



ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(5): 898-905
© 2021 TPI
www.thepharmajournal.com
Received: 28-03-2021
Accepted: 02-05-2021

N Chiranjeevi
Department of Plant Pathology,
S.V Agricultural College,
Tirupati, Andhra Pradesh, India

M Reddi Kumar
Department of Plant Pathology,
S.V Agricultural College,
Tirupati, Andhra Pradesh, India

B Padmodaya
Department of Plant Pathology,
S.V Agricultural College,
Tirupati, Andhra Pradesh, India

NC Venkateswarlu
Department of Plant Pathology,
S.V Agricultural College,
Tirupati, Andhra Pradesh, India

P Sudhakar
Department of Plant Pathology,
S.V Agricultural College,
Tirupati, Andhra Pradesh, India

R Sarada Jayalakshmi Devi
Department of Plant Pathology,
S.V Agricultural College,
Tirupati, Andhra Pradesh, India

P Aruna Sri
Department of Plant Pathology,
S.V Agricultural College,
Tirupati, Andhra Pradesh, India

Corresponding Author:
N Chiranjeevi
Department of Plant Pathology,
S.V Agricultural College,
Tirupati, Andhra Pradesh, India

Studies on Extraction, Evaluation of Crude Metabolite Extract from Endophytic *Bacillus subtilis* and Its Mechanistic Effect on Chickpea Dry Root Rot Causing Pathogen *Rhizoctonia bataticola* (Taub.) Butler

N Chiranjeevi, M Reddi Kumar, B Padmodaya, NC Venkateswarlu, P Sudhakar, R Sarada Jayalakshmi Devi and P Aruna Sri

Abstract

In the present study, crude metabolic substances from endophytic bacteria *Bacillus subtilis* were extracted by solvent extraction method and tested their bio-efficacy against *R. bataticola* by agar well diffusion assay. Among the different concentrations tested significantly higher inhibition was recorded at 100 µl (77.23 %). The microscopical examination of the crude metabolite treated mycelia of the *R. bataticola* revealed its effect on the fungal morphology. Light microscopic analysis showed that severe mycelial destruction such as breakages, vacuolations, bursting, swellings, lysis, bulged hyphal tips and hyphae, cytoplasmic aggregations etc., and the SEM images clearly explained the disruption of the fungal hyphae due to the mechanistic effect of antibiotic compounds on fungal cell wall.

Keywords: crude metabolite, inhibition, light microscopy, lysis and vacuolations

1. Introduction

Endophytic microorganisms have highly impressive features such as luxurious biodiversity and unique biosynthetic pathways. They have been reported to produce so many number of bioactive secondary metabolites which helpful in the management of plant diseases caused by plant pathogenic fungi and using of these endopytic bacteria to manage the plant diseases is ecofriendly approach. Gong *et al.* (2006) [9] extracted the five antifungal compounds from endophytic *Bacillus* strain PY-1, which are isomers of iturin A: A2, A3, A4, A6 and A7. Zin *et al.* (2007) [35] extracted crude antibiotics from potential *Streptomyces* spp. by using different solvents such as ethyl acetate, methylene chloride and n- butanol. They tested the bio efficacy of crude extracts against several plant pathogens such as *P. ultimum*, *S. sclerotiorum*, *R. solani*, *Phytophthora erythroseptica*, *F. solani* and *A. fumigatus* by agar well diffusion test. The crude extracts showed 10-85 % inhibition against all the tested pathogens.

Atta *et al.* (2009) [4] extracted crude antibiotic compounds from *Streptomyces cyaneus* with the help of n-butanol and pH was adjusted to 7. The antimicrobial compound was precipitated by petroleum ether at 5000 rpm for 15 minutes. Atta *et al.* (2011) [5] extracted the crude antibiotics from *Streptomyces* spp. AZ-NIOFD1T by using ethyl acetate solvent. N-butanol and ethyl acetate extract of the *Streptomyces* spp. culture broth supernatant inhibited the growth of several microorganisms.

Ara *et al.* (2012) [2] extracted secondary metabolite from actinomycetes by using methanol solvent. Whole culture broth and crude extract of actinomycetes were collected and tested for the antifungal activity against *F. oxysporum* and *A. alternate* by agar well diffusion test. The culture filtrate and crude extract significantly inhibited the growth of both the pathogens. The antimicrobial substances were extracted using methanol from modified nutrient glucose broth medium in which *Streptomyces* spp. Isolate had grown for five days at 28°C. The antifungal activity was assessed using broth microdilution technique against different plant pathogenic fungi. The lowest minimum inhibitory concentration of methanol fractions was 125, 61.5, 250, 500, 125 and 250 µg ml⁻¹ against *A. niger*, *Aspergillus flavus*, *B. cinerea*, *Curvularia lunata*, *Trycophyton rubrum*, and *Trycophyton mentagrophytes* respectively.

Islam *et al.* (2012) [10] extracted the crude antibiotic substance from the culture filtrate of *B. subtilis* isolate C9 by solvent extraction method with ethyl acetate and tested the antifungal activity against *R. solani* by agar well plate assay.

They observed maximum inhibition of mycelial growth by the crude extract (2 mg per well) 87.78 % compared with control (0 % inhibition). Yun-feng *et al.* (2012) [33] purified the antifungal substance from the broth culture of the *B. subtilis* B47 using acid precipitation, methanol extraction, and three-step chromatography. Based on FTIR spectrometry, amino acid composition, and MALDI-TOF-MS/MS CID analyses, they identified the antifungal substance as iturin A2, a cyclic lipopeptide antibiotic. Similarly, Nandhini *et al.* (2013) [18] observed that the potent actinomycetes were best grown in ISP-2 medium. ISP-2 media considered as suitable media for the extraction of crude compounds. Centrifugation was done for the extraction process, collected the supernatant and mixed with ethyl acetate. The extracted crude compound was dried at room temperature.

Pandey and Singh (2013) [19] extracted secondary metabolite by using chloroform and methanol as solvent. These solvents were chosen according to their checked polarity. Paul *et al.* (2013) [20] found that crude extracts of selected bacterial isolates showed antifungal activity against *B. cinerea* and among others the isolate CNU082111 performed strongest antifungal activity (inhibition zone >55mm) by paper disk method. Raja *et al.* (2013) [24] collected supernatant after centrifugation and mixed with equal amount of ethyl acetate. After shaking the sample, solvent phase was collected and evaporated. Solvent system chloroform: methanol: water was used and the Rf value was determined. Jadon *et al.* (2014) [11] found that starch casein nitrate broth was suitable for the growth of actinomycetes and production of bioactive compounds from actinomycetes. Solvent extraction method was used for the extraction of bioactive component from cultural filtrate.

Reetha *et al.* (2014) [25] extracted crude antibiotic metabolites from the bacterial biocontrol agents such as Bs 1, Bs 10, Pf 1 and Pf 2 by solvent extraction method and tested the bio-efficacy against *M. phaseolina* by inhibition zone technique (Paper disc method). They found that antibiotics produced by all the four potential bacterial isolates were effective against *M. phaseolina* and recorded reduction in growth of the pathogen ranged between 61.13 to 69.62 % over control. Kumar *et al.* (2015) [14] obtained crude antibiotic extracts from potential endophytic bacterial isolate by using different solvents such as hexane, chloroform, ethyl acetate, ethanol and methanol. After extraction they tested the bio-efficacy of crude extracts against *S. aureus*. Among them methanol and ethanol extracts showed antibacterial activity against tested pathogen with inhibition zone of 33 and 27 mm respectively. Kumari *et al.* (2015) [15] found that the extracts of bacterial metabolites in ethyl acetate inhibited the growth of *R. bataticola* in the range of 28.7-42.5 %. Smitha *et al.* (2015) [27] extracted crude antibiotics by ethanol extraction method from *B. subtilis* CaB5 isolate and tested their bio-efficacy against dry root rot (*R. bataticola*) and wilt (*F. oxysporum* f.sp. *ciceri*) causing pathogens of chickpea by agar well diffusion assay. The results indicated that crude antibiotics, at 50 µl volume inhibited the mycelial growth of *F. oxysporum* f.sp. *ciceri* by 41.6 % and *R. bataticola* by 40 %.

Muhsinin *et al.* (2016) [17] extracted secondary metabolites from two endophytic bacterial isolates by using ethyl acetate and tested the bio-efficacy against *S. aureus* by microdilution technique. Purwestri *et al.* (2016) [23] obtained bioactive compounds of endophytic bacteria from the fermentation medium using ethyl acetate extraction method. Ethyl acetate extract of IAK9 endophytic bacterial cells showed more

effective to inhibit *R. solani*, while n-hexane extract of IAK11 showed to inhibit more on *R. microporus* (Suryanto *et al.*, 2016) [29]. Ashan *et al.* (2017) extracted crude suspension of antibiotics by using different polar and non-polar solvents from *Streptomyces* strain KX852460. Among all, ethyl acetate extracted supernatant showed great potency against *R. solani* AG-3 KX852461. Ethyl acetate extracted antibiotic compounds exhibited antimicrobial activity against Gram positive, Gram negative bacteria and *C. albicans* MTCC 183 with the MIC ranging between 0.25 to 1 mg /ml (Chandrakar and Gupta, 2017) [7].

The metabolites of bacterial endophytes were extracted with methanol solvent, and the antimicrobial activity of the extracts were tested against human bacterial pathogens using agar disc diffusion method (Suresha and Jayashankar, 2019) [28].

Prapagdee *et al.* (2008) [21] observed morphological changes such as hyphal swelling and abnormal shapes in *C. gloeosporioides* or *S. rolfisii* fungi grown on potato dextrose agar that contained the culture filtrate of *Streptomyces hygroscopicus*. Kumari *et al.* (2015) [15] observed scanty growth with distorted cell surface morphology of *R. bataticola* in response to antagonistic effect of bacteria with the help of SEM analysis. Light microscopic examination of hyphal tips of antibiotic poisoned *F. oxysporum* f.sp. *ciceri* and *R. bataticola*. revealed shrunken, granulate and vesicular cytoplasm as compared to the hyaline, healthy cytoplasm of control hyphae (Smitha *et al.*, 2015) [27].

Jasim *et al.* (2016) [12] conducted light and electron microscopic analysis to see the antifungal characters of bacillomycin and surfactin antibiotics on the cell wall of *Pythium* and *Rhizoctonia* spp. From the light and electron microscopic analysis they observed hyphal lysis, vacuolations, cytoplasmic aggregations, disintegration of mycelium, shrinkage and breakages in hyphae. Purkayastha *et al.* (2018) [22] observed the significant ultrastructural changes of the hyphae like shriveling, breakage and desiccation of the pathogens (*P. aphanidermatum* and *R. solani*) under SEM when dual culture of pathogen with endophytic bacterial isolates. Zhao *et al.* (2018) [34] observed morphological abnormal changes in *P. sojae* mycelia when dual cultured with *Acinetobacter calcoaceticus* DD161 such changes include fracture, lysis, formation of a protoplast ball at the end of hyphae, and split ends.

Material and methods

Extraction of Metabolic Substances by Solvent Extraction Method

The potential root endophytic bacterial isolate CREB 37 (*B. subtilis*) was grown in one litre of modified Luria broth at 30°C for 96 hrs under continuous agitation in a rotary shaker with agitation speed of 160 rpm. Then, cell free culture filtrate was obtained by passing the bacterial broth through the bacterial proof filters. Crude metabolic substance was recovered from the cell free culture filtrate by solvent extraction with ethyl acetate. Ethyl acetate was added to the culture filtrate at ratio of 3:1 (v/v) in separating funnel and shaken vigorously for 1 hr. To obtain a crude extract, the organic layer was collected in a separate conical flask and evaporated to dryness in a vacuum rotary evaporator at 40°C (Islam *et al.*, 2012) [10].

2.2 Evaluation of Antifungal Activity of Crude Metabolite Extract by Agar Well Diffusion Technique

To test the antifungal activity of crude metabolite extract by agar well diffusion technique, different concentrations such as 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5 μ l were prepared by dissolving it in ethyl acetate solvent and poured into the wells (4 mm in dia made by cork borer) made in the DPDA agar plates in equal distance. Inoculated the virulent pathogen isolate (CRb 9) mycelia disc (6 mm) in the centre of the plate, then sealed the plates with paraffin film and the plates were incubated at 28°C for considerable time. Each concentration was replicated thrice (Islam *et al.* 2012) [10]. The inhibitory activity of each concentration was expressed as the per cent growth inhibition, compared with control ('0' concentration, solvent only used in the wells and or sterile distilled water), accordingly to formula given below.

Per cent inhibition (PI) = $C-T/C \times 100$

Where,

C = Growth of test pathogen in the control plate (cm)

T = Growth of test pathogen in the test plate (cm)

2.3 Studies on mechanistic effect of crude metabolite extract on phytopathogenic fungi by phase contrast and scanning electron microscopic analysis

For this study, both light and electron microscopic analysis was done on treated as well as control mycelia of test pathogen. For light microscopy, the mycelial changes were observed under binocular microscope. For scanning electron microscopy (SEM), hyphal samples were fixed in 2.5 % glutaraldehyde at 4°C for 2 hrs and washed 15 min in phosphate buffered saline (PBS) for four times. Then, dehydrated in gradient of ethanol for 10 min and air dried

afterwards. SEM analysis was conducted at 20 KV with a magnification of x3700 (Jasim *et al.*, 2016) [12].

Results

3.1 Extraction of Crude Metabolite Substances from Potential Endophytic Bacterial Isolate CREB 37 (*B. subtilis*) And Evaluation of Their Antifungal Activity Against *R. bataticola* In Vitro

Crude metabolite extract was obtained from potential endophytic bacterial isolate CREB 37 (*B. subtilis*) by following the procedure given in material and methods section. Total of 0.9 g of yellow-brown colour crude extract was obtained per litre of cell free culture filtrate.

Different concentrations of crude extract were prepared by dissolving in ethyl acetate and the antifungal activity was evaluated by agar well diffusion assay according to the procedure given in material and methods. The inhibitory activity of each concentration was expressed as the per cent growth inhibition. Each concentration was replicated thrice. The data was analysed using CRD and the results were presented in Table 1 and Plate 1.

Among the different concentrations tested, significantly higher inhibition was recorded at 100 μ l (77.23 %) followed by at 90 μ l (65.00 %), 80 μ l (46.11 %), 70 μ l (21.11 %) and 60 μ l (15.56 %). Least (0.00 %) was recorded at 0, 10, 20, 30, 40, 50 μ l and control (distilled water).

Based on the above results among the concentrations tested, maximum antifungal activity was recorded at 100 μ l with 77.23% inhibition followed by 90 μ l (65.00%), 80 μ l (46.11%), 70 μ l (21.11%) and 60 μ l (15.56%). Remaining all the concentrations including control exhibited no inhibition.

Table 1: *In vitro* evaluation of antifungal efficacy of crude antibiotic extract against *R. bataticola* by agar well diffusion assay

S. No	Crude extract concentrations (μ l)	Mycelial growth (cm) dia.	Inhibition (%)	Inhibition zone (cm)
1.	0	4.50 ^a	0.00 ^f (0.00) (1.00)	0.00 ^f (1.00)
2.	10	4.50 ^a	0.00 ^f (0.00) (1.00)	0.00 ^f (1.00)
3.	20	4.50 ^a	0.00 ^f (0.00) (1.00)	0.00 ^f (1.00)
4.	30	4.50 ^a	0.00 ^f (0.00) (1.00)	0.00 ^f (1.00)
5.	40	4.50 ^a	0.00 ^f (0.00) (1.00)	0.00 ^f (1.00)
6.	50	4.50 ^a	0.00 ^f (0.00) (1.00)	0.00 ^f (1.00)
7.	60	3.80 ^b	15.56 ^c (23.20) (4.06)	0.70 ^c (1.30)
8.	70	3.55 ^c	21.11 ^d (27.33) (4.70)	0.95 ^d (1.40)
9.	80	2.43 ^d	46.11 ^e (42.75) (6.86)	2.08 ^c (1.75)
10.	90	1.58 ^e	65.00 ^b (53.71) (8.12)	2.93 ^b (1.98)
11.	100	1.03 ^f	77.23 ^a (61.48) (8.84)	3.48 ^a (2.12)
12.	Control (Ethyl acetate)	4.50 ^a	0.00 ^f (0.00) (1.00)	0.00 ^f (1.00)
	C.D (P=0.05)	0.07	1.62 (1.07) (0.14)	0.07 (0.02)
	SEm (\pm)	0.03	0.56 (0.37) (0.05)	0.03 (0.01)
	C.V.	1.39	6.01 (4.26) (2.95)	6.01 (1.20)

3.2 Studies on Mechanistic Effect of Crude Metabolite Extract on Phytopathogenic Fungi by Phase Contrast and Scanning Electron Microscopic Analysis

The microscopical analysis of the crude antibiotic extract treated mycelia of the *R. bataticola* revealed its effect on the fungal morphology

(Plate 2 and 3). Light microscopic analysis revealed that lysis of hyphae, swelling (Bulging), cytoplasmic aggregation, breakage, bulged hyphal tips, vacuolation, shrinkage and severe mycelial destruction. The SEM images clearly showed the disruption of the fungal hyphae it is clearly distinguishable from that of control.

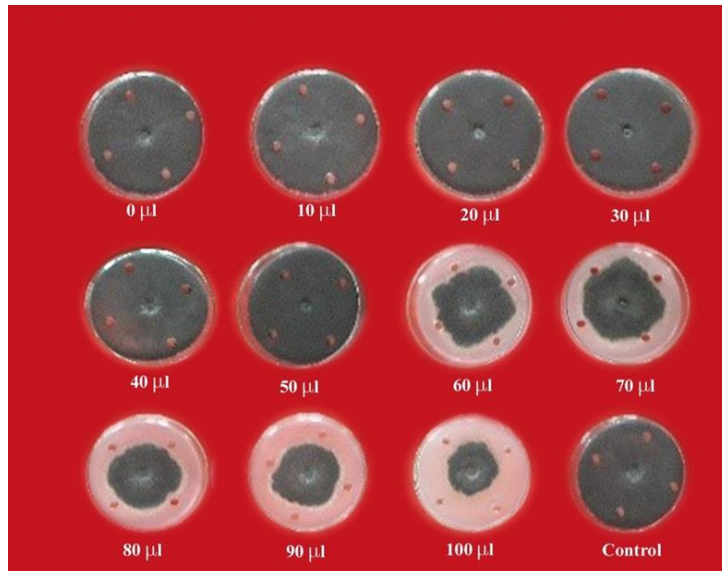
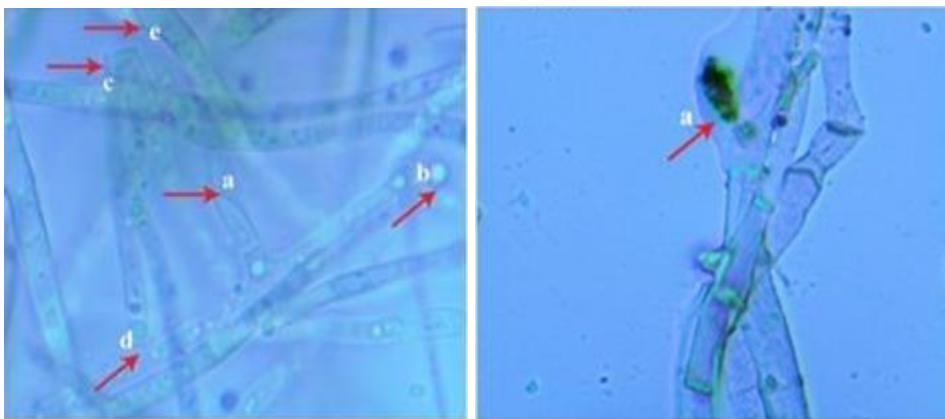
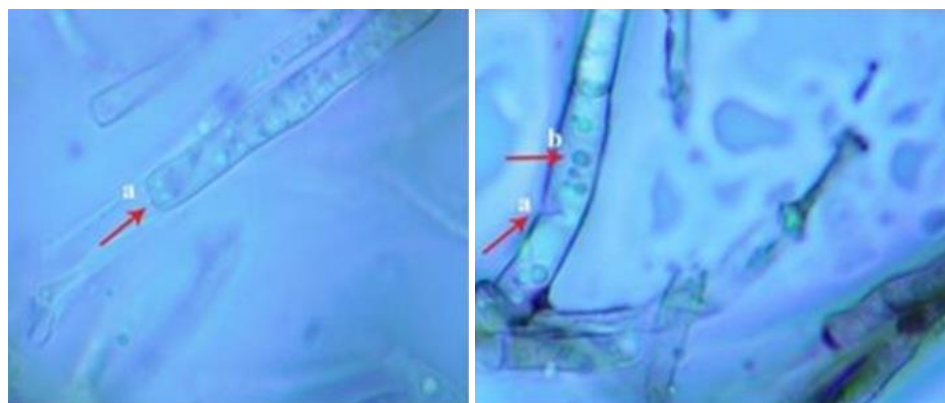


Plate 1: Testing the efficacy of crude antibiotic extract by agar well diffusion assay



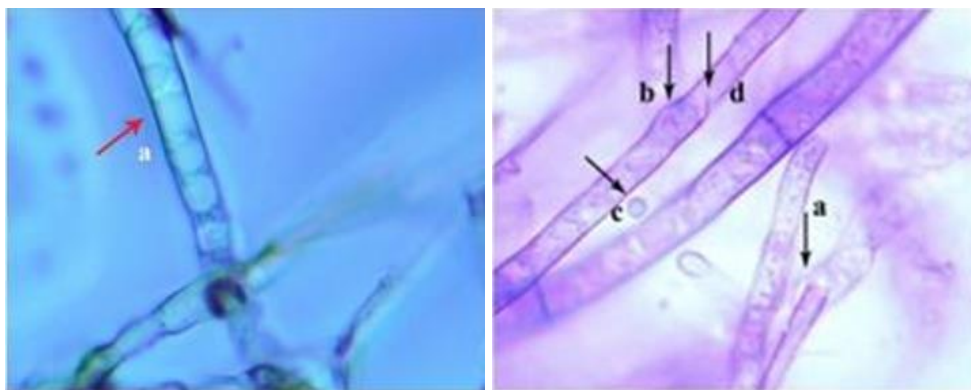
- a.** Broken hyphae
- b.** Coming out of vacuoles from broken hyphae
- c.** Shrinkage of hyphae
- d.** Completely broken hyphae
- e.** Bulging of hyphae

- a.** Cytoplasm came out from broken hyphae



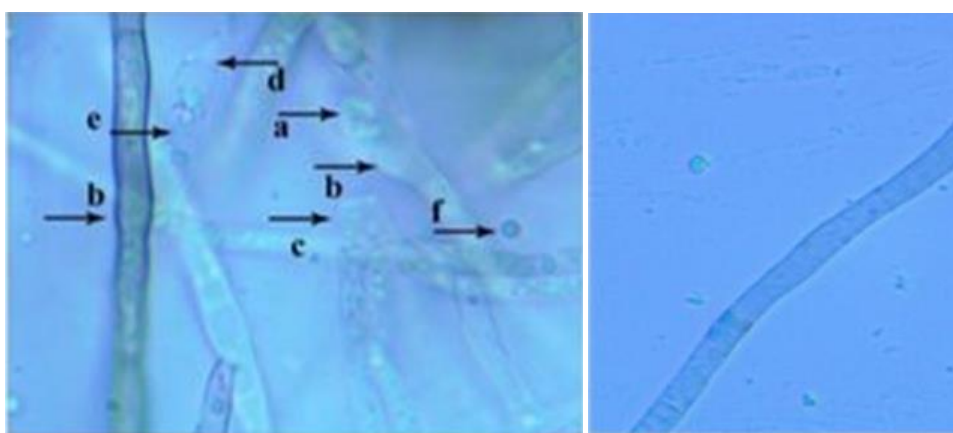
- a.** Cytoplasm and vacuoles came out from broken hyphae and emptied hyphae

- a.** Breake in hyphae
- b.** Vacuolation in hyphae



a. Cytoplasmic aggregations in hyphae

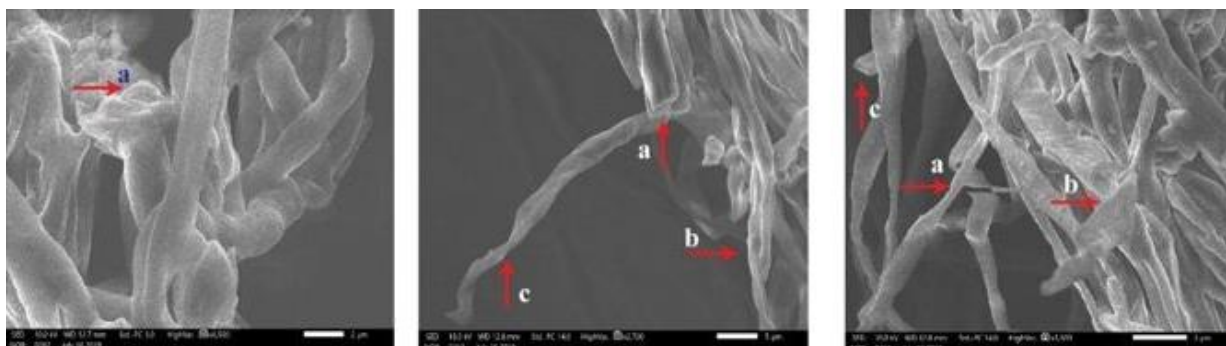
a. Completely broken hyphae
b. Bulging of hyphae
c. Coming out of vacuoles from broken hyphae
d. Break in hyphae



a. Bulged rounded tip
b. Hyphal swelling
c. Shrunken hyphae
d. Completely broken hyphae
e. Vacuolation in hyphae
f. Coming out of vacuoles from broken hyphae

a. Healthy control mycelium

Plate 2: Studies on mechanistic effect of crude antibiotic compounds on pathogen (*R. bataticola*) by phase contrast microscopic analysis



a. Hyphal aggregation

a. Broken hyphae
b. Lysed hyphae
c. Twisted hyphae

a. Lysed hyphae
b. Shriveled hyphae
c. Broken hyphae

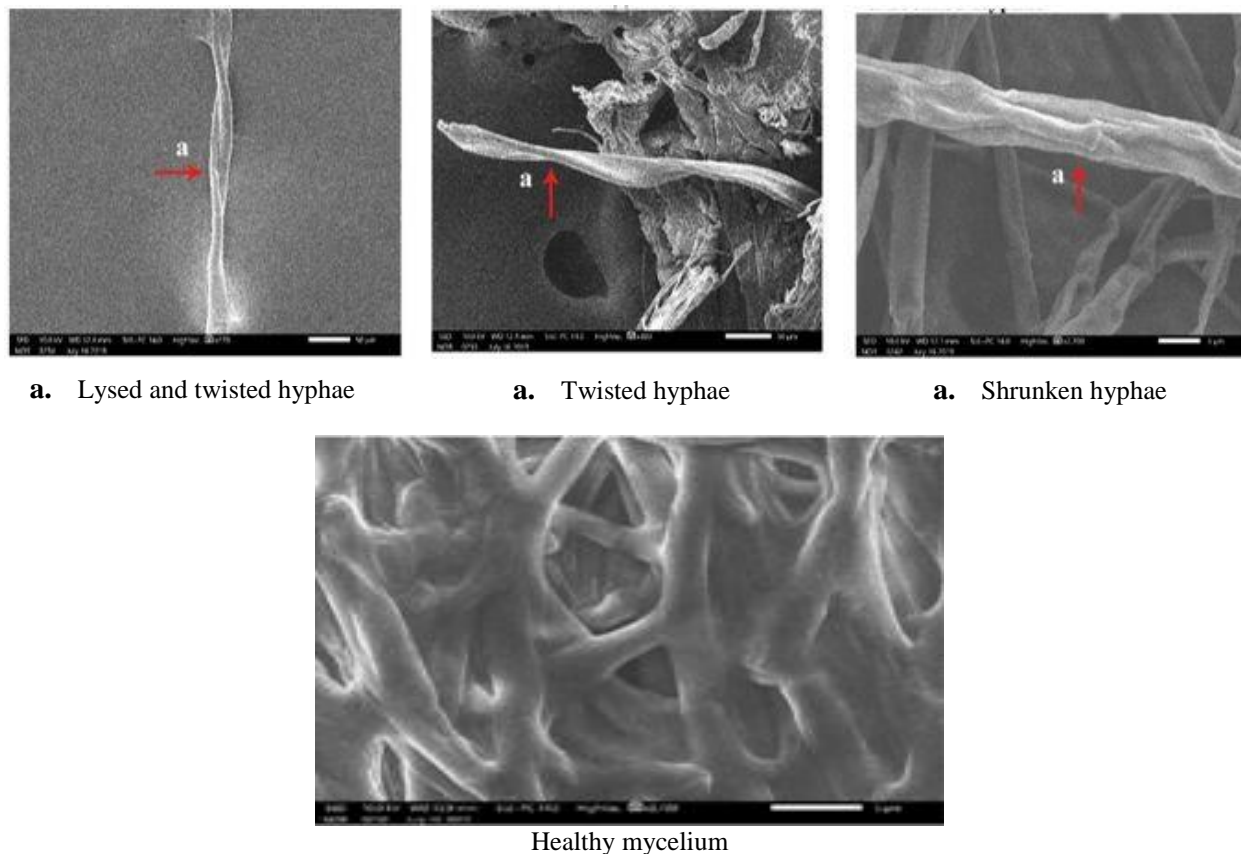


Plate 3: Studies on mechanistic effect of crude antibiotic compounds on pathogen (*R. bataticola*) by scanning electron microscopic analysis

4. Discussion

4.1 Extraction of Crude Metabolite Substances from Potential Endophytic Bacterial Isolate CREB 37 (*B. subtilis*) And Evaluation of Their Antifungal Activity Against *R. bataticola* In Vitro

At the 100 μ l concentration of crude antibiotic extract maximum inhibition in the pathogen growth was observed. The results are in agreement with the findings of Ali and Rante (2018) [1] who extracted crude antibiotic substances from potential endophytic bacterial isolates by using three different solvents such as ethyl acetate, chloroform and n-butanol and tested their efficacy against three test fungi such as *Fusarium oxysporum* KFCC 11363P, *Trichoderma reesei* NBRC 31329 and *Candida albicans* ATCC 900 by agar well diffusion technique. The ethyl acetate extract showed antifungal activity while weak to no detect by chloroform and n-hexane extract. Similarly, Ethyl acetate extract of IAK9 endophytic bacterial cells were more effective to inhibit *R. solani*, while n-hexane extract of IAK11 inhibited more on *R. microporus* (Suryanto *et al.*, 2016) [29].

The results are also accordance with findings of Smitha *et al.* (2015) [27] who extracted crude antibiotics by ethanol extraction method from *B. subtilis* CaB5 isolate and tested their bio efficacy against dry root rot (*R. bataticola*) and wilt (*F. oxysporum* f.sp. *ciceri*) causing pathogens of chickpea by agar well diffusion assay. The results indicated that crude antibiotics, at 50 μ l volume inhibited the mycelial growth of *F. oxysporum* f.sp. *ciceri* by 41.6 % and *R. bataticola* by 40 %. The results are also in line with the findings of Zin *et al.* (2007) [35], Islam *et al.* (2012) [10], Reetha *et al.* (2014) [25], Paul *et al.* (2013) [20], Chandrakar and Gupta (2017) [7] and Lal and Debnath (2018) [16].

4.2 Studies on Mechanistic Effect of Crude Metabolite Extract on Phytopathogenic Fungi by Phase Contrast and Scanning Electron Microscopic Analysis

Similar results were also obtained by Boer *et al.* (1998) [6] and Singh *et al.* (1999) [26] and who reported that chitinase and β -1,3-glucanase enzymes are able to lyse fungal cell walls and responsible for the suppression of fungal growth. Whereas, Getha and Vikineswary (2002) [8] reported that the antifungal antibiotics of *S. violaceusniger* G10 showed *in vitro* antagonistic effects against *F. oxysporum* f.sp. *cubense*, such as hyphal swelling and the inhibition of spore germination.

The present findings were in line with the studies of Wang *et al.* 1999 [32] who reported that the culture filtrate of *P. aeruginosa* K-187 causes growth aberration, hyphal swelling, and lysis of many fungi due to its high content of chitinase enzyme. According to Joo (2005) [13], *Streptomyces halstedii* AJ-7 produces extracellular chitinase and causes abnormal hyphal morphology. While, abnormal hyphal structures such as thickness and bulbous roundedness of the inhibited fungal hyphae resulting from diffusible secondary compounds have been previously reported (Taechowisan *et al.*, 2005) [30].

The present results were also in agreement with the findings of Purkayastha *et al.* (2018) [22] who observed that the nature of antagonism involved in the interaction between ETR 17 bacterial isolate and *R. solani* by scanning electron microscopy. The fungal mycelium at the interaction zone in dual culture was distorted, showing bulging, lysis and bursting of the hyphae. Abnormal hyphal structures caused due to cell wall degradation were prominent. Zhao *et al.* (2018) [34] observed morphological abnormal changes in *P. sojae* mycelia when dual cultured with *Acinetobacter calcoaceticus* DD161 such changes include fracture, lysis, formation of a protoplast ball at the end of hyphae, and split

ends. The SEM studies of interaction zone showed significant ultrastructural changes of the hyphae like shriveling, breakage and desiccation of the pathogens (*P. aphanidermatum* and *R. solani*) by PGPR *B. cereus* (RBac- DOB-S24) and endophyte *P. aeruginosa* (BacDOB-E19) (Vinayarani and Prakash, 2018) [31].

Summary and Conclusion

Total of 0.9 g of yellow-brown colour crude extract was obtained per litre of cell free culture filtrate. Different concentrations of crude extract were prepared by dissolving in ethyl acetate and the antifungal activity was evaluated by agar well diffusion assay. Among the different concentrations tested significantly higher inhibition was recorded at 100 µl (77.23 %) and least (0.00 %) was recorded at 0, 10, 20, 30, 40, 50 µl and control (distilled water).

The microscopical examination of the crude antibiotic extract treated mycelia of the *R. bataticola* revealed its effect on the fungal morphology. Light microscopic analysis revealed that severe mycelial destruction, shrinkage, lysis of hyphae, swelling (Bulging), cytoplasmic aggregations, breakages, bursting and releasing of vacuoles and cytoplasm, bulged hyphal tips and severe vacuolation. The SEM images clearly explained the disruption of the fungal hyphae in the case of the treated samples due to the mechanistic effect of antibiotic compounds on fungal cell wall and were clearly distinguishable from that of control where there was no visible disruption of the fungal cell wall.

In conclusion, the antifungal activity of crude metabolite extract was found to have mechanistic effect on cell wall of *R. bataticola* as observed by light and electron microscopic analysis. The occurrence of different target antifungal mechanisms of *B. subtilis* makes it highly attractive to be exploited for biocontrol applications.

References

1. Ali A, Rante H. Screening of endophytic bacteria producing antifungal isolated from Indonesia medicinal plant, java ginseng (*Talinum Triangulare*) (Jacq.) Willd. International Journal of Pharmacy and Pharmaceutical Sciences 2018;10(6):152-158.
2. Ara I, Bukhari N, Perveen K, Bakir M. Antifungal activity of some actinomycetes isolated from Riyadh soil, Saudi Arabia: An evaluation for their ability to control *Alternaria* caused tomato blight in green house pot trial. African Journal of Agricultural Research. 2012;7(13):2042-2050.
3. Ashan T, Chen J, Zhao X, Irfan M, Wu Y. Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by *Streptomyces* strain KX852460 for the biological control of *Rhizoctonia solani* AG-3 strain KX852461 to control target spot disease in tobacco leaf. AMB Express 2017;7(54):1-9.
4. Atta HM, Dabour SM, Desoukey SG. Sparsomycin Antibiotic Production by *Streptomyces* Sp. AZ-NIOFD1: Taxonomy, Fermentation, Purification and Biological Activities. American-Eurasian Journal of Agricultural and Environmental Sciences 2009;5(3):368-377.
5. Atta HM, El-Sehrawi MH, Awany NM, El-Mesady NI. Cirramycin-B antibiotic production by *Streptomyces cyaneus*-AZ-13Zc: fermentation, purification and biological activities. New York Science Journal. 2011; 4(2):35-42.
6. Boer DW, Gunnewiek PJA, Lafeber P, Janse JD, Spit

7. BE, Woldendorp JW. Antifungal properties of chitinolytic dune soil bacteria. Soil Biology and Biochemistry 1998;30:193-203.
7. Chandrakar S, Gupta AK. Production and characterization of actinomycin D from *Streptomyces parvulus* isolated from *Aloe vera* (L.) Burm. F. and its antimicrobial activity. International Journal of Pharmaceutical Sciences Review and Research 2017;46(1):169-175.
8. Getha K, Vikineswary S. Antagonistic effects of *Streptomyces violaceusniger* strain G10 on *Fusarium oxysporum* f. sp. *cubense* race 4: Indirect evidence for the role of antibiosis in the antagonistic process. Journal of Industrial Microbiology and Biotechnology 2002;28:303-310.
9. Gong M, Wanq JD, Zhanq J, Yang H, Lu XF, Pei Y *et al.* Study of the antifungal ability of *Bacillus subtilis* strain PY-*In vitro* and identification of its antifungal substance (Iturin A). Acta Biochem Biophys Sinica 2006;38(4):233-40.
10. Islam R, Joeng TY, Lee YS, Song CH. Isolation and Identification of antifungal compounds from *Bacillus subtilis* C9 inhibiting the growth of plant pathogenic fungi. Mycobiology 2012;40(1):59-66.
11. Jadon R, Pandey S, Singh V. Screening of potential actinomycetes isolates against selective pathogenic microorganism. International Journal of Advanced Engineering Science and Technological Research 2014;2(1):631-642.
12. Jasim B, Benny R, Sabu R, Mathew J, Radha Krishnam EK. Metabolite and mechanistic basis of antifungal property exhibited by endophytic *Bacillus amyloliquefaciens* BMB 1. Applied Biochemistry and Biotechnology 2016;7:1-15.
13. Joo GJ. Production of an anti-fungal substance for biological control of *Phytophthora capsici* causing hytophthora blight in red-peppers by *Streptomyces halstedii*. Biotechnology Letters 2005;27:201-205.
14. Kumar AG, Antony AR, Kannan VR. Exploration of endophytic microorganisms from selected medicinal plants and their control potential to multi drug resistant pathogens. Journal of Medicinal Plants Studies 2015;3(2):49-57.
15. Kumari P, Khanna V, Sharma P, Kumari S. Metabolites in control of *Rhizoctonia bataticola*. Journal of Pure and Applied Microbiology 2015;9(1):749-756.
16. Lal M, Debnath M. Recent advances in an antibacterial and antifungal natural product. International Journal of Pharmaceutical Sciences Review and Research 2018;48(2): 33-37.
17. Muhsinin S, Budiarto RM, Mulyani LN. Isolation of endophytic bacteria from plant basil (*Ocimum sanctum* L.) as antibacterials against *staphylococcus aureus*. Journal of Innovations in Pharmaceutical and Biological Sciences 2016;3(4):92-96.
18. Nandhini R, Usha ST, Selvam M. Bioactive compounds produced by *Streptomyces* strain. International Journal of Pharmaceutical Sciences 2013;2:176-178.
19. Pandey A, Singh A. A comparative study on secondary metabolites producing microbes isolated from rhizospheric & non-rhizospheric region of *Azadirachta indica* and *Oscimum tenuiflorum*. International Journal of Pharmaceutical Research and Allied Sciences 2013;2(1):36-48.

20. Paul NC, Ji SH, Deng JX, Yu SH. Assemblages of endophytic bacteria in chili pepper (*Capsicum annuum* L.) and their antifungal activity against phytopathogens *in vitro*. *Plant Omics Journal* 2013;6(6):441-448.
21. Prapagdee B, Kuekulvong C, Mongkolsuk S. Antifungal potential of extracellular metabolites produced by *Streptomyces hygroscopicus* against phytopathogenic fungi. *International Journal of Biological Sciences* 2008;4(5):330-337.
22. Purkayastha GD, Mangar P, Saha A, Saha D. Evaluation of the biocontrol efficacy of a *Serratia marcescens* strain indigenous to tea rhizosphere for the management of root rot disease in tea. *PLOS One* 2018;13(2):1-27.
23. Purwestri YA, Kartikasari N, Putri SG, Wilson W, Sembiring L. Metabolic profiling of endophytic bacteria from purwoceng (*Pimpinella pruatjan* Molkend) root and antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *AIP Conference Proceedings*, 2016, 1-7.
24. Raja A, Seetha S, Sahana V. Preliminary studies on antifungal activity of actinomycetes isolated from ice point. *International Journal of Advanced Research* 2013;1(8):17-22.
25. Reetha AK, Pavani SL, Mohan S. Hydrogen Cyanide production ability by bacterial antagonist and their antibiotics inhibition potential on *Macrophomina phaseolina* (Tassi.) Goid. *International Journal of Current Microbiology and Applied Sciences* 2014;3(5):172-178.
26. Singh PP, Shin YC, Park CS, Chung YR. Biological control of *Fusarium* wilt of cucumber by chitinolytic bacteria. *Phytopathology* 1999;89:92-99.
27. Smitha KP, Mohan R, Devadason A, Raguchander T. Exploiting novel rhizosphere *Bacillus* species to suppress the root rot and wilt pathogens of chickpea. *African Journal of Microbiology Research* 2015;9(15):1098-1104.
28. Suresha S, Jayashankar M. Antimicrobial activity of endophytic bacteria isolated from few plants of Muthathi wildlife sanctuary Mandya, Karnataka. *International Journal of Pharmaceutical Sciences Review and Research* 2019;10(5):2523-2527.
29. Suryanto D, Yeldi N, Munir E. Antifungal activity of endophyte bacterial isolates from torch ginger (*Etilingera elicitor* (Jack.) RM Smith)) root to some pathogenic fungal isolates. *International Journal of PharmTech Research* 2016;9(8):340-347.
30. Taechowisan T, Lu C, Shen Y, Lumyong S. Secondary metabolites from endophytic *Streptomyces aureofaciens* CMUAc130 and their antifungal activity. *Microbiology* 2005;151:1691-1695.
31. Vinayarani G, Prakash HS. Growth promoting rhizospheric and endophytic bacteria from *Curcuma longa* L. as biocontrol agents against rhizome rot and leaf blight diseases. *Plant Pathology Journal* 2018;34(3):218-235.
32. Wang SL, Yieh TC, Shih IL. Production of antifungal compounds by *Pseudomonas aeruginosa* K-187 using shrimp and crab shell powder as a carbon source. *Enzyme and Microbial Technology* 1999;25:142-148.
33. Yun-feng Y, Qin LQ, Gang FU, Gao-qing Y, Jian-hua M, Wei L. Identification of antifungal substance (Iturin A2) produced by *Bacillus subtilis* B47 and its effect on southern corn leaf blight. *Journal of Integrative Agriculture* 2012;11(1):90-99.
34. Zhao L, Xu Y, Lai X. Antagonistic endophytic bacteria associated with nodules of soybean (*Glycine max* L.) and plant growth-promoting properties. *Brazilian Journal of Microbiology* 2018;49:269-278.
35. Zin NM, Nurul IM, Sarmin, Ghadin N, Dayang F, Basri. Bioactive endophytic streptomycetes from the Malay Peninsula. *Federation of European Microbiological Societies Microbiology Letters* 2007;274:83-88.