to *A. cochlioides* zoospores. Zoospores approached to the roots were mostly halted before reaching the root surface. Some of them became encysted or lysed. In an earlier study, it has been demonstrated that xanthobaccin A secreted from the SB-K88 cells lyse zoospores of *A. cochlioides* at a very low doses likely to be present in the rhizosphere of SB-K88 inoculated sugar beet plants (Islam et al. 2005b). It indicates that effective colonization of SB-K88 to roots and secretion of xathobaccins from bacterial cells might interfere homing responses of zoospores on host surface and protect plants from the disease. Almost similar interference in homing events of *Pythium aphanidermatum* and *Aphanomyces euteiches* zoospores by biocontrol bacterium *Burkholderia cepacia* AMMDR1 has been demonstrated (Heungens and Parke 2000).

One of the striking findings in present study is the aggregation behavior of SB-K88 cells on and around the dead cystospores or residues of immobilized cells of *A. cochlioides*. The migration of the bacteria to the killed cystospores or residues of immobilized zoospores and their local colonization suggest that chemotactic substances could be involved. *Lysobacter* spp. are known to secrete enzymes and secondary metabolites to lyse cells of many organisms including fungi and peronosporomycetes. The SB-K88 densely colonizes both plant roots, leaves and even on the surface of hyphae (Islam et al. 2005b). Aggregation to and formation of mass of bacterial cells of a gliding bacterium SB-K88 on lysed or killed cystospores or hyphal germlings observed in present study resemble wolfpack strategy of micro-predation as described earlier (Martin 2002). In the wolfpack strategy, a number of predator cells produce hydrolytic enzymes/metabolites that degrade cell walls of targeted organism. This degradation leads to the localized availability of host cell derived

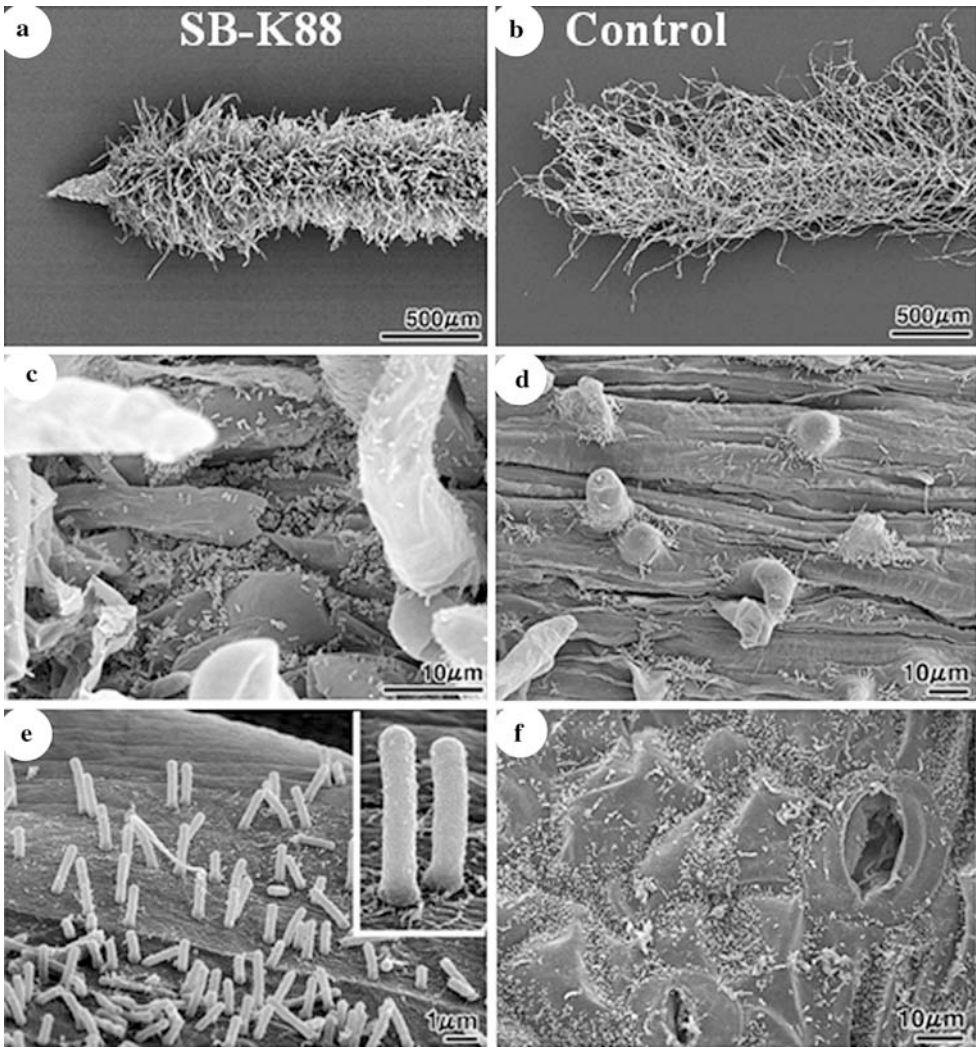

Fig. 5 Scanning electron micrographs illustrating morphology of sugar beet seedlings and colonization of *Lysobacter* sp. SB-K88 on surfaces of sugar beet seedlings upon inoculation of seeds and seedlings grown in the gellan gum-based medium. **a** Shorter root hairs in the root of a sugar beet seedling from seeds previously inoculated with SB-K88. The primary root also becomes fat. **b** Longer root hairs in the root of a sugar beet seedling free from bacterial inoculation (control). **c** Dense colonization of SB-K88 on sugar beet root. **d** Preferential colonization of SB-K88 at the bases of root hairs. **e**, **f** High-density perpendicular attachment and colonization on the sugar beet leaf surfaces after immersion into an SB-K88 bacterial suspension (ca. 10^5 CFU/ml)

nutrients. Wolfpack strategy of micro-predation has been reported earlier in gliding bacteria *Myxococcus* (Burnham et al. 1981; Fallon and Brock 1979) as well as in *Lysobacter* (Lin and McBride 1996), where the preys were also prokaryotic organisms. So far, this is first report on wolfpack-like predatory behavior of a *Lysobacter* sp. toward a eukaryotic microorganism that coexists in the same environment. A further investigation is needed to understand molecular insights of micro-predatory behavior of *Lysobacter* sp. toward the peronosporomycete phytopathogens.

An important phenomenon observed in the AC-5 interacting with SB-K88, was the dynamics of the nuclear behaviour in the hyphal cells. Similar phenomenon was observed by Deora et al. (2006) during interactions between an antagonistic bacterium *Pseudomonas jessenii* and *A. cochlioides*. Nile red staining confirmed that remarkable increase in the number of lipid bodies with characteristic globular shapes in variable sizes throughout the bacteria exposed hyphae at day 5 compared with control. This increment in the number of lipid bodies in the affected hyphae indicated a general increase in the overall hydrophobicity of the cell (Deora et al. 2006). The activity of mitochondria was remarkably increased in the hyphae affected by SB-K88 indicating higher respiratory activity in the affected hyphal cells. High density of large sized lipid bodies and mitochondria has been demonstrated earlier in the TEM sections of AC-5 hyphae confronted with SB-K88 cells on potato dextrose agar (PDA) medium (Islam 2008).

The *Lysobacter* spp. have been shown high biocontrol potentials against a number of fugal and peronosporomycetal plant diseases (Nakayama et al. 1999; Zhang and Yuen 1999; Islam et al. 2004a, 2005b; Ji et al. 2008; Postma et al. 2009). However, effect of root colonization of *Lysobacter* spp. on morphology of plant roots has not been investigated. In present study, root hairs of seedlings from seeds previously inoculated with SB-K88 had shorter and thicker root hairs than those of uninoculated control. The SB-K88 colonizes preferentially at the bases of root hairs, however, the root hairs were almost free from bacterial colonization.

Morphological alteration of root hairs due to inoculation of seeds with SB-K88 merits further investigation to clarify whether this phenomenon is linked to the resistance of roots against phytopathogens. Induced resistance as a mechanism of biological control by *Lysobacter enzymogenes* strain C3 has been reported (Kilic-Ekici and Yuen 2003).

Bacteria and unicellular eukaryotes, such as yeasts and filamentous fungi, are found together in a myriad of environments and exhibit both synergistic and antagonistic interactions (Hogan and Kolter 2002). The experimental findings of this work suggest that the intercellular interactions between *Lysobacter* sp. strain SB-K88 and *Aphanomyces cochlioides* AC-5 is a multifaceted process which might have mediated by an extensive disturbance of xanthobaccins like antibiotics and other lytic enzymes secreted from the bacteria. The combined effects evoked complex responses which lead to a series of intracellular events and ultimately death of the pathogen. Although aerial growth was not affected, however, root hairs of *Lysobacter* sp. SB-K88 colonized seedlings were shorter and thicker than those of uninoculated sugar beet seedlings. A further study is necessary to understand underlying cytochemical and molecular changes occurred in plant roots due to dense colonization of the SB-K88.

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