need to be considered. Efforts are being made at NEERI to functionalize the materials to enhance adsorption efficiency.

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Screening and isolation of cyclosporine-related compound producing soil fungi from the Western Ghats, Tamil Nadu

The fungal kingdom has enormous biodiversity, with around 70,000 known species and an estimated total of about 1.5 million species, presenting a wide scope for further research. The fungal species are known for the production of enzymes and secondary metabolites, which have not been exploited completely. The best-known fungal secondary metabolites that are subjected to commercial production are the β-lactam antibiotics. The fungal genus *Tolypocladium*, first described by Gams in 1971, encompasses fungi imperfecti occurring in soil or litter habitats. This species is well characterized by white, hyaline or bright coloured, relatively slow-growing cottony colonies[1,2].

The species *T. inflatum* is unique and important as it produces the 11-amino acid cyclic peptide compound, cyclosporine[3]. This product was identified in the 1970s originally as an anti-fungal and later exploited as an immunosuppressant, revolutionizing organ transplant surgery[4]. However, the quantity of production of these metabolites varies with strains of *T. inflatum*[5,6]. Here we report *T. inflatum*-related fungi from the soil of the Western Ghats, producing cyclosporine-related metabolite. We have isolated wild-type fungus showing a three-fold increase in cyclosporine-related metabolite production compared to the MTCC reference culture (Ti) obtained from IMTECH (Chandigarh). Furthermore, these metabolites were tested for their antimicrobial activity and compared with commercially available cyclosporine drug (*cys* Sandimmune Neoral, Novartis).

Six hundred soil samples were collected in sterile plastic bags from various places in the Shola forest, Kodaikanal hills, Western Ghats (lat. 10°12′N, long. 77°30′E), Tamil Nadu, India. The soil samples were initially dissolved in sterile water and the supernatant was subsequently plated in potato dextrose agar medium. The suspected colonies were then plated in the selective malt extract agar medium. Three isolates of *Tolypocladium* spp. were finally isolated from the suspected colonies. Fungi were identified by analysing the morphological structure, colour of the colonies (white, red, orange) and their spores by agar block technique. The microscopic and morphological characteristics of the isolates (Ti-1, Ti-2 and Ti-3) were compared with Ti received from IMTECH for confirmation.

These fungal isolates were grown on malt extract medium for 6 days at 28°C. The red and orange pigmented colonies were selected and grown in 250 ml Erlenmeyer flask containing 100 ml of malt extract broth and were incubated at 28°C with an aeration speed of 200 rpm for 14 days. The cultures of the three different isolates and Ti were inoculated in separate sets of 100 ml malt extract medium in 250 ml Erlenmeyer flask. From these cultures, samples were collected at 2 days interval from the first day till the twelfth day. The samples were centrifuged (650 *g* for 15 min), pelletized and dry biomass was estimated. The supernatant was filtered through Sartorius cellulose membrane filter (0.2 μm) to get cell-free extract for the estimation of cyclosporine.

Presence of cyclosporine was characterized by High pressured or High Performance Liquid Chromatography (HPLC) technique (Central Electrochemical Research Institute, Karaikudi).

Subsequently, it was compared with the *cys* drug for confirmation. Secondary metabolite produced by isolates *T. inflatum*, Ti-1, Ti-2, Ti-3, Ti and *cys* drug showed antimicrobial activity against a battery of microorganisms (Kirby–Bauer disc diffusion method).

For testing the antimicrobial activity, bacterial cultures such as *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Serratia marcescens* and *Salmonella* spp., and fungal cultures such as *Aspergillus niger*, *Cryptococcus neoformis*, *Penicillium chrysogenum*, *Aspergillus flavus*, *Candida albicans* and *Trichoderma virgi* were used. The lawn was made in Mueller–Hinton Agar for bacterial cultures and Potato Dextrose Agar for fungal cultures. Nitrocellulose paper discs of size 0.6 mm were made, dipped in the filtrate and placed at the centre of a petri dish plated with the test microbial cultures. The plates were then incubated at 37°C for 24 h and 24°C for 48–72 h for the bacterial and fungal plates respectively.

The minimal inhibitory concentration (MIC) for Ti-1, Ti-2, Ti-3, Ti and *cys* drug was assessed (Agar dilution technique). Results showed that the isolate Ti-3 had maximal antimicrobial activity compared to other isolates (Ti-1 and Ti-2), but lower than Ti. Both Ti-3 and Ti have more microbicidal activity than the commercially available *cys* drug (Table 1). Secondary

Table 1. Minimal inhibitory concentration of secondary metabolites

Organism	Secondary metabolites (mg/dl)				
	Ti-1	Ti-2	Ti-3	Ti	*cys* drug
Escherichia coli	17.25	15	10	12	12
Proteus vulgaris	23	24	22	16	15
Klebsiella pneumoniae	–	–	25	25	25
Serratia marcescens	30	30	23	23	18.75
Salmonella typhi	–	–	23	25	25
Aspergillus niger	17.25	25	16	16	21
Cryptococcus neoformis	17.25	20	11	16	18.75
Penicillium chrysogenum	23	20	6	8	12.5
Saccharoymces cerevisiae	25	25	19	23	25
Aspergillus flavus	25	23	20	21	25
Candida albicans	20	21	16	16	16

metabolites (cyclosporine-related) from these extracts were found to have a broad spectrum of antifungal activity and a narrow spectrum of activity against bacterial cultures. Most of the Gram-positive cultures were found to be resistant to these secondary metabolites, except *Serratia marcescens* which exhibited high susceptibility. In contrast, most of the Gram-negative bacteria showed susceptibility. Antifungal activity of these extracts revealed that the secondary metabolite from Ti showed efficient antifungal activity. Even a very low concentration (0.08 mg/ml) was found to be effective in inhibiting most of the tested fungal cultures. Isolates Ti-2 and Ti-3 showed similar antifungal activity. However, the commercially available *cys* drug exhibited poor antimicrobial and antifungal activity. The *cys* drug required a high MIC indicating its lower efficiency as an antimicrobial compared to the extracts from isolates. Molecular characterization (PCR–RFLP and RAPD) of these isolates is underway.

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A new fossil palm leaf from the Hemis Formation of Ladakh, Jammu and Kashmir, India

A field trip was undertaken during August–September 2005 to explore the Ladakh area for plant fossils. A new fossiliferous site was discovered near Shingbuk (35°27′N; 77°59′E), 12 km NNW of Tsokar (Figure 1 *a*), which yielded about ten leaf impressions. Though most of them are fragmentary in nature, two well-preserved specimens have been selected for the present study. It is interesting to note that all these specimens belong to palms and no dicot leaf has been recovered from the site.

The newly discovered fossiliferous locality (Figure 2) lies in the Indus Suture Zone which divides the Himalayas from the Karakoram Mountains as well as the Tibetan Plateau. In tectonic interpretation it can be said that a large gap of the Tethys Ocean was consumed along this zone as a result of collision of the Indian Plate with the Eurasian Plate. It is separated from the Karakoram Tethys in the north by the South Karakoram (Nubra–Shyok) Thrust and from the Himalayan Tethys in the south by the Zanskar Thrust. Marine flysch and continental deposits are found to lie in juxtaposition in the zone. The molasse horizons in the Indus Suture Zone are divisible into the southern Hemis Formation (middle–late Eocene) and the northern Kargil Formation (late Oligocene–middle Miocene), though there is an apparent lack of consensus on the issue of age range of these formations for want of age diagnostic fossil remains[1–6]. The isolated stratigraphic units of the northern molasse belt, namely Basgo, Karroo, Khuksho, Nyoma, etc. are either further subdivisions or local equivalent names of the Kargil Formation. Similarly, the Liyan molasse is equivalent to the Hemis Formation in space and time. The older Hemis Formation thrusts over the younger Kargil Formation along a south-dipping Upshi Thrust.

Leaf impressions were collected from the Hemis Formation considered as middle–late Eocene in age and characterized by rocks of either silty sandstone with fine-grained micaceous sand or greenish-grey siltstone alternating with purple-brown siltstone. The fossil remains are preserved predominantly in the finer part of the siltstone horizons (Figure 1 *b*). Although Lakhanpal *et al.*[7] have given a detailed account of the Tertiary palaeobotanical data known from Ladakh, the only known