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Endophytic isolate *Streptomyces* sp. VSMKU1023 as an effective microbe for the resistance and sensitivity of heavy metal, fungicide and antibiotics

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Abstract
On ISP2 agar medium, endophytic actinobacteria were isolated from tomato stem tissues. The selected isolate was identified as *Streptomyces* sp. VSMKU1023, based on morphology, pigment production, physiological and biochemical analyses. In this study, the isolate VSMNKU1023 was evaluated for resistance and sensitivity to metals, fungicides, and antibiotics. Copper, iron, nickel, and zinc resistance was highest in VSMKU1023, whereas silver and cadmium resistance was lowest. In comparison to the control, it is extremely sensitive to arsenic, lead, mercury, and selenium. In comparison to control, *Streptomyces* sp. VSMKU1023 was extremely resistant to low concentrations of mancozeb but very sensitive to high concentrations. Antibiotics were also crucial for the management of several plant infections. For example, the selected isolate VSMKU1023 was showed to be highly resistant to streptomycin and kanamycin, moderately resistant to tetracycline and rifampicin, but less resistant to ampicillin and nalidixic acid than the control.

Keywords: Tomato stem tissues, *Streptomyces sp.* VSMKU1023, heavy metals, fungicide tolerance and antibiotic resistance/sensitivity

1. Introduction
Plants are continually exposed to many types of stress, both biotic and abiotic, which cause changes in their metabolism and functioning, lowering their productivity and resulting in huge agricultural production losses (Rejeb *et al*., 2014) [1]. Abiotic stress refers to environmental stresses that modify plant physiology and have a negative impact on plant development and productivity. In most plant species, growth and yield decreases are projected to be greater than 50% and 70%, respectively, severely harming all crops (Wang *et al*., 2003) [2]. Drought, salt, and heavy metal toxicity are all examples of abiotic stress.
Metals are naturally occurring soil elements that are essential for the growth and survival of all known life species. However, due to their persistence, non-degradability, and high levels of aggregation in living tissues, their elimination has become a pressing necessity in recent decades (Djinni and Djoudi, 2022) [3]. Heavy metal ions induce toxicity and cell damage in living creatures by causing oxidative stress, which affects cell organelles and causes biomolecule confirmational alterations (Tchounwou *et al*., 2012) [4]. Some metals can cause chromosomal changes (beryllium), growth rate suppression (antimony), cell lysis (silver), or enzyme inactivation (even at low concentrations) (arsenic). Nickel, chromium, copper, zinc, mercury, lead, and cadmium are just a few metals that are hazardous to people and the environment (El-Gendy and El-Bondkly, 2016, Ayangbenro and Babalola, 2017, Timkova *et al*., 2018) [5-7]. As a result of the increase and intensification of industrial operations such as chemical fertiliser use and mining, heavy metal pollution has become a severe health concern. Heavy metal contamination produces a wide range of symptoms and organ damage (Oztürk *et al*., 2014, Djinni and Djoudi, 2022) [8, 3]. New microorganism-based processes such as bioaccumulation and bio sorption have been presented as a feasible alternative to reduce the amount of heavy metals in the environment because existing technologies are ineffectual and expensive (Locatelli *et al*., 2016, Timkova *et al*., 2018) [9, 7]. Microorganisms adapt and become exceedingly metal resistant when exposed to polluted environments with varied amounts of heavy metal absorption (Saberi and Hassan, 2014) [10]. Because these bacteria share many behavioural and chemical characteristics, molecular-based techniques have recently been employed to precisely identify them (McTaggart *et al*., 2010, Rahdar *et al*., 2021) [11, 12].

Soil is a wonderful site to explore for environmentally significant chemicals to isolate and identify since it is a natural repository for bacteria and their antimicrobial compounds (Dancer, 2004) [13]. Actinobacteria are a large and diverse group of aerobic, filamentous, spore-forming, Gram positive bacteria belonging to the Actinomycetales order with a high metabolic adaptability, especially their rapid ability to colonise a wide range of substrates, making them good candidates for inorganic and organic compound bioremediation (Baz, 2017, Bankar and Nagaraja, 2018) [14, 15]. *Streptomyces* sp. is the most important group of soil bacteria for producing antibiotics of agronomic and clinical use, with *Streptomyces* producing around 6,000 chemicals (Kavitha *et al*., 2010, Hossain and Rahman, 2014, Harikrishnan *et al*., 2016) [16-18]. Because of their metal detoxifying methods, they have received a lot of interest as heavy metal bioremediations. *Streptomyces* is noted for its ability to produce a wide range of siderophores, which are necessary for bacterial growth (Park and Kim, 2002, Polti *et al*., 2014, Harikrishnan *et al*., 2014, Djinni and Djoudi, 2022) [19-21, 3]. Heavy metal-induced adaptive responses, such as resistance and keeping heavy metals at an optimal sub-toxic level, are instances of stress in this organism (Schmidt *et al*., 2005) [22]. Furthermore, when iron deficiency in the agriculture field, the majority of *Pseudomonas* sp, *Streptomyces* sp, and *Bacillus* spp produce siderophore to alleviate the iron production (Shanmugaiah *et al*., 2006, Harikrishnan *et al*., 2014, Shanmugaiah *et al*., 2008) [23, 21, 24]. Overuse of pesticides in modern agriculture has resulted in a plethora of problems, including pollution, environmental degradation, and the creation of resistant strains (Fox *et al*., 2007, Singh and Chhatpar, 2011, Shanmugaiah *et al*., 2010) [25-27]. In agricultural fields, integrated pest management (IPM) by microorganisms like Fluorescent pseudomonads, *Streptomyces* spp, *Bacillus* spp and *Trichoderma* sp has evolved as a cost-effective and ecologically acceptable alternative to utilising chemical pesticides to treat plant diseases (Nithya *et al*., 2020, Harikrishnan *et al*., 2016, Shanmugaiah *et al*., 2009) [28, 18, 29]. In this context, we wanted to assess the biochemical features, heavy metal, fungicides and antibiotics tolerance and sensitivity of *Streptomyces* sp. VSMKU1023 from various habitats, in order to improve soil fertility.

2. Materials and Methods

2.1 Culture Conditions

The endophytic actinobacterial isolate VSMKU1023 was isolated from tomato stem tissues that was obtained from Madurai Kamaraj University, Department of Microbial Technology, School of Biological Sciences. *Streptomyces* sp. VSMKU1023 was cultivated for 5 to 7 days at 37 °C in ISP2 medium (International Streptomyces project 2 media, which contains dextrose – 4 gm/l, yeast extract – 4 gm/l, malt extract – 10 gm/l, and agar – 20 gm/l).

2.2 Morphological characterization of VSMKU1023

The selected endophytic isolated VSMKU1023 was identified based on Gram staining, culture morphology and growth characteristics on various media. Employing various mediums, such as spore shape, substrate and aerial hyphae, pigment generation, and colony characteristics on ISP medias (ISP-1, ISP-2, ISP-3, ISP-4, ISP-5, ISP-6, and ISP-7) were used to test growth response and chromogenesis, as well as potato dextrose agar, nutritional agar, bennet's medium, glucose medium, and Luriabertani agar medium, as listed in the table 1.

2.3 Biochemical characterization of isolate VSMKU1023

The identification of selected isolate was carried out by various biochemical tests according to Bergey's Manual of Determinative Bacteriology (Harikrishnan *et al*., 2014) [21]. The isolate VSMKU1023 morphology, physiochemical analysis were performed with slight modification methodology (Harikrishnan and Shanmugaiah, 2012) [30].

2.4 Hydrolytic enzymes and secondary metabolites production by VSMKU1023

The quality of hydrolytic enzymes such as gelatinase, amylase, cellulase, protease, pectinase and chitinase was tested by the method of (Shanmugaiah *et al*., 2008) [24]. On plates, enzyme assays were done, and the existence of a zone signified a favourable result. Phosphate solubilization and nitrate reduction were also investigated.

2.5 Effect of heavy metal resistance and sensitivity of VSMKU1023: The heavy metal resistance and sensitivity of VSMKU1023 was scrutinized by well diffusion method (Varatharaju *et al*., 2020) [31] with commercially available heavy metals such as arsenic, cadmium, copper, iron, lead, mercury, nickel, selenium, silver, and zinc at various concentrations. Before making the wells, the 5 day old culture of *Streptomyces* sp. VSMKU1023 was swabbed on the surface of the ISP2 agar and allowed to dry completely for 10 minutes. A sterile cork borer was used to create 9 mm wells on ISP2 medium. The above-mentioned heavy metals were then introduced in to wells with various quantities like 2, 4, 6, 8, and 10 mM/ml. At 30°C, the plates were incubated until bacterial growth was visible. The outcomes were monitored and documented at the end of the incubation period.

2.6 Fungicide resistance and sensitivity of VSMKU1023

By using the well diffusion method, fungicide resistance and sensitivity test for *Streptomyces* sp. VSMKU1023 was performed using commercially available fungicides such as carbendazim and mancozeb at various doses. Before making the well, the 5 days old *Streptomyces* sp. VSMKU1023 was swabbed on the surface of the ISP2 agar medium and allowed to dry completely for 10 minutes. A sterile cork borer was used to create 9 mm wells on ISP2. After that, different concentrations of fungicides like 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 mM/ml were added in to the wells. At 30 °C, the plates were incubated until *Streptomyces* sp. VSMKU1023growth was visible. The diameter of each zone was measured and recorded at the end of the incubation period. The diameter of the inhibitory zone was used to determine the sensitivity and resistance profiles, which were then evaluated.

2.7 Antibiotic resistance/sensitivity of VSMKU1023

By using the well diffusion method, antibiotic resistance/sensitivity of *Streptomyces* sp. VSMKU1023 was carried out using commercially available antibiotics such as Ampicillin, Kanamycin, Nalidixic acid, Rifampicin, Streptomycin and Tetracycline at different concentrations ranging from 10-80mM. Before making the well on ISP2, the 5 day old isolate VSMKU1023 was swabbed on the surface of the ISP2 agar medium and allowed to dry

completely for 10 minutes. A sterile cork borer was used to create 9 mm wells on ISP2 agar medium. After that, the antibiotics were introduced in to the wells with various concentrations like 10, 20, 30, 40, 50, 60, 70, and 80 mM/ml. At 30 °C, the plates were incubated until *Streptomyces* sp. growth was evident. The outcomes were monitored and documented at the end of the incubation period.

3. Results

3.1 Identification of selected isolate VSMKU1023

Our chosen isolate VSMKU1023 is gram-positive, non-motile, catalase oxidase, Indole, citrate, endospore, urease, and H2S generation are all positive reactions, but MR, VP, and nitrate reduction are all negative reactions. The synthesis of hydrolytic enzymes such protease, gelatinase, chitinase, and pectinase indicated a positive result. When cultivated in the appropriate medium, our isolate reacted positively to the majority of carbon and nitrogen sources. Furthermore, the selected isolate was recognised as *Streptomyces* sp. based on morphology, white to grey pigment production, physiological, and other biochemical studies (Table 1).

3.2 Heavy metal resistance / sensitivity of *Streptomyces* sp. VSMKU1023

Our strain of *Streptomyces* sp VSMKU1023 showed a wide range of resistance and sensitivity to heavy metals. Our isolate demonstrated great resistance to zing, iron, and copper, but modest resistance to silver nickel, and cadmium, when tested against ten distinct heavy metals. When compared to a control, the chosen isolate VSMNKU1023 was found to be particularly sensitive to arsenic, lead, mercury, and selenium (Table 2).

3.3 Fungicide resistant and sensitivity of *Streptomyces* sp. VSMKU1023

The chosen isolate *Streptomyces* sp. VSMKU1023 was found to be resistant to carbendazim and mancozeb at 100 and 200 Mm, but less resistant at 300 and 400 Mm, compared to the control. Our isolate VMKU1023, on the other hand, was extremely sensitive to the remaining concentrations of both fungicides. Tolerant to tested fungicides. Amongst the fungicides, against mancozeb culture showed maximum resistance till 600 mM of fungicide whereas, our strain can resist carbendazim to concentration of 400mM (Table 3).

3.4 Antibiotic resistance/sensitivity

Streptomyces sp. VSMKU1023, an endophytic isolate, was highly resistant to streptomycin and kanamycin, moderately resistant to tetracycline and rifampicin, but less resistant to ampicillin and nalidixic acid than the control strain (Table 4).

4. Discussion

Actinomycetes can be found in soil, water, decomposing plants, and animals, among other places (Harikrishnan *et al*., 2014, Rahdar *et al*., 2017, Rahdar *et al*., 2021) [21, 32, 12]. *Streptomyces* species are long filamentous, Gram-positive aerobic members of the Actinomycetales order, which belongs to the Actinobacteria class, with a DNA G-C deliberation of 69±78 mol%. *Streptomyces* spp are most frequent soil bacteria and they are capable for producing extracellular enzymes and antibiotic chemicals (Crawford *et al*., 1993, Singh and Chhatpar, 2011) [33, 26]. Plant growth can be helped by nitrogen fixation in the environment, mineral solubilization, and the production of siderophores or phytohormones by *Streptomyces* sp (Dimkpa *et al*., 2008, Chang and Yang, 2009, Harikrishnan *et al*., 2014) [34, 35, 21]. *Streptomyces* strains can thus act as both a biocontrol agent and a plant development promoter (Harikrishnan *et al*., 2014) [21]. In addition, their metabolic variety, mycelia growth habit, rapid growth rate, colonisation of semi-selective substrates, and genetic manipulation make them ideal for soil inoculation. Their ability to create desiccation-resistant spores also helps them disseminate, persist, and formulate, making them suitable biocontrol agents in different environmental conditions (Benimeli *et al*., 2007, Singh and Chhatpar 2011, Harikrishnan *et al*. 2014) [36, 26, 21]. In this study, the promising isolate *Streptomyces* sp. VSMKU1023, was investigated. Because the colony that grew on ISP2 agar plates was slow growing, aerobic, white to grey in colour, and contained different coloured aerial and substrate mycelia with diffusible pigment, The selected isolate was recognised as belonging to the genus *Streptomyces* sp. based on biochemical, physiological, hydrolytic enzyme synthesis, and carbon and nitrogen source utilisation. The spore chain is spiral, according to Atta *et al*., (2015) [37] analysis of the actinomycete isolate's identification, morphological features, and microscopic research. The previous analysis indicated that the spore mass is light grey on ISP-media, with a warty spore surface, light yellowish brown substrate mycelium, and diffusible pigment, this coherence with our report (Suneetha and Raj, 2011, Harikrishnan *et al*., 2014, Kurnianto *et al*., 2020) [38, 21, 39]. As according Islam *et al*., (2014) [40] microscopically observations and staining properties revealed that *Streptomyces* sp. were Gram positive and non-acid fast, having filamentous, branching, and coenocytic mycelia. Similar tests were performed on our *Streptomyces* sp. VSMKU1023, which was Gram positive and possessed filamentous and coenocytic mycelia. As previously proven, *Streptomyces* spp. were both catalase positive and responded well to nitrate reduction, as well as casine and starch hydrolyser (Islam *et al*. 2014) [40]. The methyl red test revealed that VSMKU1023 was negative. The utilization of carbon nitrogen sources were also play an important role for the characterization of a beneficial microbe to survive towards biotic and abiotic factors (Harikrishnan *et al*., 2016) [18]. The findings of morphological and biochemical examinations were used to make this decision. In this present study, isolate VSMKU1023 was actively utilized nutrient sources like lactose, fructose, potassium nitrate, sodium nitrate and ammonium sulphate (Table 1).

Heavy metal resistant bacteria are commonly found in contaminated locations and in soils that naturally contain high quantities of heavy metals. Previous research revealed that one of the *Streptomyces* strains could grow on 85.2mmol/l nickel, the highest nickel resistance ever discovered to our knowledge (Van Nostrand *et al*., 2007, Schmidt *et al*., 2009) [41, 22]. In our study indicated that highly resistance to zing, iron, and copper, but modest resistance to silver nickel, and cadmium, when tested against ten distinct heavy metals. When compared to a control. However, the chosen isolate VSMNKU1023 was found to be sensitive to arsenic, lead, mercury and selenium. In coincide with that of our results, the previous reports

were recorded that lead, mercury and cadmium are toxic in agricultural land even at low concentration. Further, heavy metal accumulation was noted that all the biochemical and physiological functions like C_3 and C_4, respiration and transpiration was entirely going to affect. Hence, the plant production and entire biomass has been reduced (Varatharaju *et al*., 2020) [31]. Heavy metals such as cadmium and selenium can be conveyed by gene transformation between microorganisms in a polluted environment, resulting in the transfer of sensitivity genes (Malik and Jaiswal, 2000) [42]. Cadmium accumulation on agricultural soil, on the other hand, disrupts the production of several hydrolytic enzymes.

Our forefathers have been using fungicides in our agricultural forms on a regular basis in recent years. To manage soil-borne plant diseases induced by biotic factors such as bacterial and fungal pathogens in vegetable cropping systems. Toxic fungicide residues can linger in soils for long periods of time, degrading beneficial microbes and potentially polluting agricultural land (Gámiz *et al*., 2017) [43]. In this continuation our current study notified that the selected endophytic isolate *Streptomyces* sp. VSMKU1023 was found to be resistant to carbendazim and mancozeb at 100 and 200 Mm, but less resistant at 300 and 400 Mm, compared to the control. Our isolate VMKU1023, on the other hand, was extremely sensitive to the remaining concentrations of both fungicides (Table 3). Aside from these research, the toxic effects of fungicides on radish growth and development, as well as whether or not fungicide-tolerant PGPR could attenuate such fungicidal toxicity, are yet unknown. As a result, we identified fungicide-tolerant PGPR and examined its fungicidal toxicity suppression and plant growth-promoting activities in white radish cropping to cover these information gaps (Khan *et al*., 2020) [44].

The overuse of chemical fungicides also puts native beneficial soil microbes' survival and physiological functions in jeopardy (Zhang *et al*., 2017) [45]. A group of free-living bacteria that colonise the rhizosphere and provide benefits to root physiology and growth (Shanmugaiah *et al*., 2015) [46]. Fungicides also have a negative impact on microbial respiration, biomass production, diversity, and function, as well as the function and composition of soil enzymes Ju *et al*., 2017, Singh, 2015) [47, 48]. Hexaconazole (HEXA), for example, has been found to have a negative impact on a variety of plant metabolic activities (Kengar and Patil, 2017) [49]. Furthermore, the use of fungicides on a regular basis has resulted in the emergence of resistance among fungal phytopathogens. As a result, we could maintain our environment and save our agriculture lands got healthy bacteria by reducing the use of fungicides in our agricultural plant growth and protection from various biotic causes.

Among six different antibiotics, our selected endophytic *Streptomyces* sp. VSMKU1023 was highly resistant to streptomycin and kanamycin, moderately resistant to tetracycline and rifampicin, but less resistant to ampicillin and nalidixic acid than the control strain (Table 4). According to our findings, microorganisms such as *Pseudomonas stutzeri* ST6 found to be resistant to heavy metals and antibiotics in different agricultural fields (Barbieri *et al*., 2001) [50]. In contrast to our findings Varatharaju *et al*. (2020) [31] showed *Pseudomonas* sp. VSMKU4035 resistance to tetracycline, moderate resistance to streptomycin and nalidixic acid, however less resistant to ampicillin. Despite this, when compared to control isolates, our isolate is extremely sensitive to rifampicin. Furthermore, antibiotic-resistant microbes were prevalent in the Indian ecosystem, particularly in agriculture and water bodies (Malik *et al*., 2002, Ansari *et al*., 2008) [51, 52]. We were able to determine the stability and viability of antagonistic microbes in the rhizosphere of various agriculture crops based on resistance and sensitivity.

Table 1: Biochemical characterization of *Streptomyces* sp. VSMKU1023

Biochemical Test	Characteristics
Gram`s staining	+
Catalase	+
Oxidase	+
Motility	Non motile
Indole	+
Methyl red	-
Voges proskaeur	-
Simmon citrate	+
Endospore	+
Urease	+
H_2S	+
Nitrate Reduction	-
Citrate	+
Casein	+
Starch	+
Gelatin	+
Chitin	+
Pectin	+
Sucrose	+
Lactose	+
Fructose	+
Potassium nitrate	+
Sodium nitrate	+
Ammonium sulphate	+

-; Negative, +; Positive

Table 2: Heavy metal resistance/ sensitivity of *Streptomyces* sp. VSMKU1023

S. No.	Heavy metal	*Streptomyces* sp. VSMKU1023 at different concentration (mM)				
		2	4	6	8	10
1	Arsenic	-	-	-	-	-
2	Cadmium	++++	+++	+	+	+
3	Copper	+	++	+++	++++	++++
4	Iron	+	+++	++++	++++	++++
5	Lead	-	-	-	-	-
6	Mercury	-	-	-	-	-
7	Nickel	-	+	++	+++	+++
8	Selenium	-	-	-	-	-
9	Silver	+	+	+	++	++
10	zinc	+++	+++	+++	++++	++++

-: Sensitive, +: Less resistance, ++: Moderate resistance, +++: High resistance, ++++: Very high resistance

Table 3: Fungicide tolerance of *Streptomyces* sp. VSMKU1023

Fungicide	Concentration (mM)	ZOI (cm)
Carbendizum	100	+++
	200	+++
	300	++
	400	+
	500	-
	600	-
	700	-
	800	-
	900	-
	1000	-
Mancozeb	100	+++
	200	+++
	300	++
	400	++
	500	+
	600	+
	700	-
	800	-
	900	-
	1000	-

-: Sensitive, +: Less resistance, ++: Moderate resistance, +++: High resistance

Table 4: Antibiotic susceptibility of *Streptomyces* sp. VSMKU1023

S. No.	Antibiotics	*Streptomyces* sp. VSMKU1023 at different concentration (mM)							
		10	20	30	40	50	60	70	80
1	Ampicillin	++	++	+	+	+	-	-	-
2	Kanamycin	++	+++	+++	+++	+++	+++	+++	+++
3	Nalidixic acid	++	++	++	+	-	-	-	-
4	Rifampicin	++	+++	++	++	+	-	-	-
5	Streptomycin	++	+++	+++	+++	++++	++++	++++	++++
6	Tetracycline	+++	+++	++	++	+	+	+	+

-: Sensitive, +: Less resistance, ++: Moderate resistance, +++: High resistance, ++++: Very high resistance

5. Conclusion

The development of metal and fungicidal toxic resistance bacteria is crucial for the protection of microbes and plants against numerous biotic and abiotic stimuli. As a result, endophytic *Streptomyces* spp could be a good bioremediation inoculum for metals, fungicides and antibiotics.

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