Summary

For every community to function properly there must be a system to ensure that every home has enough food to eat. Plant diseases, on the other hand, are making this difficult. Numerous fungal phytopathogens cause serious plant diseases and decrease agricultural production and quality. In addition to their ability to serve as biocontrol agents, actinomycetes can stimulate plant development. *Streptomyces* sp., the actinobacteria explored in this study, are well-known nutrient boosters, siderophore producers, and sulphate solubilizers. The present work aims to isolate *Streptomyces* sp. from the rhizosphere soil of *Cajanus cajan* grown in the Indian state of Gujarat and test them against *Fusarium udum*, the known causal agent of Fusarium wilt in *Cajanus cajan*.

Soil and sediment samples from various locations within Gujarat were collected to isolate actinomycetes therein. Also, rhizospheric soil samples of about two month's old pigeon pea from Lasundra, Vadodara were collected. A total of 165 actinomycetes from 8 soil samples were isolated and characterized. Three promising isolates were identified to species levels using 16S rDNA sequence analysis as *Streptomyces sp.* S-107, S-280, and S-9. The morphological characterization of the strains confirmed their status generically as *Streptomyces*. Morphologically, the isolates were gram-positive, filamentous, and branched. The sequences of the identified isolates were deposited in GenBank (NCBI) with accession numbers MK610729.1, MK158952.1, and MK610795.1.

The PGP potential of the three strains were carried out by investigating their phosphate solubilization potential, IAA production, siderophore production, ammonia, HCN, β-glucanase, and chitinase production. In addition to those, ACC deaminase assay was also carried out. From our study, S-9 had the highest zone of inhibition (34), compared to S-107 (32) and S-208 (24). The strains exhibited the potential for β-glucanase, p-solubilization, and IAA production. However, none of the strains produced chitinase, which may be because of the method used. The ACC deaminase was also verified, and S-9 exhibited the maximum ACC deaminase.

Streptomyces sp.S-9 was investigated for its antifungal efficacy against *Fusarium udum* using standard protocols. Scanning electron microscope was used to characterize the effect S-9 posed on *F. udum*morphology. The bioactive compounds responsible for the acclaimed activity of S-9 was identified and purified using high throughput tools, viz; TLC, LC-MS, ^{1}H

NMR, and FTIR. Phenotypically, there was morphological alterations on the hyphae of *F. udum* treated with the extract of *Streptomyces* sp.S-9 under SEM indicates that the bacterial extract had a remarkable effect on the growth and development of the fungus. This among others comfirms that S-9 could serve as a potential biocontrol agent for protecting pigeon pea against the wreckage of *Fusarium* wilt.

The extract of S-9 subjected to bioautography using TLC contained a bioactive compound at Rf 0.46 which was capable of inhibiting the growth of *F. udum*. The extract was also subjected to LC-MS analysis. Four novel compounds with prominent peaks were identified in ES+ and ES- of the chromatographic tool. The identified compounds may be respoinsible for the antifungal activity of S-9. The compounds so identifed were 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-. However, no prior information on the identified compounds as antifungal, but this study will serve as a basis for their isolation and eventual application in fungal disease management.

The media component that can produce the optimum amount of antifungal compounds present in the *Streptomyces* sp.S-9 was optimized statistically using the one factor at a time (OFAT) method via response surface methodology (RSM) with central composite design (CCD). However, we found out that the nutrient sources must be enhanced to enhance the production of bioactive compounds, especially the carbon and nitrogen sources. The optimal production, the concentration of mannitol and glycine must be increased to 4.6g/L. Using CCD, 13 runs were done to examine the interactions between mannitol and glycine to establish effective metabolite production.

The biocontrol agent, *Streptomyces* sp. S-9 was developed into powder formulation to investigate its potential as a biocontrol agent and growth promoting agent (PGA) in natural *in vitro, in situ,* and *ex-situ.* 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.*

Seed bacterization entailed the inoculation of *C. cajan* seeds in the prepared treatments of S-9 and some selected known biocontrol agents of microbial and chemical origin and arbuscular mycorrhiza. The bacterization had effects on the shoot and root length of the test plant after germination. The combination of 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 S-9 reduced wilt incidence on the field. We found that seed dressing using S-9 resulted in better wilt control than chemical fungicide (Bavistin). The application of S-9 as a biocontrol agent increases the average number of pods per plant, pod yield, and total grain yield of *C. cajan.*

Streptomyces sp.S-9 was also examined for compatibility with arbuscular mycorrhizal fungus (AMF), *Rhizophagus irregularis.* The S-9 stimulated germination and mycelial development from *R. irregularis* spores *in vitro* and *in situ,* Root colonization was observed in inoculated *C. cajan.* There was evidence of colonization by visualization of intra-radicular hyphae and arbuscular formation through the cortical cells.

There was increase in ethylene production and proline accumulation. Also, accumulation of $H_2O_2$and MDA increased significantly 72 hours after the inoculation of *F. udum.* However, in the PGA-treated plant, $H_2O_2$and MDA accumulation was relatively low indicating a reduction in blotch-induced oxidative damage.

The whole genome sequence of the isolated actinomycetes was carried out using standard protocol. Our study found out that *Streptomyces* specied 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 belong to genus *Streptomyces*. The genome comparison with reference bacteria with validly published names available in GenBank and the PubMLST e-database indicated that the strains were mostly 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