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A Study on Cyclosporine a Production by Fungal Cells

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Abstract: Cyclosporin A (CyA) is found as an antibiotics, exhibiting immunosuppressive and antifungal properties. Cyclosporine A has been synthesized in large quantities by fermentation process using various fungal species such as *Tolypocladium, Trichoderma, Fusarium Penicillium and Aspergillus* spp. Submerged liquid fermentation (SLF) and solid state fermentation (SSF) have been used successfully to produce the Cyclosporin A. The SSF is alternative to submerged fermentation for production of value added products like antibiotics. In solid-state fermentation (SSF), growth of microbes is occurred without free flowing aqueous phase. It provides low availability of water and reduces the possibilities of contamination by bacteria and yeast. SSF can provide higher yields and other advantages for this product. Due to the concentrated nature of the substrate, smaller reactors could be used in SSF. An industrial point of view, it is necessity to obtain a suitable and economic medium and process for higher production of CyA. CyA is produced by using *Tolypocladium inflatum* in solid state fermentation.

Keyword: *T. inflatum, Cyclosporin A, fungal, antibiotics, yields, SSF*

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I. Introduction:

Cyclosporines are reported as a member of the group of cyclic peptides and are made up of 11 amino acids. Cyclosporine A is the major component of the cyclosporines and showing distinguishes at carbon number 2 in type of amino acid from other cyclosporines. It is the only member of this group used clinically (Anjum et al., 2012). *Trichoderma polysporum has been used to* produce Cyclosporine A by submerged fermentation as aerobic fungi (Dreyfuss, et al., 1976). In Solid state fermentation (SSF) rotary drum bioreactor is used to provide the better aeration and mixing of solid substrate, which resulted to achieve the higher productivity, yield and concentration for metabolites. Production of meroparamycin by *streptomyces* sp. strain MAR01 is reported. They The rice, wheat bran, Quaker, bread, and ground corn as solid substrates used and also screened for their ability to support meroparamycin production in solid-state fermentation (El-Naggar et al., 2009). Impact of cheap solid substrates as a carbon and nitrogen sources on CyA and ochratoxin A antibiotic production by *Tolypocladium inflatum* and two species of *Penicillium* respectively has been studied and it has reported that it can reduce current market price of these antibiotics (Rao et al., 2012). The formation of CyA has been reported under different fermentation conditions (including selection of the cultivation medium, fermentation time course, inoculum nature and size, and pH value) which affect physiological condition of *T. inflatum*. It is influenced by carbon and nitrogen sources and cultivation condition. Analysis of fungal morphology and differentiation of *Tolypocladium inflatum* mycelium is shown a relation with CyA formation. And kinetics of the process, in terms of associated metabolite formation with biomass growth is also played role in CyA formation and cyclosporine A has been produced by biosynthetic pathways via use cyclosporine synthetase enzyme. (Survase et al., 2008). While there are numerous report on the use of these compounds in clinical studies, the microbiological aspects of cyclosporine A has been less well documented. CyA has application of in medical field in transplantation surgery and treatment in autoimmune disease (Christians, and Sewing, 1993). Enhance the yield and productivity of CyA is achieved by the use of cheap solid substrates with better mixing and aeration of solid nutrient. The kinetic study of hyphal growth pattern with metabolite formation rate in solid state fermentation can help for using cheap solid substrate medium. CyA is also reported to produce by static fermentation and also synthesized enzymically (Billich, and Zocher, 1987). Rotary drum bioreactor is for mixing and aeration of solid nutrients which enhance the Cyclosporine A production. By using low cost solid substrates as solid medium would reduce market price of Cyclosporin A (current price of High Purity is in the range of US $1,000 - 1,500 / Kilogram) (Ellaiah et al., 2002). Here author will study the production cyclosporine A and do the analysis of media components on it.

Cyclosporine A

Cyclosporine A is the major component of the cyclosporines, which distinguishes from other cyclosporines by the type of amino acid at carbon number 2. It is the only member of this group used clinically (Tehmina et al., 2012).

Cyclosporine is found as an anti-fungal antibiotic with narrow range spectrum drug. All the cyclosporine has been made from *Tolypocladium inflatum* cultures without it ever reaching the sexual state. *Tolypocladium inflatum* is an asexual ascomycete fungus originally isolated from a Norwegian soil sample, produces cyclosporine A (Hodge et al 1996). Immunosuppression property of cyclosporine A was found by J. F. Borel in 1976. In 1983, cyclosporine was approved for clinical use to prevent graft rejection in transplantation (Borel, 1986). Most of the surgical problems of allograft transplantation had already been solved by that time. Before it, the standard method of achieving immunosuppression had been done with a combination of azathioprine and corticosteroids (Rang et al., 2000). Pure undissolved cyclosporine powder was given in gelatin capsules. The drug was not absorbed and trials were stopped until absorption from the gastrointestinal tract could be achieved. Cyclosporine A is sufficiently non-toxic and powerful as an immunosuppressant to make an attractive candidate for clinical investigation in patients receiving organ grafts (Borel, 1986; Christians and Sewing, 1993).

Production of Cyclosporin A

Production of Cyclosporine A has been occurred by submerged fermentation by using local isolate of *Penicillium fellutanum* and medium used for it, composed of glucose, 5%; peptone, 1%; KH2PO4, 0.5% and KCl, 0.25% (w/v). And *Penicillium fellutanum* (FCBP 937) isolated from Guava fruit showed 6.18 µg/ml as a concemtration (Anjum et al., 2012). The production of Cyclosporin A is found by submerged liquid and solid state fermentation (i.e. by using the solid substrate) by *Tolypocladium* sp. *Tolypocladium inflatum* as an organism of choice gives more yield and is recently used process. *Tolypocladium inflatum* strains when grown on moist wheat bran produced 310–459 mg of Cyclosporin-A kg of bran−1. *Tolypocladium inflatum* ATCC 34921 has produced the 459 mg of Cyclosporin-A /kg of bran compared with improvement of production concentration i.e.1031±27 mg of Cyclosporin-A/ kg of bran by subjecting the spores to different mutagenic treatments. The mutated strain of Tolypocladium inflatum DRCC 106 has produced better improvement in concentration i.e. 4843 mg Cyclosporin-A /kg of bran keep under optimum fermentation conditions in 10 days. They has grown on wheat bran medium containing millet flour 20%, jowar flour 10%, zinc sulphate 0·15%, ferric chloride 0·25% and cobaltous chloride 0·05% with optimal inoculum (60%), initial moisture content (70%), initial bran pH- 2·0 and incubation temperature 25°C (Murthy et al., 1999).

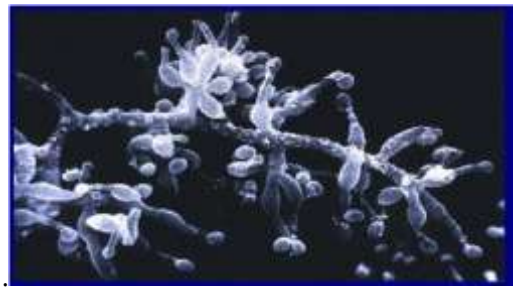

Figure 1. Scanning electron micrograph of *Tolypocladium inflatum (*Murthy et al., 1999)

Optimized fermentation media composition has enhanced production of CyA. Statistical optimization of CyA production is done on a semi synthetic medium using *T. inflatum.* But changes in morphology of fungal species have been found to affect the quantity or concentration of particular fermentation product formation (Dobson et al., 2008,). Different microbial morphology is reported in many fermentation processes depending on culture conditions and the genotype of the strain. *Streptomyces avermitilis* strain pellet morphology is found more favorable for avermectin production (Peng et al., 2008). Control of suitable fungal morphology of *Rhizopus chinensis* strain has helped to obtain higher lipase productivity in submerged fermentation. Maximum yield of geldanamycin by *Streptomyces hygroscopicus* is found by limiting its appropriate size of pellet formation (Dobson et al., 2008). Sekar et al has reported the increase the yield of Cy A by hydrolysing the wheat bran using dilute HCl. He has used the different solvents for the optimization of extraction of Cyc A from the fermented bran. Optimization of the production of cyclosporin A by solid state fermentation has been reported and studied the several parameters including (a) tray fermentation with and without perforation (b) thickness of solid substrate bed (c) type of inoculum (d) size of inoculum (e) effect of relative humidity for the optimum production of Cyclosporin A (Sekar et al., 1997; Sekar and Balaraman, 1998). Survase et al has worked on statistical optimization of Cyclosporin A production on a semi-synthetic medium using *Tolypocladium inflatum* MTCC 557. They used to optimize the fermentation conditions for maximum production of cyclosporin A (CyA) via screening of various carbon and nitrogen sources by response surface methodology (RSM) (Survase et al., 2009).

Design of rotating drum bioreactor

The fermentor would consist of a 250-500 mL glass roller bottle (Ø 5-14-28 cm) connected to a filtered air supply. Air flow is measured by a rotameter and then is sterilized by passing through a 0.45 µm cellulose filter. The humidifier system is based on a glass column filled with glass beads (3-5mm) and sterilized distilled water. The air is introduced in the roller bottle through a syringe, which is assembled to a barbed wire to remove the solid stuck in the bottle. Suitable

reactor parameters for the desired biological, chemical and physical (macrokinetic) system is based on bioreactor design. The kinetic system includes microbial growth and metabolite production. The bioreactor must be designed to both promote formation of the optimal morphology of the organism and to eliminate or reduce contamination by unwanted organisms or mutation of the organism. Bioreactor design involves the bioreaction parameters, including controlled temperature, optimum pH, sufficient substrate, water availability, oxygen (for aerobic processes), product and byproduct removal. Different types of cheap solid substrates can be used as solid substrates. It may be starchy substrate (e.g. wheat bran, rice) or soluble sugars containing substrates (e.g. sweet sorghum, sugar-beet, pineapple peel waste). These substrates have different impact or effect on CyA productivity and *T. inflatum* growth when uses as solid medium in rotary drum bioreactor. To perform the solid state fermentation we need to take following considerations such aeration (liter/min), mixing of nutrient media (in rpm), components of nutrient media, inoculum size (in %) and age (in hours), size of solid bed (length, width and height), incubation period (days) temperature and pH. For starch substrates, it is necessary to go for acid hydrolysis (by use of dilute HCl) for better solublization. These parameters would help to measure the rate of consumption or utilization (Stuart et al., 1999).

II. Conclusions:

Cyclosporin A (CyA) has shown an immunosuppressive and antifungal properties. Cyclosporine A is synthesized by fermentation process using various fungal species Submerged liquid fermentation (SLF), solid state fermentation (SSF) and enzymatic hydrolysis method have been applied to produce the Cyclosporin A. The SSF is good for production of value added products like antibiotics and growth of microbes has reported without percentage of aqueous phase. It helps in reduction of contamination by bacteria and yeast with higher yields and other advantage. CyA is produced by using *Tolypocladium inflatum* in solid state fermentation suitable by using economic medium and process for higher production. Rotary drum bioreactor designed is to promote formation of the optimal morphology of the organism with elimination of contamination by unwanted organisms or mutation of the organism.

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