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Isolation of *Streptomyces rochei* from the rhizospheric soil of *Ocimum tenuiflorum* (tulsi) of Karad, Maharashtra, India, and its 16S rRNA based identification

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Abstract

A soil sample (T42.2) was collected from a rhizosphere of *Ocimum tenuiflorum* (tulsi) of Maharashtra, India and diluted to 10^{-2} after which, it was spread on Glycerol Aspargine Agar. The colony with actinobacteria like morpholoigical characters represented thread like structures with the cells attached to each others with a Gram Positive nature. Biochemical characters were catalase negative, starch and Triple Sugar Iron utilization positive. The culture was then processed for molecular identification based on their partial sequences of 16S ribosomal RNA genes which was identified as *Streptomyces rochei*. It showed 98.45% identity with partial 16S ribosomal rRNA gene sequence of *Streptomyces rochei* strain NRRI B-2410. Its evolutionary analysis was performed with Maximum Parsimony Method by constructing a phylogenetic tree showing its closest clade with JF304944.1:3-769 *Streptomyces* sp. BN-6. The study revealed that *Streptomyces rochei* has a habitat in the rhizosphere soil of *Ocimum tenuiflorum* (tulsi) for its further medical applications. Further, the study showed the need of increased species level identification data of *Streptomyces* genus in NCBI database to prevent misidentifications.

Keywords: *Streptomyces rochei*, *Ocimum tenuiflorum*, 16S rRNA, Maximum Parsimony Method, phylogenetic tree.

1. Introduction

Actinobacteria are present in the rhizosphere regions of soil, fresh water, and marine ecosystem. Gram positive actinobacteria have considerable potential of excellence of producing large quantity of the secondary metabolites which are bioactive in nature (Jose, P. A., Maharshi, A., & Jha, B. 2021). They have branching unicellular nature and they are well known for production of the antibiotics (Gopinath, B. V., & Singaracharya, M. A. 2012).

Some of them with a novel structure and a considerable biological activity are broadly applied in medicines and industry along with the agriculture (Uyeda, M., et al. 2001, Kurniawati, N., et al. 2016, Li. X., et al.2021a). The soils in the forest have organisms which show antifungal activity (Azish, M., et al. 2020). From the actinomycetes group, a genus known as *Streptomyces,* has a diverse range of metabolic abilities leading to their consideration as a bacteria of a higher value in industry as well as in natural environments (Kumar, M., et al. 2023). In addition, *Streptomyces* produce 80% of the antibiotics (Yun, T. Y. et al. 2018). Moreover, many bioherbicides, insecticides as well as antifungal compounds are produced by *Streptomyces* (Barka, E. A., et al. 2016).

The activity of *Streptomyces* against fungi was observed in many studies (Rey, T., & Dumas, B. 2017, Tamreihao, K., et al. 2018, Sholkamy, E. N., et al. 2020, Gebily, D. A., et. al 2021). In addition, the genus *Streptomyces* produces the bioactive natural products (Sapna Devi et al. 2023). Besides, this genus has an ability to make antifungal compounds, antihypertensive medicines, antitumor compounds, and the materials that acts against viruses (Omura, S et al. 2001, Khan, S. T., et al. 2011, Patzer, S. I., & Braun, V. 2010, Procópio, R. E. D. L., et al. 2012, Franco-Correa, M., et al. 2010).

Further, *Streptomyces rochei* strain HF391 has potential antifungal activity against *A. fumigatus* (Hadizadeh, S., et al. 2015), which is a human pathogenic fungi (Denning, D. W. 1998, Morgan, J. et al. 2005), and against *Fusarium oxysporum* (Kanini, G. S., Katsifas et al. 2013, E., E., A., A., T., & M., E. 2022) that is reported to cause human infections (Zhang, Y., et al 2020). In addition, *Streptomyces* species was reported from the rihizospheric soil of *Ocimum tenuiflorum* (Ayswaria, R., et al. 2023). However, the presence of *Streptomyces rochei* in the same habitat has been not studied yet. Therefore, in the present study, the *Streptomyces rochei* was isolated from the rhizospheric soil of *Ocimum tenuiflorum* (tulsi) and identified by 16S rRNA gene sequence for its further medical applications. Furthermore, the study reported the need of high species level identification data in NCBI database regarding *Streptomyces* species to perform robust and reliable identifications.

2. Materials and Methods

Isolation

A Rhizospheric soil sample of *Ocimum tenuiflorum* (tulsi) was collected from Karad, Maharashtra State of India The collected sample was diluted in distilled water at a ratio of 1 gram per 100 milliliters, and serial dilutions were subsequently performed up to 10^{-2}. The diluted test sample was then spread onto Glycerol Aspargine Agar plate (Meenakshi, S., et al. 2024) using the spread plate technique. Following this, the plates was incubated at 28^0C (Kumar, N., et al 2010) for 4-6 days (Shepherd, M. D., et al. 2010). The colonies displaying the morphological characteristics of actinobacteira were isolated, purified, and subjected to molecular identification by cultivating it in the slant (figure 1) through Progenome Life Science, Sambhajinagar, Maharashtra, India.

Characteristics

The biochemical tests (Triple Iron Sugar (Cappuccino JG, Sherman N. Microbiology.2004), Catalase (Lertcanawanichakul, M., & Sahabuddeen, T. 2023), Starch hydrolysis (Collee JE, et al 1996) were performed along with microscopic and Gram's nature analysis (Benson HJ. 1994) of the isolated culture.

DNA Extraction, Polymerase Chain Reaction, cycle sequencing, DNA sequencing, Sequence alignment and assembly

The DNA of isolated culture was extracted by Nucleospin Microbial DNA Kit and its quality was checked by 1% agarose gel electrophoresis. Partial fragment of 16S ribosomal rRNA gene was amplified by 27_F and 1492_R primers. A single discrete PCR amplicon band was observed by 1.2 % agarose gel after electrophoresis. The PCR amplicons was purified to remove contaminants. Forward and reverse DNA sequencing reactions of PCR amplicon was carried out using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. PCR product was processed for cleanup to remove unincorporated nucleotide and residual primers using Exonuclease-I and Shrimp Alkaline phosphatase enzyme followed by cycle sequencing reactions using BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.).

The Cycle sequencing was followed by a sequencing cleanup by ethanol precipitation followed by dissolving template in HiDi formamide and bidirectional sequencing in ABI 3730 Genetic analyzer. PCR product was then processed for direct bi-directional sequencing using ABI PRISM 3730×1 Genetic Analyzer (Applied Biosystems, USA). Consensus sequence of 16S ribosomal rRNA gene was generated from forward and reverse sequences using Bioedit software. The 16S ribosomal rRNA gene sequence was used to carry out Basic Local Alignment Search Tool (BLAST) with the database of NCBI.

The resulting DNA sequence was aligned using CLUSTALW in MEGA 11, manually trimmed and edited to obtain complete sequence. The confirmation of species was made by the sequence similarity score. Homology searches were carried out using the BLASTn program against the NCBI GenBank database (https://blast.ncbi.nlm.nih.gov/Blast. cgi).

3.Result

Morphological and Gram's Characters with biochemical features

The isolated culture showed grey colored colonies (Kumar, M., et al. 2023) which were hard to remove from the agar (figure 1). Under 45X objective of the microscope, the culture showed thin thread like structures with cells attached to each other (figure 2). The Gram staining of a culture displayed Gram positive nature (figure 3). The Triple Sugar Iron test showed development of reddish black color without gas production as compared to control in which no color change reported (figure 4). The catalase test was found to be negative (figure 5a) whereas starch hydrolysis was found to be positive (figure 5b).

Figure 1: *Streptomyces rochei cultured in the slant with Glycerol Aspargine Agar.*

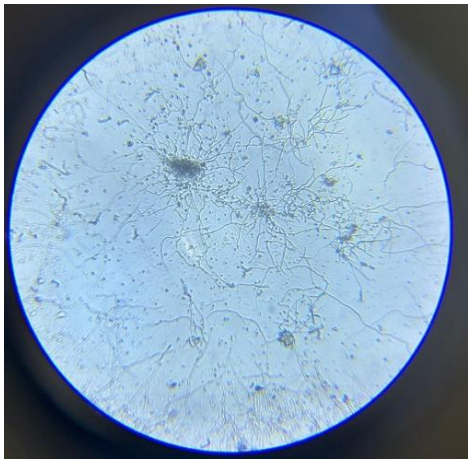

Figure 2: *The culture showed thread like structure Under 45X objective showing cells attached with each other.*

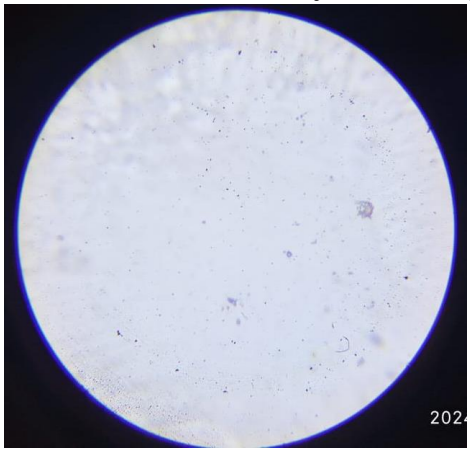

Figure 3: *Gram positive nature of the isolated culture.*

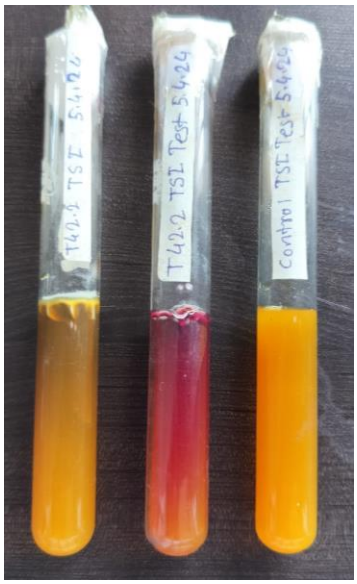

Figure 4: *Triple Sugar Iron Utilization Test of Streptomyces roche in the duplicate test tubes.*

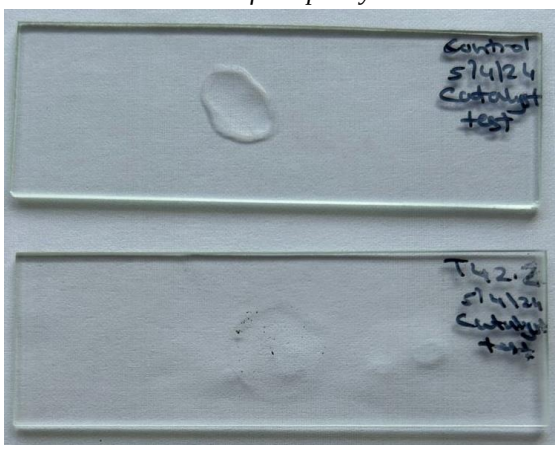

Figure 5a*: Catalase test of Streptomyces roche.*

Figure 5b*: Streptomyces rochei hydrolysed starch medium to produce zone of clearance (from right-first and second plate). In the control plate (from right, first plate), no zone of clearance was reported since there was no Streptomyces rochei culture.*

4.Result

Partial 859 base pair (bp) sequence of 16S rRNA gene of *Streptomyces roche* was submitted in the database of National Center for Biotechnology Information (NCBI) with accession number PP856680.

5.Evolutionary analysis by Maximum Likelihood method

The phylogenetic analysis of the obtained partial 16S rRNA gene sequence of *Streptomyces rochei* was performed (figure 6). The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura K. and Nei M. 1993). The bootstrap consensus tree inferred from 500 replicates (Felsenstein J. 1985) is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test 500 replicates are shown next to the branches (Felsenstein J. 1985). Initial tree (s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. This analysis involved 21 nucleotide sequences. There were a total of 859 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura K., Stecher G., and Kumar S. 2021). The *Streptomyces rochei* represented by sample ID SEQ-24-0210-T42.2-27F showed the closest clade with JF304944.1:3-769 identified as *Streptomyces* sp.

6. Discussion

In the study, the DNA of *Streptomyces rochei* was extracted by Nucleospin Microbial DNA Kit and its quality was checked by 1% agarose gel electrophoresis. Partial fragment of 16S ribosomal rRNA gene was amplified by 27_F and 1492_R primers. Similar observation was reported by Azish, M., et al. (2020) who used primers 27F and 1492R for amplification of 16S rRNA gene sequence of *Streptomyces angustmyeticus* (H-57) and *Streptomyces libani* (I-65). To add, Gopinath, B. V., & Singaracharya, M. A. (2012) amplified 16S rDNA fragment by 16S rDNA universal forward primer 8F in contrast the 27_F primer used in the study for the amplification of forward DNA strand. However, authors used 1492R as a primer to amplify the reverse DNA strand of 16S rDNA identical to the conducted study.

Moreover, Elsilk, S. E., et al. (2022) reported that novel *Streptomyces rochei* MS-37 isolated from marine ecosystem showed a separate clade in the phylogenetic tree when analyzed with *Streptomyces* sp. NK01103 (LC48879), *Streptomyces* sp. strain T.S.A.KP (MN 911386), *Streptomyces rochei* strain BF3A1 (OM746935), *Streptomyces rochei* strain APS29 (MH021962), *Streptomyces rochei* UAE 1-3 (MN795133), *Streptomyces maritimus* strain UP1A-1 (MT279914), and *Streptomyces rochei* strain SCSIO ZJ89 (MF104551). Authors found that *Streptomyces rochei* strain SCSIO ZJ89 (MF104551) formed closest clade to *Streptomyces rochei* strain MS-37. In contrast, our study showed that *Streptomyces rochei* strain (indicated as SEQ-24-0210-T42.2-27F in the phylogenetic tree) formed the closest clade with JF304944.1:3-769 *Streptomyces* sp. BN-6 based on 16S riobosomal RNA gene partial sequence phylogeny analysis (figure 6). This appeals the need of species level identification of the *Streptomyces* sp. before submission to the NCBI DNA sequence database to avoid misidentification practices.

5.Conclusion

The rhizospheric soil of *Ocimum tenuiflorum* (tulsi) belonging to Karad, Maharshtra, India possesses 16S ribosomal rRNA gene sequence identification based *Streptomyces rochei* strain. There is a requirement of preventing misidentifications of *Streptomyces* species by increasing the data of species level identifications in NCBI database.

Figure 6: *The phylogeny analysis of the Streptomyces rochei with equivalent 16S gene sequences deposited in NCBI nucleotide database.*

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Conflict of Interest

Authors declare that no conflict of interest exists among them.

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Ethical Statement

No ethical guidelines were violated during the research.

References

1. Azish, M., Shams Ghahfarokhi, M., & Razzaghi Abyaneh, M. (2020). Optimization of the antifungal metabolite production in *Streptomyces libani* isolated from northern forests soils in Iran. *Current medical mycology*, *6*(4), 20–26. https://doi.org/10.18502/cmm.6.4.5333.
2. Ayswaria, R., Alfred, J., Gopalan, G., & Krishna, R. (2023). Endophytic Streptomyces isolated from Ocimum tenuiflorum and Catharanthus roseus and its efficacy test to develop antibacterial fabric for wound caring. *The Journal of The Textile Institute*, *114*(4), 530-541.
3. Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H. P., ... & van Wezel, G. P. (2016). Taxonomy, physiology, and natural products of Actinobacteria. *Microbiology and molecular biology reviews*, *80*(1), 1-43.
4. Benson HJ. Morphological applications. Sixth edition. Wm C Brown Publishers, 1994, 447.
5. Cappuccino JG, Sherman N. Microbiology. A laboratory manual. 6th edition. Pearson education. 2004, 491.

6. Collee JE, Frase AG, Marmion BP, Simmons A. Mackie and McCartny. Practical Medical Microbiology. 14th edition. Churchill living stone, 1996, 978.
7. Denning, D. W. (1998). Invasive aspergillosis. *Clinical infectious diseases*, 781-803.
8. Devi, S., Verma, J., Sohal, S. K., & Manhas, R. K. (2023). Insecticidal potential of endophytic Streptomyces sp. against Zeugodacus cucurbitae (Coquillett)(Diptera: Tephritidae) and biosafety evaluation. *Toxicon*, *233*, 107246.
9. E., E., A., A., T., & M., E. (2022). Characterization of Promising Antifungal Bioactive Compounds from Endophytic Actinobacterium, Streptomyces rochei OM182844. *Egyptian Academic Journal of Biological Sciences, G. Microbiology*, *14*(2), 29-39. doi: 10.21608/eajbsg.2022.256512.
10. Elsilk, S. E., Khalil, M. A., Aboshady, T. A., Alsalmi, F. A., & Ali, S. S. (2022). *Streptomyces rochei* MS-37 as a Novel Marine Actinobacterium for Green Biosynthesis of Silver Nanoparticles and Their Biomedical Applications. *Molecules (Basel, Switzerland)*, *27*(21), 7296. https://doi.org/10.3390/molecules27217296.
11. Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.
12. Franco-Correa, M., Quintana, A., Duque, C., Suarez, C., Rodríguez, M. X., & Barea, J. M. (2010). Evaluation of actinomycete strains for key traits related with plant growth promotion and mycorrhiza helping activities. *Applied Soil Ecology*, *45*(3), 209-217.
13. Gebily, D. A., Ghanem, G. A., Ragab, M. M., Ali, A. M., Soliman, N. E. D. K., & Abd El-Moity, T. H. (2021). Characterization and potential antifungal activities of three Streptomyces spp. as biocontrol agents against Sclerotinia sclerotiorum (Lib.) de Bary infecting green bean. *Egyptian Journal of Biological Pest Control*, *31*, 1-15.
14. Gopinath, B. V., & Singaracharya, M. A. (2012). Identification of Streptomyces rochei (MMKK5_1492R_S0068) by using 16S rDNA Sequencing Isolated from Coal Fields of Andhra Pradesh. *JOURNAL OF PURE AND APPLIED MICROBIOLOGY*, *6*(1), 381-385.
15. Hadizadeh, S., Forootanfar, H., Shahidi Bonjar, G. H., Falahati Nejad, M., Karamy Robati, A., Ayatollahi Mousavi, S. A., & Amirporrostami, S. (2015). Antifungal activity of terrestrial *Streptomyces rochei* strain HF391 against clinical azole-resistant *Aspergillus fumigatus*. *Current medical mycology*, *1*(2), 19–24. https://doi.org/10.18869/acadpub.cmm.1.2.19.
16. Jose, P. A., Maharshi, A., & Jha, B. (2021). Actinobacteria in natural products research: Progress and prospects. *Microbiological Research*, *246*, 126708.
17. Kanini, G. S., Katsifas, E. A., Savvides, A. L., & Karagouni, A. D. (2013). Streptomyces rochei ACTA1551, an indigenous Greek isolate studied as a potential biocontrol agent against Fusarium oxysporum f.sp. lycopersici. *BioMed research international*, *2013*, 387230. https://doi.org/10.1155/2013/387230.
18. Kurniawati, N., Meryandini, A., & Sunarti, T. C. (2016). Introduction of actinomycetes starter on coffee fruits fermentation to enhance quality of coffee pulp. *Emirates Journal of Food and Agriculture*, 188-195.
19. Kumar, N., Singh, R. K., Mishra, S. K., Singh, A. K., & Pachouri, U. C. (2010). Isolation and screening of soil Actinomycetes as source of antibiotics active against bacteria. *International Journal of Microbiology Research*, *2*(2), 12.
20. Khan, S. T., Komaki, H., Motohashi, K., Kozone, I., Mukai, A., Takagi, M., & Shin-ya, K. (2011). Streptomyces associated with a marine sponge Haliclona sp.; biosynthetic genes for secondary metabolites and products. *Environmental microbiology*, *13*(2), 391-403.
21. Li, X., Jing, T., Zhou, D., Zhang, M., Qi, D., Zang, X., ... & Xie, J. (2021). Biocontrol efficacy and possible mechanism of Streptomyces sp. H4 against postharvest anthracnose caused by Colletotrichum fragariae on strawberry fruit. *Postharvest Biology and Technology*, *175*, 111401.
22. Lertcanawanichakul, M., & Sahabuddeen, T. (2023). Characterization of *Streptomyces* sp. KB1 and its cultural optimization for bioactive compounds production. *PeerJ*, *11*, e14909. https://doi.org/10.7717/peerj.14909
23. Morgan, J., Wannemuehler, K. A., Marr, K. A., Hadley, S., Kontoyiannis, D. P., Walsh, T. J., ... & Warnock, D. W. (2005). Incidence of invasive aspergillosis following hematopoietic stem cell and solid organ transplantation: interim results of a prospective multicenter surveillance program. *Medical mycology*, *43*(Supplement_1), S49-S58.
24. Meenakshi, S., Hiremath, J., Meenakshi, M. H., & Shivaveerakumar, S. (2024). Actinomycetes: Isolation, Cultivation and its Active Biomolecules. *Journal of Pure & Applied Microbiology*, *18*(1).
25. Ōmura, S., Ikeda, H., Ishikawa, J., Hanamoto, A., Takahashi, C., Shinose, M., ... & Hattori, M. (2001). Genome sequence of an industrial microorganism Streptomyces avermitilis: deducing the ability of producing secondary metabolites. *Proceedings of the National Academy of Sciences*, *98*(21), 12215-12220.

26. Patzer, S. I., & Braun, V. (2010). Gene cluster involved in the biosynthesis of griseobactin, a catechol-peptide siderophore of Streptomyces sp. ATCC 700974. *Journal of bacteriology*, *192*(2), 426-435.
27. Procópio, R. E. D. L., Silva, I. R. D., Martins, M. K., Azevedo, J. L. D., & Araújo, J. M. D. (2012). Antibiotics produced by Streptomyces. *Brazilian Journal of Infectious Diseases*, *16*, 466-471.
28. Rey, T., & Dumas, B. (2017). Plenty is no plague: Streptomyces symbiosis with crops. *Trends in plant science*, *22*(1), 30-37.
29. Shepherd, M. D., Kharel, M. K., Bosserman, M. A., & Rohr, J. (2010). Laboratory maintenance of Streptomyces species. *Current protocols in microbiology*, *Chapter 10*, Unit–10E.1. https://doi.org/10.1002/9780471729259.mc10e01s18.
30. Sholkamy, E. N., Ahmed, M. S., Yasser, M. M., & Mostafa, A. A. (2020). Antimicrobial quercetin 3-O-glucoside derivative isolated from Streptomyces antibioticus strain ess_amA8. *Journal of King Saud University-Science*, *32*(3), 1838-1844.
31. Tamura K. and Nei M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512-526.
32. Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution https://doi.org/10.1093/molbev/msab120.
33. Tamreihao, K., Nimaichand, S., Chanu, S. B., Devi, K. A., Lynda, R., Jeeniita, N., & Ningthoujam, D. S. (2018). Acidotolerant Streptomyces sp. MBRL 10 from limestone quarry site showing antagonism against fungal pathogens and growth promotion in rice plants. *Journal of King Saud University-Science*, *30*(2), 143-152.
34. Uyeda, M., Mizukami, M., Yokomizo, K., & Suzuki, K. (2001). Pentalenolactone I and hygromycin A, immunosuppressants produced by Streptomyces filipinensis and Streptomyces hygroscopicus. *Bioscience, biotechnology, and biochemistry*, *65*(5), 1252-1254.
35. Yun, T. Y., Feng, R. J., Zhou, D. B., Pan, Y. Y., Chen, Y. F., Wang, F., ... & Xie, J. H. (2018). Optimization of fermentation conditions through response surface methodology for enhanced antibacterial metabolite production by Streptomyces sp. 1-14 from cassava rhizosphere. *PloS one*, *13*(11), e0206497.
36. Zhang, Y., Yang, H., Turra, D., Zhou, S., Ayhan, D. H., DeIulio, G. A., ... & Ma, L. J. (2020). The genome of opportunistic fungal pathogen Fusarium oxysporum carries a unique set of lineage-specific chromosomes. *Communications biology*, *3*(1), 50.