SUMMARY
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*Tolyphocladium* sp. was cultivated in four different media in shake flasks and it was found that the growth and cyc A yield were comparatively less in synthetic media than in complex media. Addition of the precursor amino acids valine and aminobutyric acid influenced the yield of cyc A. While medium 4 containing peptone as a nitrogen source supported good growth of the fungus, cyc A yield was maximum in medium 3 containing casein acid hydrolysate as a nitrogen source.

Diffusional and mechanical properties of alginate beads made out of different concentrations of alginate and different strength of calcium chloride indicated that beads made of 2% alginate possessed good mechanical and diffusional properties. The mechanical and diffusional properties were found to be influenced more by the concentration of the alginate used rather than the strength of the calcium chloride used for curing them.

A model for the production of immobilized biocatalysts was designed which was capable of producing 2-3 kg of biocatalysts under sterile conditions using a peristaltic pump.

The stationary phase mycelium at 30gm wet weight /100ml of 2%
alginate was used for the production of the immobilized biocatalyst. Cyc A was produced in packed bed reactors under batch and recycle mode. As the substrate was recycled, the yield of cyclosporine A also increased up to 3 cycles. This indicated that increasing the number of cycles leads to better utilization of the amino acids for biotransformation. However, beyond a certain level there was no increase in the yield which may be due to feed back inhibition by the product.

Immobilized spores when used in packed bed reactors under recycle mode also produced cyclosporine A to levels similar to that obtained with mycelium immobilized system.

The half-life of the packed bed reactor was found to be 180 days.

The cyc A obtained from the immobilized system was found to be identical with the standard both in its chemical and biological properties. The HPLC profile and IR spectrum of both the compounds were identical and their amino acid composition after hydrolysis and TLC analysis showed similarity in their amino acid composition.

The antifungal activity of cyc A obtained from the immobilized system was checked using Aspergillus niger. The biological potency of the cyc A obtained from the immobilized system and standard cyc A were compared by performing skin grafting experiments in rats. The
studies indicate that administration of both the compounds resulted in the acceptance of skin grafts and the toxicity symptoms associated with the administration of both the preparations were similar.

Two enzymes, SAM synthetase and Cyclosporine synthetase complex involved in the synthesis of cyclosporine were purified and characterized.

As the mycelium grows and enters the stationary phase, SAM synthetase activity was also found to increase with corresponding increase in the yield of cyc A. This indicates a positive role of this enzyme in the synthesis of cyclosporine. This enzyme may supply the S-adenosyl methionine which is the methyl donor to the cyclosporine synthetase during the synthesis of cyclosporine.

These two enzymes were extracted from the mycelium and purified. Biochemical studies performed with the purified SAM synthetase indicate that its molecular weight was approx. 28 kDa. The enzyme had an optimum pH between 7-9 with a maximum activity at 8.0. The enzyme was not stable at higher temperatures (temperature above 0 40 C) and had maximum activity at 37 C.

The enzyme required magnesium ions for its activity although addition of manganese restored the activity to some extent. The enzyme was inhibited by iodoacetic acid, EDTA and sodium fluoride and
required -SH groups for its activity.

Cyclosporine synthetase activity was detected based upon the binding of C-leucine to the enzyme. Amino acid binding studies indicate that leucine binds to the enzyme both covalently and non-covalently. First, an intermediate aminoacyl-enzyme complex is formed after which the adenylate bound amino acid is transferred to the enzyme-bound sulhydryl group to form a thio-ester bond.

Methyl leucine did not compete with leucine for the binding sites on the enzyme indicating the enzyme specificity for amino acids and not their methyl amino acids.

Methyltransferase activity based on the conversion of amino acid to their corresponding methyl amino acids was detected in the cyclosporine synthetase complex. Analysis of methyl amino acids in the enzyme supernatant and the TCA precipitate indicated that methyl amino acids were bound to the enzyme and thus methylation takes place only after the binding of the amino acids to the enzymes by thio-ester bond.

Methyl amino acids were not detected in the mycelial amino acid pool which further indicates the enzyme specificity for the amino acids and also that methylation of the amino acids occurs only after the amino acid is bound to the enzyme complex.
SAM synthetase activity was not detected in the cyclosporine synthetase complex which indicates that two different enzymes operate for the synthesis of cyclosporine and that they are compartmentalized inside the mycelium.