ABSTRACT

Streptomyces sp.of Actinomycetes thrives in the rhizosphere of plants with a wide range of effects on plants development and physiological processes and has a protective role against plant diseases. In order to develop wide spectrum of biocontrol agents from actinomycetes against *Fusarium udum,* a common *Cajan cajan* fungal pathogen, isolation, characterization, and antifungal activity of *Streptomyces* sp. from rhizospheric soil was studied.

A total of eight soil samples were collected from different places in the Gujarat region of India. Actinomycetes were isolated from the soil samples collected from the rhizosphere of *Cajanus cajan* plant in Lasundra, Vadodara, Gujarat, India, using standard protocols. Also, an established procedure was employed to extract and sequence the genomic DNA of the selected isolated Actinomycetes. Various biocontrol and plant growth-promoting (PGP) traits viz; phosphate solubilization, indole acetic production, siderophore production, and many more, were evaluated in the selected isolates using established protocols. The isolated actinomycetes were screened against the fungal pathogen of *C. cajan, Fusarium udum.* The antifungal compounds responsible for the acclaimed activity were identified, bioauthographed, and purified using TLC, LC-MS, ^{1}H -NMR, and FTIR. The optimization of the antifungal compounds present in the actinomycetes was carried out using response surface methodology (RSM) with a central composite design (CCD) according to a one-factor-at-a-time (OFAT)-based screening experiment. The biocontrol agent, *Streptomyces* sp. S-9 was developed into powder formulation to investigate its potential as a biocontrol and growth-promoting agent (PGA) *in vitro, in situ,* and *ex-situ.* Seed bacterization entailed the inoculation of *C. cajan* seeds in the prepared treatments of *Streptomyces* sp. S-9 and some selected known biocontrol agents of microbial and chemical origin and arbuscular mycorrhiza were studied to document its effect on the growth of *C, cajan*. The whole-genome sequence of *Streptomyces* sp. S-9 and transcriptomic analysis of healthy and *Fusarium*-infected *C. cajan* was carried out using standard protocols.

In our study, 165 actinomycetes from eight soil samples collected from different regions of Gujarat were isolated. The isolated actinomycetes were later reduced to three based on morphology and molecular characterization. The three isolates were identified using 16S rRNA amplification and sequenced. The sequences were deposited in GenBank (NCBI) with accession

numbers MK610729.1, MK158952.1, and MK610795.1. The molecular characterization based on the 16S rRNA indicated that all the isolates belong to the genus *Streptomyces.* The strains are plant growth-promoting agents based on their IAA and siderophore production attributes. All three isolates are also phosphate solubilizers. Using the scanning electron microscopy (SEM), four weeks old culture of *Streptomyces* sp. S-9 in ISP-3 media also affirmed that it belongs to the *Streptomyces* genus. The S-9 posed a serious threat to the morphology of *F. udum*, which indicates its biocontrol effectiveness. Bioautography of *Streptomyces* sp. S-9 via immersion TLC revealed that at Rf 0.46, a bioactive compound inhibited the growth of *F. udum.*

The solvent extract of *Streptomyces* sp. S-9 , after being subjected to LC-MS contained 2-(4-Chloro-3,5-dimethyl-1H-pyrazol-1-yl)-N-(3,5-difluoro-4-iodophenyl) acetamide, methyl (3S,4R)-4-methoxy-3-[(2S)-2-[5-[4-[4-[2-[(3R)-3-(phenylcarbamoyl)-2-bicyclo[2.2.1]heptanyl]-3H-pyrrol-4-yl]phenyl]phenyl]-1H-imidazol-2-yl]pyrrolidine-1-carbonyl]pentanoate, in ESI+, and (5E)-3-[(4-bromophenyl)methyl]-5-[[4-[(2,4-dichlorophenyl)methoxy]-3,5-diiodophenyl]methylidene]imidazolidine-2,4-dione and 3,3'-{4-[(3β,5α)-8-Methylcholestan-3-yl]-1,1-butanediyl}bis(5-chloro-6-hydroxybenzoic acid) in ES-.

In optimizing the bioactive constituents of *Streptomyces* sp. S-9, we found out that the nutrient sources must be enhanced to improve the production of bioactive compounds, especially the carbon and nitrogen sources. For optimal production of bioactive compounds, the concentration of Mannitol and glycine must be increased to 4.6g/L. The antifungal activity obtained using 4.6g/L mannitol and glycine was 37.50, the highest out of the 13 runs examined.

The *in vitro* antagonism assay of the powdered formulation revealed that at 100mg/ml, the formulation exhibited remarkable inhibition against *F. udum*, thus, a potential antifungal against Fusarium wilt infection of *Cajanus cajan.* The combination of *Streptomyces* sp. S-9 and *Rhizophagus irregularis* enhanced shoot and root lengths of *C. cajan* upon germination. Meanwhile, inoculation with *F. udum* did not enhance the root and shoot lengths. The treatment of *C. cajan* seeds with *Streptomyces* sp. S-9 reduced wilt incidence on the field. We found out that seed dressing using *Streptomyces* sp. S-9 resulted in better wilt control than chemical fungicide (Bavistin). The application of *Streptomyces* sp. S-9 as a biocontrol agent increases the average number of pods per plant, pod yield, and total grain yield of *C. cajan.*

The whole genome of *Streptomyces* sp. S-9 was submitted to NCBI with Bioproject ID PRJNA695540 and Biosample ID SAMN17616131 respectively. Our study found out that *Streptomyces sp.* S-9 that was isolated contains a genome size of 142393, GC content 72.60%, total genes 6872, non-coding gene, 86, rRNA genes 9, and tRNA 77. The phylogenetic analysis confirmed that S-9 belongs to the genus *Streptomyces*. MLST analyses were performed in effort to characterize and identify the isolate resulting in a new ST and it classified as ST93. The genome comparison with reference bacteria with validly published names available in GenBank and the PubMLST e-database indicated that the strains mainly were related to *Streptomyces hygroscpicus*.

The purpose of the experiment conducted is to see the changes in the expression of the same genotypes under two different conditions that is Control and infected with Fungus. A total of 1338 differentially expressed genes (DEGs) were recognized, comprising 584 up-regulated genes and 755 down-regulated genes. All raw data from the DGE library sequencing has been deposited in SRA (NCBI BioSample Accessions Number: SAMN20060467 and SAMN20060468).All clean reads were deposited in NCBI SRA metadata with accession numbers SRR15059311 and SRR15059312. The raw reads for all the Illumina sequenced transcriptome used for the analysis have been deposited to NCBI with the BioProject ID PRJNA743724. Clustering of the DEGs utilizing Kyoto Encyclopedia of Genes and Genomes (KEGG) indicated that transcripts associated with phenylpropanoid biosynthesis, transporters, transcription factors, hormone signal transduction, glycosyltransferases, exosome, and MAPK signaling might be involved in wilt conditions.