IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

Exploitation of novel uncultivable microbes for biotechnological application by mimicking natural environment using i-Chip: A prelude study

Mahesh Anumalla[a,b§], Uma RajeswariBatchu[b §], Chandrasekhar Cheemalamarri[c], Bhima Bhukya[a], Prakasham Reddy Shetty [b*]

[a]Department of Microbiology, Osmania University, Hyderabad-500007, India.

[b]Organic synthesis and Process Chemistry, CSIR- Indian Institute of Chemical Technology, Hyderabad- 500 007, India.

[c]Energy and Environmental Engineering, CSIR- Indian Institute of Chemical Technology, Hyderabad- 500 007, India.

§ - These authors contributed equally.

Abstract

The search of novel bioactive compounds has been one of the major task world-wide due to their potential to synthesize unique molecules which help in preventing and treating various diseases and disorders as well as combat the developing antimicrobial resistance (AMR). The present study explored the bioactive potential of uncultivable actinobacteria isolated by i-Chip method from six soil samples of Tirumala region, India and preliminary selected; based on the antimicrobial, anti-oxidant and xanthine oxidase (XO) inhibition potential. These isolates were further identified according to growth, cultural and microscopic features and confirmed by 16S rRNA sequencing. Among them two potent bioactive isolates belong to *Streptomyces* species designated as *Streptomyces costaricanus* IICT-RSP396 and *Streptomyces* sp. IICT-RSP475 respectively. The crude extract of *Streptomyces costaricanus* IICT-RSP396 has shown potent antifungal activity against various candida species, whereas, *Streptomyces* sp. IICT-RSP475 has exhibited XO inhibition potential indicating its preliminary potential in treating the uric acid associated diseases. In conclusion, the unexplored region of Tirumala combined with novel *i*-Chip cultivation technique discovered a novel uncultivable *Streptomyces* sp. IICT-RSP475 of potent XO inhibition potential. Further exploitation of this strain metabolite may pave the way for discovery of novel therapeutic compounds of microbial origin for human welfare.

Key words: *i*-Chip, XO inhibition, *Streptomyces costaricanus* IICT-RSP396, *Streptomycessp*. IICT-RSP475, Tirumala region.

1. Introduction

Microbial entities are widespread globally across the terrestrial and aquatic environment. Despite the overwhelming richness of microbial diversity on the earth, only a few microbial species (less than 1%) are cultivable under *in vitro* conditions either by supplementing the synthetic or natural media components and others remain unexplored. Ever since the above remarks, the concept of "uncultivable microorganisms" has been a challenging topic for researchers. In real terms, uncultivable microorganisms are metabolically active in their natural environment, however, the lack of appropriate knowledge on their growth requirements (nutritional and physical), growth rate, ecological role, and their interaction with abiotic-biotic components limits the growth of soil bacteria in standard laboratory conditions [1]. Conventional screening technologies have been seems to be leading to the isolation of regular microbial entities capable of generating already been extensively studied and established metabolites [2]. Owing to the hidden potential of the uncultivable strains (bacteria and archaea) as a source of novel metabolites of biotechnological and pharmaceutical application, investigations have been progressed to develop novel cultivation strategies globally [3].

Towards this, several effective strategies have been implemented to isolate and cultivate novel unexplored microbes from different ecological niches. Out of these strategies, *in situ* cultivation (simulating natural environments) is the most promising one as it accesses periodically the natural chemical components essential for replication of previously uncultivable microbes. The development of *i*-Chip, diffusion chambers [4], solid substrate membrane system [5], Microtitre plate method [6], Single-cell encapsulation combined with flow cytometry [7], Hollow-fiber membrane chamber (HFMC) [8], double encapsulation method [9] are the few of effective prevailing approaches for *in situ* cultivation of uncultivable microbes.

Microbial metabolites remain a major source of global therapeutics since long ago. Exploring these untapped biological resources encourages future drug discovery to mitigate life-threatening diseases globally. India's the most distinctive asset of bio-diversity could be one of the best treasure for the exploration of unique microbial isolates. Soil generally afford very complex habitat to the residing microorganisms mainly due to its geographical variation that lead to isolation of microbes with unique potential of innovative and diverse chemical metabolites [10]. Among various soil habituated microbes, actinobacteria widely subjugated for the exploitation of the industrially / health sector essential secondary metabolites and biocatalyst.

In the present investigation, the virgin soil samples from several unexplored ecological niches of Tirumala region, India have been screened-out to segregate actinobacteria producing metabolites of anti-microbial, anti-oxidant, anti-gout potential. As of now, these ecological niches have been under-explored for microbial diversity and their metabolite potential. The objectives of the present study is to isolate uncultivable microbial isolates of biotechnological importance from Tirumala forest region of India using *i*-Chip as a new isolation and cultivation tool.

2. Materials and Methods

A total of 16 soil samples were brought to the lab from diverse sites within Tirumala region, Andhra Pradesh, India i.e National Park as per procedure described by Prashanthi et al. (2021) [11]. The collected samples were transported to CSIR-IICT laboratory and preserved in cold cabinet at 4 ºC until further studies. All the chemicals and reagents used in the present study were of analytical grade and procured from Sigma Aldrich (USA), Hi-media laboratories (India) and Merk (USA).

2.1 Isolation of bacteria by novel cultivation techniques

The collected soil samples were coarsely grounded, sieved and kept for overnight air-drying followed by bacterial isolation using modified *i*-Chip technique developed in our laboratory [12]. The microcolonies isolated from the discrete micro agar chambers of *i*-Chip central plate were transferred to Actinomycetes Isolation Agar (AIA) containing nystatin and cycloheximide (40 and 50 µg/mL, respectively) to inhibit growth of unwanted bacteria and fungi and incubated at 35°C for one week. The colonies were purified by re streaking and preserved as glycerol stocks at -80ºC.

2.2 Screening of microbial isolates for bioactive properties

The isolated actinobacterial cultures were screened for bioactivity profile i.e. antimicrobial, antioxidant and xanthine oxidase (XO) inhibition potential.

2.2.1 Anti-microbial activity

The screening for antibacterial activity was performed by using Giant colony techniques as described by Madigan et al., (1997) against pathogenic bacteria and fungi i.e. *Bacillus subtilis* MTCC 121, *Candida glabatra* MTCC 3019 *and Candida albicans* MTCC 4748, *Escherichia coli* MTCC 739, *Mycobacterium smegmatis*, *Pseudomonas aeruginosa* MTCC2453, and *Staphylococcus aureus* MTCC 96 [13].

The potent strains from primary screening with good inhibition against pathogens were further subjected for secondary screening by using agar-well diffusion method against various gram positive (*B. subtilis* and *S. aureus*), gram negative (*E. coli* and *P. aeruginosa*), acid fast (*M.smegmatis*) bacteria and yeast (*C.glabatra* MTCC 3019 and *C.albicans* MTCC 4748) [14] using standards streptomycin and miconozole.

2.2.2 XO inhibition activity

XO inhibition screening of culture filtrates was performed using a revised qualitative Xanthine-Nitroblue Tetrazolium chloride (NBT) plate assay technique [15]. DMSO and Allopurinol (2 mM) were used as a negative- and positive- control, respectively. The appearance of blue color halo zone in control wells depicts the XO activity.

2.2.3 Anti-oxidant activity

The antioxidant activity of culture filtrates was assessed by studying the free radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) reagent described by Moon and Terao (1998) with minor modifications [16]. DPPH, L- Ascorbic acid were kept as a positive and negative control, respectively. The radical scavenging activity was measured as a decrease in the absorbance of DPPH .

DPPH radical scavenging activity = $1 - \frac{At}{Ac}$ X 100

Ac - Absorbance of control, At- Absorbance of test

2.3 Characterization of bioactive isolates

2.3.1 Morphological characterization

The isolates with bioactive potential were subjected for identification by studying cultural and microscopic characteristics [17]. The cultural characteristics were studied by growth pattern, colony color, color of the aerial and substrate mycelia on different ISP media (ISP1 to ISP7). The cultures were streaked on above mentioned broth and incubated up to 7 to 10 days at 28°C for the given characteristics. The structure of the mycelia was studied by Scanning Electron Microscope (SEM).

2.3.2 Molecular characterization

The molecular characterization of strains were performed at Bioserve Biotechnologies Pvt Ltd, Hyderabad, India using 16S rRNA ribotyping method.

2.4 Fermentation studies and extraction of crude metabolites from bioactive actinobacterial sp

Fermentation of selected isolates were conducted in starch casein broth, ISP-1 to ISP-7 broth at pH 7.0 and incubated at 30° C at 250 rpm agitation for 5 days. The fermented cell free broth and cell biomass was separated by centrifugation (REMI C-24BL, India) at 10,000×g. The obtained cell free-broth was subjected for liquid-liquid extraction to isolate the bioactive compound according to [18] using ethyl acetate and water in the ratio of (1: 2 v/v). Then organic phase (ethyl acetate) containing bioactive compound was separated and evaluated for activity to confirm the maximum compound had been extracted. Finally, the interested compound containing solution was concentrated by subjecting to a rotary vacuum evaporator (Heidolph Rotacool, Germany) under reduced pressure and for bioactivity studies.

2.5 Bioactive potential of crude extracts

The preliminary screening of culture filtrate of *S. costaricanus* IICT-RSP396 had shown potent anti-fungal activity against *Candida* species hence, the crude extract was tested against various *Candida* species *in vitro* by the agar well diffusion method [19] along with miconazole as control. Thereafter, the minimum inhibitory concentration (MIC) was calculated for the most sensitive test pathogens by broth dilution method. The study was performed against fungal pathogenic strains like, *C. albicans* MTCC 227, *C. albicans* MTCC 4748, *C. albicans* MTCC 3018, *Candida albicans* MTCC 4718, *C. albicans* MTCC 7315, *C. caseri* MTCC 1962, *C. glabrata* MTCC 3019 and *C. krusei* MTCC 3020.

The screening studies revealed qualitative XO inhibition potential of *Streptomyces* IICT-RSP475. Therefore, the crude extract percentage of XO inhibition was studied by modified uric acid estimation assay [20]. Allopurinol was used as a positive control. The reactions were executed using 50 mM Tris buffer with 0.03 µM enzyme and 500 µM xanthine as a substrate in the 96-well flat bottom microtiter plate (Tarsons, India) using a multimode plate reader (TECAN, Austria) at 295 nm.

3. Results

3.1 Isolation and screening of actinobacterial isolates

The collected soils have been characterized by reddish brown to brown color, highly acidic to neutral in nature and non-saline. Generally, these are low in organic carbon, nitrogen and medium in potassium and phosphorous but sufficient in Sulphur content [22]. This has been considered as an enriched environment for actinobacteria notably Streptomyces species exhibiting bioactive potential as related with previous research [23]. Hence, these soil samples from Tirumala region of India were explored for actinobacteria (Fig 1). A total of 61 strains were isolated and screened for anti-microbial, anti-oxidant and XO inhibition potential (Table 1). The highest number of isolates (16) have been isolated from Pulibonu and Talakona, followed by 9 from Kalyani Dam, Balapalli and Tirumala Mallela Manda, 6 from Srivari Padalu, 2 from Ankalamma and one from Tirumal Meg Road. Out of these isolates, 13 exhibited anti-bacterial activity, 4 revealed anti-fungal activity, 7 isolates showed anti-oxidant activity, while one isolate inhibited XO in primary screening (Table1)

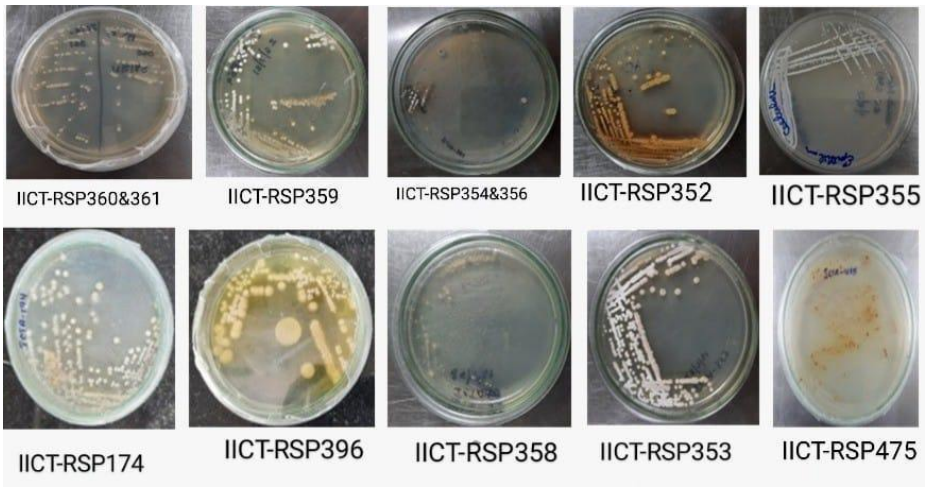


Fig1: Few Actinobacteria examples of isolated from Tirumala region using i-Chip method.

Table1: Few examples of Bioactive isolates collected from Tiruma region.

S No	Name of the Soil	Sample Type	Area	Isolates	Total Isolates	Bioactive Isolates	longitude and latitude
1	TK-1	Water	TALAKONA	350, 351, 353, 356, 359, 360, 385,387,371	9	5	13°48'41.0"N 79°12'55.3"E
2	TK-2	Soil	TALAKONA	454,459,461,462,701, 702, 703	7	3	13°48'39.1"N 79°12'57.4"E
3	BP-1	Soil	BALAPALLI	388,389,390,391,392, 397,451,452	8	2	13°48'38.5"N 79°24'16.6"E
4	BP-4	Wood	BALAPALLI	386	1	1	13°48'16.4"N 79°25'04.4"E
5	TSP-1	Soil	TIRUMAL SRIVARI PADALU	399,398,455,456,457,458	6	2	13°40'46.2"N 79°19'58.8"E
6	KDM-1	Water	KALYANI DAM	393,396, 460,459,464, 394, 395, 450	8	2	13°39'32.2"N 79°16'20.7"E
7	SMM-1	Rock	TIRUMALA MALLELA MANDA	465, 466, 467, 471, 472, 476	6	1	13°41'46.2"N 79°19'54.1"E

8	SMM-3	Soil	TIRUMALA MALLELA MANDA	468,469,470	3	1	13°41'39.7"N 79°19'56.7"E
9	PB-1	Soil	PULIBONU	372,373,374,375,376,377,378,379,380,381,382,383,384, 473, 474, 475	16	2	13°41'53.6"N 79°15'39.3"E
10	ANK	Wood	ANKALAMMA	481,482	2	1	13°40'43.6"N 79°15'57.0"E
11	TMR	Soil	TIRUMALA MEG ROAD	480	1	1	13°43'01.2"N 79°20'46.7"E

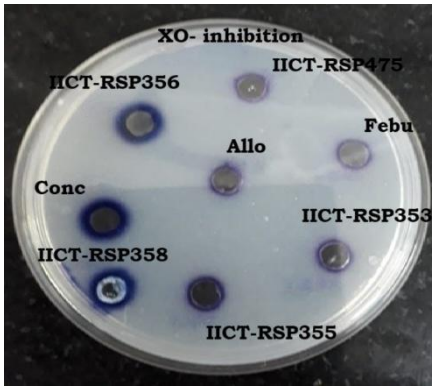
Based on primary screening, nine isolates were selected for secondary screening (5 for anti-bacterial activity, 3 for anti-fungal activity and one isolate for XO inhibition-activity) (Fig 2A). Due to poor anti-oxidant activity of isolates further studies were limited anti-fungal and XO inhibition profiling.

Out of bioactive isolates, 1 isolate exhibited moderate antibacterial activity against *B.subtilis*, one against *Mycobacterium smegmatis*, 4 against *staphylococcus aureus*, 4 against *E.coli*, 4 against *Pseudomonas aeruginosa*. Surprisingly, IICT-RSP396 exhibited potent antifungal activity against *Candida albicans* (Table 2) whereas, IICT-RSP 475 exhibited potent xanthine oxidase inhibition in secondary screening (Fig 2B). Therefore, IICT-RSP396 and IICT-RSP475 were further selected for cultural and molecular characterization and bio-active potential.

Table2: The antimicrobial activity of selected isolates against test pathogen.

S No	Test Strain	Zone of inhibition (mm)															
1	**Isolate**	351	352	354	359	371	378	385	394	396	398	399	451	452	467	702	703
2	***Candinaalbicans***	–	–	–	–	12	21	18	–	22	–	–	–	–	–	–	–
3	***Bacillus subtilis***	18	18	19	18	–	22	6	–	17	12	11	16	14	–	–	–
4	***Pseudomonas aeruginosa***	10	12	15	16	–	–	–	16	–	22	–	15	18	–	18	19
5	***Escherichia coli***	14	14	15	13	–	–	–	–	–	–	–	–	–	–	–	–
6	***Mycobacterium smegmatis***					–	–	–	14	–	–	15	–	–	18		

A



B

Fig2: Secondary screening of selected strains. A. Antifungal activity of IICT-RSP396, B. XO inhibition potential of IICT-RSP475.

3.2 Characterization of Potent actinobacterial strains

3.2.1 Cultural characterization

The cultural characteristics of potent bioactive actinobacterial isolates i.e. microbial growth including its pattern on agar plate, and pigment production were evaluated on International *Streptomyces* Project (ISP) media and the obtained results were tabulated (Table 3). The SEM images and agar plate cultures were represented in fig 3.

Table 3: Microbial growth characteristics of IICT-RSP475 on different ISP media

ISOLATES	MEDIA	CULTURAL CHARCTERISTICS				
		GROWTH	SUBSTRATE MYCELLIUM	AERIAL MYCELLIUM	SPORES	PIGMENT PRODUCTION
StreptomycesCostaricanusIICT-RSP396	ISP1	Luxuriant	Deeply penetrated	Sporulated	present	Golden brown
	ISP2	luxuriant	Deeply penetrated	Sporulated	Present	Golden brown
	ISP3	Luxuriant	Deeply penetrated	Sporulated	Present	Golden brown
	ISP4	Luxuriant	Deeply penetrated	Sporulated	present	No pigment
	ISP5	luxuriant	Deeply penetrated	Sporulated	Present	Golden brown
	ISP6	luxuriant	Deeply penetrated	Sporulated	Present	Golden brown
	ISP7	poor	Deeply penetrated	Non-Sporulated	Absent	Golden brown
*Streptomyces*spIICT-RSP475	ISP1	*Good*	Penetrated to culture medium	Light reddish brown	Absent	No pigment
	ISP2	*Luxuriant*	Penetrated to culture medium	Dark red	Present	Red pigment
	ISP3	Luxuriant	Penetrated to culture medium	Brownish white	Present	Weak melanoidpigment
	ISP4	Luxuriant	Light brown	Brownish white	Present	Melanoidpigment
	ISP5	good	Reddish brown	Red	Present	Reddish pigment
	ISP6	good	Light brown	Light brown	Absent	No pigment
	ISP7	Luxuriant	Dark brown	Whitish brown	Present	Melanoidpigment

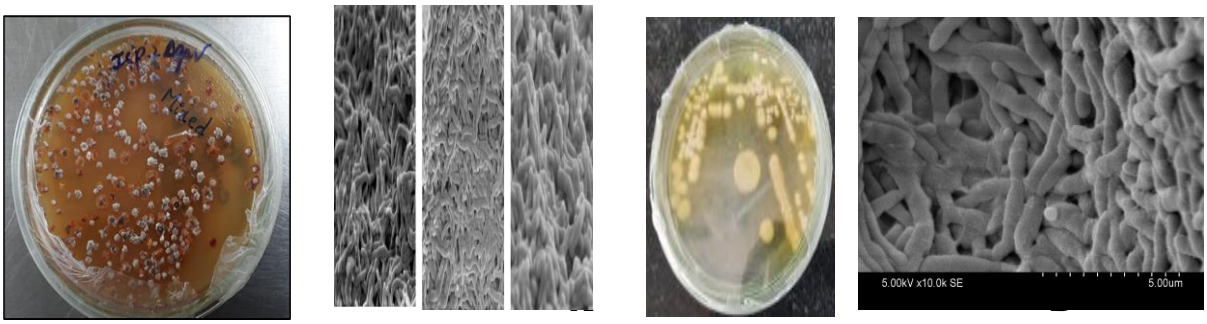


Fig 3: Morphology of selected strains: A)Plate culture and SEM image of ICTA-475 B) Plate culture and SEM image of IICTRSP-396

3.2.2 Molecular Ribotyping

The obtained data from the 16S rRNA sequencing and phylogenetic tree evaluation depicted that the selected strains belongs to the genus *Streptomyces*, the 16S rRNA genome sequence of the strain showed similarity with different *Streptomyces* strains. Based on the sequence homology, the strain IICT-RSP396 showed identity with both *Streptomyces rochi* 7434AN4 [21] and *Streptomyces costaricanus* K21C32,

however, the release of soluble yellow pigment was similar as like *Streptomyces costaricanus* K21C32 therefore our strain IICT-RSP396 was identified as *Streptomyces costraticanus* IICT-RSP396. Surprisingly, the strain IICT-RSP475 has shown sequence resemblance with previously unidentified species of *Streptomyces* sp. strain IC12A and also MM108 and also two more strains of *Streptomyces* sp BH-MK-02 & L4_7_907R. The closest relative of *Streptomyces* sp. IICT-RSPS475 was *Streptomyces aureus* strain NBRC 100912 and BT63. Based on these comparisons, the isolates IICT-RSP396 was identified as *S. costaricanus* IICT-RSP396, whereas IICT-RSP475 was *Streptomyces* sp. IICT-RSP475. The strains were maintained in the in-house culture collection of the laboratory and designated as *S. costaricanus* IICT-RSP396 and *Streptomyces sp.* IICT-RSP475.

3.3 Fermentation and extraction of crude metabolites from identified actinobacterial species

The fermentation studies of *S. costaricanus* IICT-RSP396 and *Streptomyces* sp IICT-RSP475 were performed and the obtained fractions were evaluated qualitatively by subjecting to thin-layer chromatography (TLC) using hexane and ethyl acetate (60:40) as mobile phase and visualized under UV chamber. The yield of the crude metabolite from *S. costaricanus* IICT-RSP396 and *Streptomyces* sp IICT-RSP475 fermentation broth was found to be 3.0 and 0.25 g/L, respectively.

3.4 Bio-activity potential of crude extracts

The crude extract of *S. costaricanus* IICT-RSP396 had shown potent anti-fungal activity against *Candida* species (Fig 4). From the antimicrobial data it was inferred that, the crude depicted noteworthy antifungal functionality against all the studied species of *Candida.* However, the potent inhibition was exhibited against *Candida caseri* MTCC1962, *Candida krusei* MTCC3020 and *Candida albicans* MTCC4748. The MIC of the compounds were given in the Table 4.

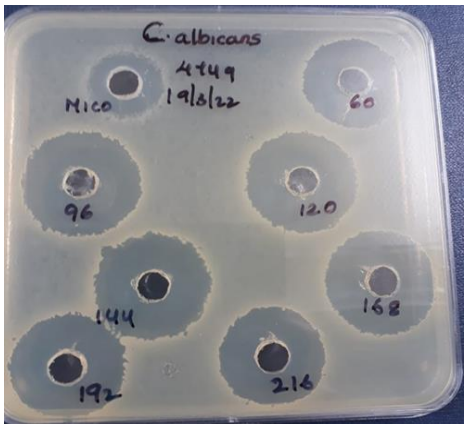


Fig 4: anti-fungal activity against *Candida* species

Table 4: The Minimum Inhibitory Concentration (MIC) of the *S. costaricanus* crude extract against *Candida* species.

Name of the organism	MIC (µg/ml)
Candida caseri	0.39
C. krusei	0.195
C. albicans	0.39

The results of Uric acid estimation assay revealed potent XO inhibition of *Streptomyces* IICT-RSP475 crude extract. It has shown 98% inhibition of XO at a concentration of 50 µg/ml (Fig 5).

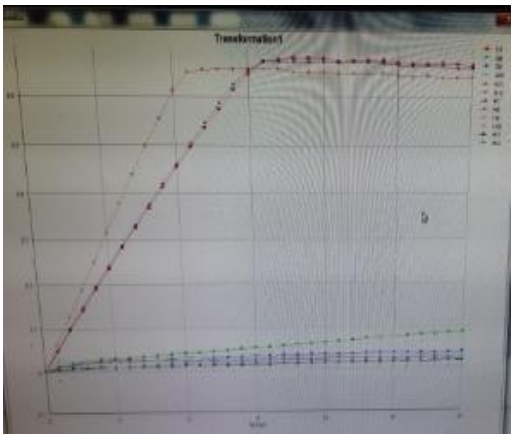


Fig 5: XO inhibition of *Streptomyces* IICT-RSP475 crude extract.

4. Discussion

The present study emphasizes the need of isolation of novel microbial strains for biotechnological application in view of the constant AMR evolution. *i*-Chip mediated isolated uncultivable actinobacteria from soil samples collected from various locations of Tirumala Bamboo forest area (Talakona) revealed their preliminary imperative role and may pave the way to develop novel drugs for uric acid associated diseases.

The exploration of bioactive actinobacterial species from the Tirumala region has been under-explored in the previous literature. The present study explored the 16 isolates with antimicrobial potential, 4 isolates with anti-oxidant potential and 1 isolate with XO inhibition potential which is significant. This might be due to implementation of novel *i*-Chip method of isolation that could attribute to isolation and cultivation of bioactive uncultivable microorganisms [24]. The findings of present study demonstrated antimicrobial potential of actinobacterial strains against gram-positive bacteria &*Mycobacterium* species, antioxidant potential and XO inhibition potential.

Owing to its potent antifungal activity and XO inhibition potential, strains IICT-RSP396 & IICT-RSP475 were further characterized by cultural characterization and ribotyping. Surprisingly, the growth of IICT-RSP475 on different ISP media their growth characteristics were inconsistent indicating its un-cultivability and need of development of novel media. The ribotyping analysis revealed, the strain IICT-RSP396 has shown sequence similarity with *S.costaricanus*, whereas, IICT-RSP475 was closely related to unknown *Streptomyces* sp. Strain indicating its novel nature. The MIC of *S.costaricanus* IICT-RSP396 crude extract against *Candida* species indicated potent anti-fungal activity. The % XO inhibition of *Streptomyces sp.*IICT-RSP475 indicates potent XO inhibitory activity. These results promoting the Tirumala region as a source of novel bioactive actinobacteria and *i*-Chip as a prospective tool for uncultivables isolation.

5. Conclusions

The present study indicate the imperative role of isolation of promising actinobacterial strains from different virgin areas by using laboratory modified *i*-Chip method. The different bioactivities of crude extract explored the diversity of isolated actinobacterial strains. The crude extract of the strains, IICT-RSP396 and IICT-RSP475 (*S.costaricanus*) and IICT-RSP396 (*Streptomyces* sp. IICT-RSP475) showed potent antifungal activity against various *Candida* species and XO inhibition potential, respectively. It suggests the

pharmaceutical potential of two isolates and warrants further investigation to isolate pharmacologically active moiety. Currently our group are involved in whole genome sequencing and prediction of secondary metabolite biosynthetic gene clusters (smBCGs) of Novel *Streptomyces* sp. IICT-RSP475 using antiSMASH followed by isolation and chemical characterization of pharmacologically active compounds. Our successive publication would explore the draft genome potential as well wet lab chemical characterization of pharmacologically active compound from Novel *Streptomyces* sp. IICT-RSP475.

6.Acknowledgments

URB and PRS thank Council for Scientific and Industrial Research (CSIR), New Delhi for funding under the Emeritus Scheme, grant number 21(1102)/20/EMR-II. Authors thanks to Dr. Linga Banoth,, Ms Tejaswi, and Ms Padmasri O for their constant support. The communication number issued by CSIR-IICT for this article is **IICT/Pubs./2025/118.**

7.Statements and Declarations

8.Competing interests

The authors declare no financial and non-financial competing interests for the present work.

9.Funding

This work was supported by the funding agency Council for Scientific and Industrial Research (CSIR) under the Emeritus scheme, grant number 21(1102)/20/EMR-II.

10.Conflicts of interest

The authors declared that there are no conflicts of interest.

11. Author's contribution

The study conception and design was performed by Dr. Prakasham Reddy Shetty, Dr.Bhima Bhukya. Data collection by Dr. Chandrasekhar Cheemalamarri, Experimental work and analysis were performed by Mahesh Anumalla and Dr Uma Rajeswari Batchu.

References:

1. Stewart E J. Growing unculturable bacteria. *J. Bacteriol.* 2012; 16: 4151–4160.
2. Okami, Y., and Hotta, K. (1988). "Search and discovery of new antibiotics," in Actinomycetes in Biotechnology, eds M. Goodfellow, S. T. Williams, and M. Mordarski (London: Academic Press), 37–67).
3. VanDorst JM, Hince G, Snape I & Ferrari BC. Novel culturing techniques select for heterotrophs and hydrocarbon degraders in a Sub antarctic soil. *Sci Rep* 2016; **7**: 36724anDorst JM, Hince G, Snape I & Ferrari BC. Novel culturing techniques select for heterotrophs and hydrocarbon degraders in a Sub antarctic soil. *Sci Rep* 2016; **7**: 36724.
4. Kaeberlein T, Lewis K, Epstein SS. 2002. Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. *Science* 2002; 296:1127–1129.
5. Svenning MM, Wartiainen I, Hestnes AG, Binnerup SJ. Isolation of methane oxidising bacteria from soil by use of a soil substrate membrane system. FEMS Microbiology Ecology. 2003 Jun 1;44(3):347-54.
6. Rappe MS, Connon SA, Vergin KL, Giovannoni SJ. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. *Nature* 2002; 418: 630–633.
7. Zengler K, Toledo G, Rappé M, Elkins J, Mathur EJ, Short JM, Keller M. Cultivating the uncultured. Proceedings of the National Academy of Sciences. 2002 Nov 26;99(24):15681-6.

8. Aoi Y, Kinoshita T, Hata T, Ohta H, Obokata H, Tsuneda S. Hollow-fiber membrane chamber as a device for in situ environmental cultivation. Applied and environmental microbiology. 2009 Jun 1;75(11):3826-33.
9. Ben-Dov E, Kramarsky-Winter E &Kushmaro A. An *in situ* method for cultivating microorganisms using a double encapsulation technique. *FEMS MicrobiolEcol* 2009; 68: 363–371.
10. Singh V, Haque S, Singh H, Verma J, Vibha K, Singh R, Jawed A, Tripathi CK. Isolation, screening, and identification of novel isolates of actinomycetes from India for antimicrobial applications. Frontiers in microbiology. 2016 Dec 6;7:1921).
11. Prashanthi R, G K S, S K, L M. 2021. Isolation, characterization, and molecular identification of soil bacteria showing antibacterial activity against human pathogenic bacteria. J Gene EngBiotechnol 19:120).
12. Anumalla M, Batchu UR, Singh NK, Bhukya B, Reddy Shetty P. Draft genome analysis of Delftiatsuruhatensis IICT-RSP4, a strain with uricase potential isolated from soil. Indian Journal of Microbiology. 2025 Jan 7:1-8.
13. Madigan,M.T.,Martiko,J.M.,and Parker, J. (1997).“Antibiotics: isolation andcharacterization,”in *Brock Biology of Microorganisms*, *8thEdn.*,edM.T. Madigan (New Jersey:Prentice-Hall International Inc.),440–442).
14. Valgas C, De Souza S.M, Smânia E.F.A., et al. Screening methods to determine antibacterial activity of natural products. Braz. J. Microbiol. 38 (2007) 369–380.
15. Kapoor, N., Saxena, S., 2016. Xanthine oxidase inhibitory and antioxidant potential of Indian Muscodor species. 3 Biotech 6, 248 .https://doi.org/10.1007/s13205-016-0569-5.
16. Moon JH, Terao J. 1998. Antioxidant activity of caffeic acid and dihydrocaffeic acid in lard and human low-density lipoprotein. J. Agric. Food Chem. 46: 5062-5065).
17. Shirling ET, Gottlieb D. Methods for characterization of Streptomyces species. International Journal of Systematic and Evolutionary Microbiology. 1966 Jul;16(3):313-40.
18. Schugerl K. Solvent extraction in biotechnology: Recovery of primary and secondary metabolites, (1994) Springer Verlag, Berlin.
19. Wu, R. Y. (1984). Studies on the Streptomyces SC4. II Taxonomic and biological characteristics of Streptomyces strain SC4. Bot. Bull. Acad. Sin. 25, 111–123.
20. Chang, WS., Lee, YJ et al., 1993. Inhibitory effects of flavonoids on xanthine oxidase. *Anticancer Research.* 13, 2165-2170.
21. Nindita Y, Cao Z, Fauzi AA, Teshima A, Misaki Y, Muslimin R, Yang Y, Shiwa Y, Yoshikawa H, Tagami M, Lezhava A. The genome sequence of Streptomyces rochei 7434AN4, which carries a linear chromosome and three characteristic linear plasmids. Sci Rep. 29 (2019) 9(1):1-1.
22. Rao PC. Characterization and Classification of Bamboo (Dendrocalamusstrictus and Bambusa bamboos) Supporting Soils in Talakona Forest Area of Chittoor District in Andhra Pradesh. The Andhra Agricultural Journal. 2013;60(1):157-64.
23. Shekar P, Radhakrishnan M, Gopikrishnan V, Suresh A. Characterization and antimicrobial potential of soil actinobacterium TFA1 isolated from Talakona forest, Andhra Pradesh. Journal of Applied Pharmaceutical Science. 2017 Mar 30;7(3):202-6.
24. Nichols D, Cahoon N, Trakhtenberg EM, Pham L, Mehta A, Belanger A, Kanigan T, Lewis K, Epstein S. Use of ichip for high-throughput in situ cultivation of “uncultivable” microbial species. Applied and environmental microbiology. 2010 Apr 15;76(8):2445-50.

List of Figures

List of Tables