General Medicine: Open Access
ISSN: 2327-5146

khan, Gen Med (Los Angeles) 2017, 5:4
DOI: 10.4172/2327-5146.1000294

Review Article Open Access

Cyclosporin A Production from *Tolipocladium inflatum*

Nida Tabassum Khan*

Department of Biotechnology, Faculty of Life Sciences and Informatics, Balochistan University of Information Technology Engineering and Management Sciences, (BUITEMS), Quetta, Pakistan

***Corresponding author:** Nida Tabassum Khan, Department of Biotechnology, Faculty of Life Sciences and Informatics, Balochistan University of Information Technology Engineering and Management Sciences, (BUITEMS), Quetta, Pakistan, Tel: 03368164903; E-mail: nidatabassumkhan@yahoo.com

Rec date: July 12, 2017; **Acc date**: August 4, 2017; **Pub date**: August 10, 2017

Abstract

The demand for this drug in the US market is estimated at $450 million. Cyclosporin A demand is increasing worldwide because of its enhanced anti-fungal and anti-viral potency that it can be used to treat diseases like canine skin disease and rheumatoid arthritis. Cyclosporin A is easily produced from the fungus *Tolipocladium inflatum* by the process of fermentation in a bioreactor under optimum reaction conditions to obtain maximum yield of the antibiotic. Currently attempts have been made to increase its solubility to enhance its absorption in the body.

Keywords: *Tolipocladium inflatum*; Cyclosporin A; Submerged culture fermentation

Introduction

Tolipocladium inflatum is an ascomycete fungus, a pathogen of beetle larvae and best known as the producer of the anti-fungal antibiotic cyclosporin A. This fungus is a prolific producer of bioactive secondary metabolites with potential applications in medicine and agriculture. It was originally isolated from Norwegian soil sample. *Tolipocladium inflatum* is the asexual state whereas the sexual state *Elaphocordyceps subsessilis* is the pathogen of beetles and is widely distributed in the soil [1] (Table 1).

Kingdom	Mycota
Sub-kingdom	Diakarya
Phylum	Ascomycota
Sub-phylum	Pezizomycotina
Class	Sordariomycetes/Pyrenomycetes
Sub-class	Sordariomycetidae
Order	Hypocreales
Family	Clavicipitaceae
Genus	Tolipocladium
Species	*T. inflatum*

Table 1: Classification of *Tolipocladium inflatum.*

Morphology

During the asexual phase of its life cycle, *Tolipocladium inflatum* lives as saprotrophs in soil. Asexual mode of reproduction is by means of conidia, positioned on phialides with inflated bases. While reproduce sexually by producing ascospores through the fusion of opposite mating type spores [2].

Cyclosporin A

This drug possesses low toxicity to mammals, anti-viral and anti-fungal properties. It is the most commonly prescribed immunosuppressive drug for the treatment of patients with organ transplantation, autoimmune diseases including AIDS, owing to its superior T-cell specificity and low levels of myelotoxicity. Organisms like *Tolipocladium inflatum*, *Fusarium solani*, *Neocosmospora varinfecta,* and *Aspergillus terreus* are well known producers of cyclosporin A. It possesses anti-fungal properties. Cyclosporin A consisted of 11 hydrophobic amino acids. It is neutral and very soluble in all organic solvents. Molecular weight of cyclosporine A 1202.6 g/mol with UV absorption of 215 nm [3] (Figure 1).

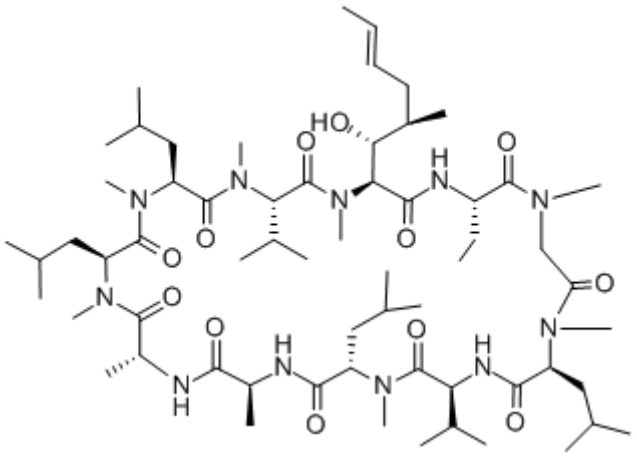

Figure 1: Chemical structure of Cyclosporin A and Molecular formula: $C_{62}H_{11}1N_{11}O_{12}$ [4].

Mode of action

Cyclosporin A is a non-polar cyclic oligopeptide produced by the fungus *Tolipocladium inflatum.* Cyclosporin A is an important immunosuppressive agent that inhibits the functioning of several

proteins that are involved in the activation of T-cells at the level of mRNA transcription thus affecting T-lymphocytes. Cyclosporin A binds with its intracellular receptor cyclophilin to form a unique compex. This complex then binds to calcineurin which is a calcium and calmodulin dependant protein phosphatases, inhibiting its enzymatic activity. It was found that cyclosporin A suppresses the replication of hepatitis C virus genome in cultured hepatocytes. It can also inhibit IL2 resulting from T cell activation through calcineurin inhibition and also block cytochrome c release from mitochondria [5] (Figure 2).

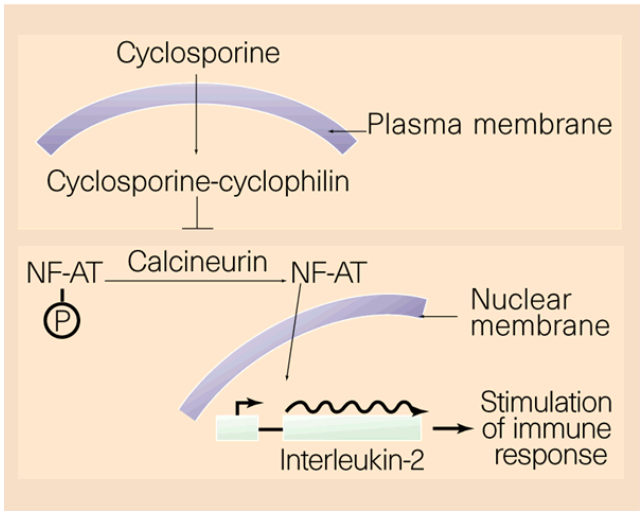

Figure 2: Mode of action of cyclosporin A [6].

Methods of production

Cyclosporin A is commonly produced by the following given methods:

- Submerged culture fermentation
- Static fermentation
- Solid state fermentation
- Enzymatic synthesis [7]

Requirements of fermentation process for Cyclosporin A production

- Carbon source i.e., glucose, sucrose, maltose, glycerol etc. It influences morphological differentiation of the fungus *Tolipocladium inflatum.*
- Organic and inorganic nitrogen sources i.e., casein peptone, bactopeptone, yeast extract, xylose, galactose, beef extract, malt extract etc.
- Amino acids like L-valine, DL amino butyric acid, L-leucine, glycine, DL methionine etc.
- Solvents like concentrated hydrochloric acid, acetonitrile, sodium hydroxide, n-butyl acetate, sulphuric acid etc.
- Salts like sodium chloride, calcium chloride, zinc sulphate, magnesium sulphate, magnesium chloride, ferric chloride, Tween 20 etc.
- Agar

Fermentation conditions

- Organism subculture incubated at 25 degree centigrade for 7 or 12 days
- Fungal agar disc from 7 days old culture or 12 days old culture
- 100 ml of autoclaved broth medium
- Incubation conditions for cultured flask at 30 degree centigrade for 7 days
- Agitation rates at 150 and 200 rpm
- pH range of the medium 2 to 9
- Aerobic conditions [8,9]

Experimental Procedure

Fungal agar disc from 7 days old culture or 12 days old culture was inoculated into 100 ml of autoclaved broth medium in a rotatory shaker that contains all the essential nutrients required for the fungal growth under appropriate incubation conditions of 30 degree centigrade for 7 days.

Then it is transferred to a fermentation bioreactor with proper aeration i.e., dissolved oxygen supply since *Tolipocladiun inflatum* grows under aerobic conditions. Probe indicators and pH meter detect temperature and pH changes respectively and is automatically adjusted to optimum conditions [10].

During the theoretical phase of the fermentation reaction when maximum production of cyclosporin takes place as indicated by the curve analysis techniques that monitors the ongoing fermentation reaction (Figure 3).

After that it is followed by a decline phase in which cyclosporin production slows down because of depletion of one or more critical carbon sources used which act as limiting reagents.

The desired drug is then separated and purified by filtration and distillation i.e., downstream processing [11].

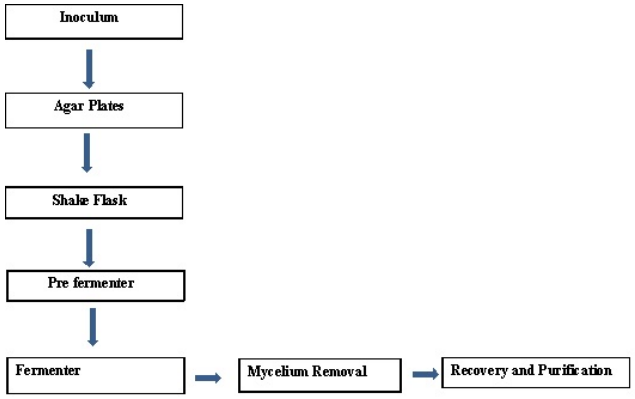

Figure 3: General outline of fermentation process.

Current research and development of Cyclosporin A

Attempts have been made in order to increase the level of absorption of Cyclosporin A in the body. Therefore different approaches had been used to solubilize the highly hydrophobic cyclosporin A. So extensive study of cyclosporin A structure will help

the researchers to understand its highly hydrophobic nature which in turn will aid in devising strategies or methods to solubilize this drug. Work has been carried out that highlighted the immunosuppressive nature of this drug by using different dosage levels in different model organisms [12]. Further it was revealed that Cyclosporin A selectively inhibits lymphocytes proliferation at the same time does not affects somatic cells proliferation. Series of experiments were conducted by using applications of genetic engineering to genetically transform the fungus in order to increase its growth rate for large scale production of cyclosporin A [13,14]. For this purpose various genetically modified strains of *Tolipocladium* are under study. Besides, optimization of fermentation process by controlling and maintaining optimum reaction conditions is another effort to increase the yield of the obtained product. Currently new oral cyclosporin A has been developed for patients with rheumatoid arthritis [15-17].

Uses of Cyclosporin A

- Prevention of tissue rejection in case of organ transplantation e.g. Kidney, liver, heart and bone marrow transplants.
- Acts as T-lymphocytes suppresses and also inhibits interleukins.
- Used in combination with other immunosuppressant and steroid medications.
- Use to treat canine skin disease and rheumatoid arthritis [18].

Side effects of Cyclosporin A

- Causes metabolic alterations in liver, muscle and adipose tissue, which may contribute to the development of dyslipidemia and insulin resistance [19]
- Acute kidney injury
- Hyperlipidemia (especially hypertriglyceridemia)
- Hypertension
- Gastrointestinal upset and headaches[20]
- Hypertrichosis [21]
- Increase testosterone level [22]
- Gingival overgrowth [23]

Conclusion

Cyclosporin A is one of the mostly used drugs that are easily produced from a biological organism such as fungi. This drug is used to treat patients with organ transplantation, canine skin disease and rheumatoid arthritis.

References

1. Svarstad H, Bugge HC, Dhillion SS (2000) From Norway to Novartis: Cyclosporin from *Tolypocladium inflatum* in an open access bioprospecting regime. Biodivers Conserv 9: 1521-1541.
2. Hodge KT, Krasnoff SB, Humber RA (1996) *Tolypocladium inflatum* is the anamorph of *Cordyceps subsessilis*. Mycologia 88: 715-719.
3. Dreyfuss M, Härri H, Hofmann H, Kobel H, Pache W, et al. (1976) Cyclosporin A and C. European J Appl Microbiol 3: 125-133.
4. Wenger RM (1982) Cyclosporin A. Biomedical Journal 3: 19-31.
5. Wenger RM (1989) Pharmacology of cyclosporin. Pharmacol Rev 41: 243-247.
6. Wenger RM (1984) Total synthesis of 'cyclosporin A' and 'cyclosporin H', two fungal metabolites isolated from species *Tolypocladium inflatum* Gams. Helvet Chimica Acta 67: 503-515.
7. Wenger RM (1986) Synthesis of cyclosporin and analogues: Structural and conformational requirements for immunosuppressive activity. Prog Allergy 38: 46-64.
8. Petcher TJ, Weber HP, Rüegger A (1976) Crystal and molecular structure of an iodo-derivative of the cyclic undecapeptide cyclosporin A. Helv Chim Acta 59: 1480:157.
9. Rang HP, Dale MM, Ritter JM (1999) Pharmacology (4th edn) Churchill Livingstone Publishers, London.
10. Stähelin HF (1996) The history of cyclosporin A (Sandimmune®) revisited: Another point of view. Experientia 52: 5-13.
11. Kahan BD (1984) Cyclosporine: Nursing and paraprofessional aspects. Grune and Stratton, NY, USA.
12. Hassan MM, Al-Yahya MA (1987) Analytical profiles of drug substances. 16: 145-206.
13. Borel JF (1986) Ciclosporin and its future. Prog Allergy 38: 9-18.
14. Bach JF (1999) The contribution of cyclosporine A to the understanding and treatment of autoimmune diseases. Transplantation Proceedings 31: 16S-18S.
15. Cavanak T, Sucker H (1986) Formulation of dosage forms. Prog Allergy 38: 65-72.
16. Borel JF, Feurer C, Gubler HU, Stähelin H (1976) Biological effects of cyclosporin A: A new antilymphocytic agent. Agents Actions 6: 468-475.
17. Schran HF, Robinson WT, Abisch E, Niederberger W (1986) Bioanalytical considerations. Prog Allergy 38: 73-92.
18. Calne RY, White DJ, Thiru S, Evans DB, McMaster P, et al. (1979) Cyclosporin A in patients receiving renal allografts from cadaver donars. Lancet 2: 1323-1327.
19. Fuhrmann A, Lopes PC, Sereno J, Pedro J, Espinoza DO, et al. (2014) Molecular mechanisms underlying the effects of cyclosporin A and sirolimus on glucose and lipid metabolism in liver, skeletal muscle and adipose tissue in an *in vivo* rat model. Biochem Pharmacol 88: 216-228.
20. Foley C, Leonard N, Wynne B (2015) Cutaneous pseudolymphoma: A rare side effect of cyclosporine. J Am Acad Dermatol 72: e85-e86.
21. Rosmarin DM, Lebwohl M, Elewski BE, Gottlieb AB (2010) Cyclosporine and psoriasis: A 2008 National Psoriasis Foundation Consensus Conference. J Am Acad Dermatol 62: 838-853.
22. Cutolo M, Giusti M, Villaggio B, Barone A, Accardo S, et al. (1997) Testosterone metabolism and cyclosporin A treatment in rheumatoid arthritis. Br J Rheumatol 36: 433-439.
23. Dongari A, McDonnell HT, Langlais RP (1993) Drug-induced gingival overgrowth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 76: 543-548.