J. Microbiol. Biotechnol. (2010), **20**(7), 1086–1091
doi: 10.4014/jmb.1001.01006
First published online 18 May 2010

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Gellan Gum as Immobilization Matrix for Production of Cyclosporin A

Survase, Shrikant A.*, Uday S. Annapure, and Rekha S. Singhal

Food Engineering and Technology Department, Institute of Chemical Technology, University of Mumbai, Matunga, Mumbai 400 019, India

Received: January 8, 2010 / Revised: March 24, 2010 / Accepted: April 12, 2010

This study explored the use of gellan gum as an immobilization matrix for the production of cyclosporin A (CyA) by immobilized spores and mycelia of *Tolypocladium inflatum* MTCC 557. Different carriers, such as gellan gum, sodium alginate, celite beads, and silica, were tested as immobilization carriers, along with the role of the carrier concentration, biomass weight, number of spore-inoculated beads, and repeated utilization of the immobilized fungus. The maximum CyA production was 274 mg/l when using gellan gum [1% (w/v)], and a mycelial weight of 7.5% (w/v) supported the maximum production of CyA. Additionally, the addition of a combination of L-valine (6 g/l) and L-leucine (5 g/l) after 48 h of fermentation produced 1,338 mg/l of CyA when using gellan gum. The immobilized mycelia beads were found to remain stable for four repetitive cycles, indicating their potential for semicontinuous CyA production.

Keywords: Cyclosporin A, gellan gum, immobilization, fermentation, *Tolypocladium inflatum*

Cyclosporin A (CyA) is a cyclic undecapeptide that exhibits a variety of biological activities, including anti-inflammatory, immunosuppressive, antifungal, and antiparasitic properties [11]. It is also an effective immunosuppressant in organ transplantation and in the treatment of autoimmune diseases [17].

The immobilization of whole cells can serve as a means for the immobilization of multienzyme conjugating systems, as microbial cells contain metabolic systems that mediate complicated reactions. Among the many advantages of whole-cell immobilization, eliminating the tedious and time-consuming procedures required for extracting and purifying the product is the most important, whereas other advantages include convenience in handling, lower susceptibility to contamination, and ease of product separation. Nondividing immobilized cells only require maintenance energy, and the yield is generally greater than that obtained from submerged fermentation. In fungal cell fermentations, highly branched hyphal filaments result in suspension cultures with high viscosities and a reduced mass transfer. Immobilization also facilitates the use of a dense cell population without any influence on the rheological properties of the suspending medium [18].

The production of CyA using free cells of *Tolypocladium inflatum* in shake flasks has already been described by Abdel-Fattah *et al*. [1] and Agathos *et al*. [2]. Different carrier materials have also been used by other researchers to immobilize the spores and mycelia for the production of CyA. For example, Chun and Agathos [8] studied the immobilization of *T. inflatum* conidia into porous celite beads; Sekar and Balaraman [28] used sodium alginate as the carrier material for the production of CyA in a packed bed reactor under batch and continuous flow modes; Sallam *et al*. [25] studied the immobilization of *A. terreus* spores and mycelia in sodium alginate for the production of CyA; Chun and Agathos [9] compared the physiological and environmental effects of CyA production using suspended and immobilized cells of *T. inflatum*, and found a significant difference in the precursor flow between the immobilized and free cell systems; and Lee *et al*. [21] developed an efficient sporulation/immobilization procedure to shorten the time and number of steps of sporulation.

Celite consists of highly porous diatomaceous beads composed of 90% silica plus some other inorganic oxides. However, because of its inertness and unique interconnected pore structure suitable for the physical entrapment of mycelial cells, there is growing interest in the use of celite beads as a biosupport [5, 16]. Silica is a polymer of silicic acid, consisting of SiO_4 interlinked in a tetrahedral fashion, which gives it the stoichiometry of SiO_2. Silica gel is a porous, granular form of silica, synthetically manufactured from sodium silicate or silicon tetrachloride or a substituted chlorosilane/orthosilicate solution [15]. Sodium alginate is

**Corresponding author*
Phone: +91-022-24145616; Fax: +91-022-24145614;
E-mail: shrikantraje1@rediffmail.com

the sodium salt of alginic acid, which is a linear copolymer with a homopolymer of (1-4)-linked β-D-mannuronate and its C-5 epimer α-L-guluronate residues, respectively, covalently linked together in different sequences or blocks. Owing to its biocompatibility and simple gelation with divalent cations, such as Ca^{2+}, sodium alginate is widely used for cell immobilization and encapsulation [14, 24, 25].

Gellan gum is a high-molecular-weight anionic linear exopolysaccharide produced by pure culture fermentation from *S. paucimobilis* [4]. It is already used for the controlled release of drugs, such as paracetamol [19] and cimetidine [22]. Sun and Griffiths [29] also described the viability of *B. infantis* immobilized in a mixture of gellan and xanthan beads, where bacteria isolated from infant feces were immobilized in polysaccharide gel beads (2.5% gellan gum, 0.25% xanthan gum) using a two-phase dispersion process [10]. Moreover, Wang *et al*. [32] investigated the degradation of carbazole by immobilized *Sphingomonas* sp. strain XLDN2-5 cells, and found gellan gum to be a better immobilization material than agar, alginate, or κ-carrageenan, whereas Ferrance [12] reported on the use of gellan gum beads as a novel substrate for protein immobilization and immobilized protein activity measurements.

However, as yet, there would seem to be no report on the use of gellan gum as a carrier for the immobilization of *T. inflatum* for the production of CyA. Accordingly, the present study investigated different immobilization parameters for gellan gum beads, including the carrier concentration, biomass concentration, and reusability.

MATERIALS AND METHODS

Materials

The glucose, yeast extract, casein peptone, bactopeptone, malt extract, sodium alginate, celite 545, and agar were procured from Himedia Ltd, Mumbai, India. The salt, including sodium chloride and calcium chloride, the amino acids, including L-valine, L-leucine, glycine, DL-amino butyric acid, and DL-methionine, and the solvents, including acetonitrile, *n*-butyl acetate, sodium hydroxide, concentrated hydrochloric acid, and sulfuric acid, were all purchased from S. D. Fine Chemicals Ltd. Mumbai, India. The silica gel was procured from Sisco Research Laboratories Pvt. Ltd, India. All the solvents used were of AR grade, except for acetonitrile, which was of HPLC grade. The standard CyA (authentic sample) was a gift sample courtesy of RPG Life Sciences Ltd., Mumbai, India and the gellan gum, Kelcogel, was generously provided by C. P. Kelco, U.S.A.

The *T. inflatum* MTCC 557 (indicated as *Beauveria nivea* in the MTCC catalog) was procured from MTCC, Chandigarh, India. The culture was maintained on agar slants containing 2% malt extract and 0.4% yeast extract (MYA), pH 5.4, at 4°C, after being grown for 12 days at 24°C.

Spore Propagation

A series of agar slopes of a malt extract–yeast extract agar (MYA) medium was inoculated with the pure culture. The inoculated slants were then incubated for 12 days at 25°C. The spores were harvested by the addition of 10 ml of sterile distilled water to each slant and gentle scraping of the agar surface under aseptic conditions.

Spore Immobilization Using Gellan Gum Carrier

The method reported by Sallam *et al*. [25] was used for the immobilization of the spores with sodium alginate and gellan gum as the carrier. Aliquots of the spore suspension (10^8–10^9 spores/ml) were added to the carrier solution. The carrier spore mixtures were then added dropwise to a sterile 2% calcium chloride solution, resulting in fine pellets with diameters ranging from 2–3 mm. The beads were allowed to harden for 1 h. A number of beads (100 beads/100 ml medium) were then added to the production medium, and incubated at 180 rpm for 14 days at 25±2°C.

Spore Immobilization Using Celite and Silica Carrier

The method adopted by Gbewonyo and Wang [13], and Chun and Agathos [8] was used for the immobilization of the spores. The celite beads were washed with distilled water several times and heated in a furnace overnight at 600°C, and then autoclaved for 1 h and dried at 121°C for 30 min. The spore suspensions (prepared as described before) were added to the dry celite beads based on a ratio of 2:1 (v/v), and stirred at 200 rpm for 2 h, and the supernatant was decanted to obtain the celite–spore mixture. Finally, aliquots of a sterile semisynthetic medium (SSM) consisting of (g/l) glucose (58.46), casein peptone (8.66), KH_2PO_4 (4.48), and KCl (3.23), pH 5.6, were added aseptically to the immobilized celite beads dispensed in 250-ml flasks. A similar procedure was followed for the silica. The inoculated beads were then incubated at 180 rpm for 14 days at 25±2°C.

Mycelia Immobilization

The mycelia immobilization using gellan gum was performed using the method reported by Schlosser *et al*. [26] and Schmuader *et al*. [27]. Samples of the wet mycelia were added to a sterile carrier solution. The carrier–mycelia mixture was then added dropwise to a 2% calcium chloride solution to obtain spherical beads (3–4 mm diameter). These beads were then transferred to a 250-ml flask containing SSM and allowed to grow on a rotary incubator shaker at 180 rpm for 14 days at 25±2°C.

Optimization of Immobilization Parameters

Different carriers were initially screened for the maximum production of CyA by immobilizing *T. inflatum* spores. The carrier that supported the maximum production was then selected for further studies. In addition, the concentration of gellan gum was varied between 0.25% and 1.25% (w/v), and the effect of the immobilized spores and mycelia on the production of CyA was evaluated.

The effect of the mycelia concentration on the CyA production was studied by immobilizing various quantities [2% to 12.5% (w/v)] of wet biomass using gellan gum as the carrier. All the spore-loaded beads were transferred to the production medium and the flasks incubated on a rotary incubator shaker at 180 rpm for 14 days at 25±2°C. The CyA production was studied by inoculating different numbers of spore-loaded beads (25–100 beads per 100 ml medium).

The effect of adding an optimized combination of L-valine (6 g/l) and L-leucine (5 g/l) on the production of CyA was also studied. Additionally, the mycelia-immobilized beads, prepared using gellan gum as the carrier, were evaluated for repetitive cycles of CyA

production. The beads were separated after every fermentation cycle and transferred to a fresh production medium. The free cells in the fermentation medium were checked for CyA production.

Analytical Determinations

CyA extraction and estimation. The culture broth was homogenized to ground the beads using a high-speed stirrer. The CyA extraction from the culture broth was then carried out according to the method of Agathos *et al*. [2]. A 10-ml sample of the homogenized broth was extracted with an equal volume of *n*-butyl acetate. Before extracting the sample, a concentrated solution of NaOH was added to reach a concentration of 1 N and heated at 60°C for 30 min. The mixed sample was then kept on a rotary shaker (180 rpm) for 24 h. After centrifuging, the extract was filtered using Whatman filter paper (No.1) and then a Pall 0.2-μm membrane filter (Ultipor N_{66} Nylon 6; 6 membranes) to give a clear extract. One ml of the extract was evaporated to dryness under a vacuum, and the dried extract dissolved in an equal volume (1 ml) of HPLC−grade acetonitrile. Twenty μl of the sample was then analyzed for the CyA content using an HPLC (Jasko system) fitted with a reverse-phase column Waters Sperisorb ODS (C_{18} octadecyl silane, 250×4.6 mm ID) using the method described by Survase *et al*. [30]. The mobile phase consisted of acetonitrile and water in a ratio of 70:30 with a flow rate of 1 ml/min. The temperature of the column was maintained at 70°C and the HPLC profile was monitored at 210 nm.

Estimation of biomass. The free cells and cells that had leaked from the matrix were collected by centrifugation at 3,000 rpm for 10 min, dried at 80°C to a constant weight, and reported as the dry cell weight (DCW). The biomass immobilized on the beads was measured by drying the beads at 80°C and subtracting the weight of the dried beads without immobilization.

Data analysis. The differences among the CyA yields, DCWs, and bead sizes were evaluated using NCSS software (Version 12.0.1 for Windows, 2003) with Duncan's multiple ranges.

Results and Discussion

When screening different entrapment matrices (celite, silica gel S, sodium alginate, and gellan gum) to immobilize *T. inflatum* spores for the production of CyA, the celite beads were found to support the maximum production (302 mg/l), followed by the sodium alginate (279 mg/l) and gellan gum beads (255 mg/l). Thus, the results indicated that the yield of CyA was affected by the nature of the entrapping carrier. The immobilization mechanism with celite and silica was adsorption and physical entrapment, whereas that with sodium alginate and gellan gum was entrapment within the gel. Chun and Agathos [8, 9] previously reported on the use of celite beads as an immobilization material, whereas Sallam *et al*. [25] and Sekar and Balaraman [28] reported on the use of sodium alginate as a carrier material for the production of CyA. Although the gellan gum provided a comparatively lower CyA yield, it was still selected for further experiments, as this is the first report on using gellan gum as an immobilization carrier for CyA production. Moreover, the use of gellan gum made it possible to study the effect of the immobilization of both spores and mycelia, as the immobilization of mycelia is difficult in the case of celite beads and silica gel.

The effect of the gellan gum concentration [0.25−1.25% (w/v)] on the production of CyA was also studied, and a 1% concentration was found to give the maximum production of CyA when using the spores (257 mg/l) and mycelia (274 mg/l) (Fig. 1). At lower and higher carrier concentrations, the production of CyA was lower than the optimum (1% gellan gum). This may have been because the beads with a lower carrier concentration were fragile and broke during the fermentation period, whereas the beads with a higher carrier concentration were hard enough to resist the diffusivity of the nutrients. Wang *et al*. [32] reported on the use of gellan gum [1% (w/v)] for immobilizing *Sphingomonas* sp. strain XLDN2-5 cells to degrade carbazole, whereas other investigators have selected 3% (w/v) alginate as a suitable matrix for the immobilization process [3, 7, 25]. Thus, the concentration of gellan gum would seem to be important for the viability and enzymatic activity of the immobilized cells. The carrier concentration may also be important for the pore size, bead stability, and optimum oxygen diffusion.

Fig. 2 demonstrates the effect of different immobilized mycelia weights on the production of CyA. The best CyA yield of 271 mg/l with gellan gum was obtained when using 7.5% (w/v) of wet fungal mycelia. The CyA production did not increase significantly with a further increase in fungal mycelia. Sallam *et al*. [25] previously reported that 20 g of *A. terreus* mycelia immobilized in sodium alginate per 100 ml of medium gave the maximum production of 105.7 mg/l CyA. Fig. 3 shows the effect of the number of inoculated spore-loaded beads on the production of CyA, where the addition of 100 beads per 100 ml of production medium gave the maximum production of 257 mg/l with gellan gum. The CyA production did not increase significantly with a further increase in the number of beads added.

Fig. 4 demonstrates the effect of the time of the addition of an optimized combination of L-valine and L-leucine on the CyA production using the immobilized system. Survase *et al*. [31] previously reported on the optimal combination of amino acids for the maximum production of CyA, where the addition of L-valine and L-leucine produced 556 mg/l CyA, in contrast to 113 mg/l without the addition of the amino acids. These amino acids presumably play one or more roles, such as precursor, inducer, and/or developmental regulator. Lee and Agathos [20], Balakrishnan and Pandey [6], and Nisha *et al*. [23] also reported that the addition of L-valine and L-leucine in combination increased the production of CyA several fold when compared with the yields obtained without the addition of amino acids.

In the present study, the addition of L-valine (6 g/l) and L-leucine (5 g/l) after 48 h of fermentation produced 1,338 mg/l of CyA, compared with 1,035 mg/l when the amino acids

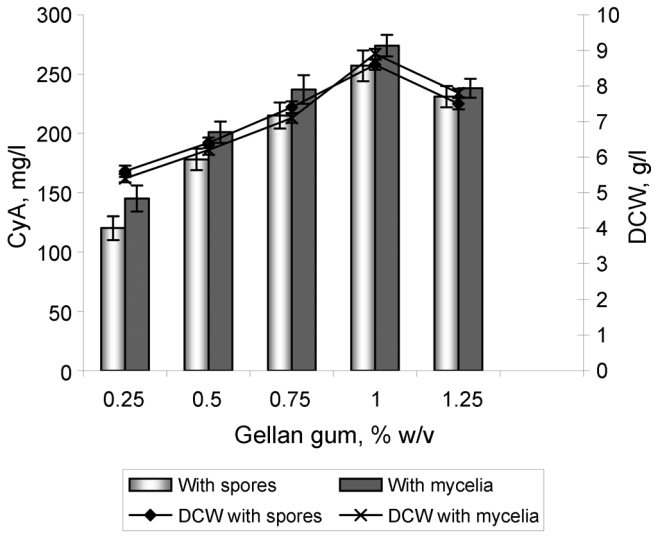

Fig. 1. Effect of gellan gum concentration [0.25–1.25% (w/v)] on production of CyA by immobilized *T. inflatum* MTCC 557 spores and mycelia.
Values were found to be significantly different, as measured by Duncan's multiple-comparison test.

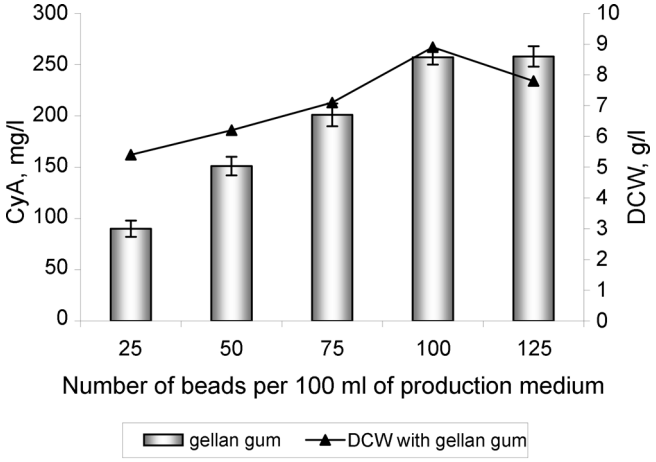

Fig. 3. Effect of number of inoculated spore-loaded beads on production of CyA.
CyA yields with 100 and 125 beads per 100 ml production medium were not statistically significant, as measured by Duncan's multiple-comparison test.

were added at the time of inoculation using gellan gum beads. Lee and Agathos [20], and Balakrishnan and Pandey [6] also reported that the addition of amino acids after 18 h and 20 h, respectively, gave better yields than their addition at the beginning of fermentation. Moreover, they found that the addition of amino acids in the exponential growth phase was more beneficial for metabolite production.

Table 1 illustrates the reusability of the *T. inflatum* cells immobilized in gellan gum beads for CyA production. The reusability of the immobilized cells was achieved by aseptic removal of the fermentation medium and replacement with a fresh medium for CyA production. Here, the free cells were analyzed for CyA production, while the beads were transferred to the new production medium. The free cells were also used to measure the DCW. A CyA production of 546 mg/l was obtained after the first batch when using the gellan gum beads (Table 1), and increased to 897 mg/l after the second batch. However, after the second cycle, the production was found to decrease, possibly because of the increased size of the beads and their consequent disruption, followed by more cell leakage. After four cycles using the gellan gum beads, the beads were all

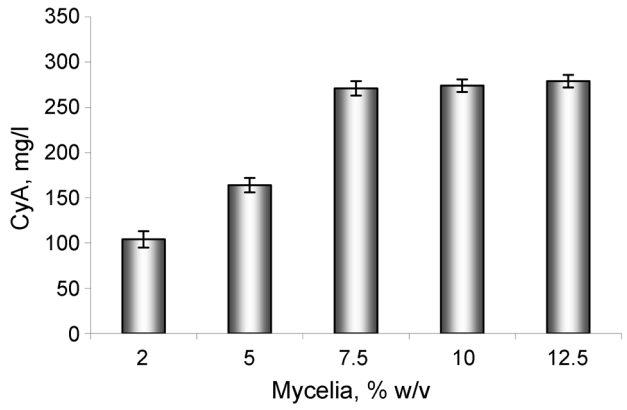

Fig. 2. Effect of different immobilized mycelia weights on production of CyA.
CyA yields with 7.5%, 10%, and 12.5% (w/v) mycelia were not found to be significantly different, as measured by Duncan's multiple-comparison test.

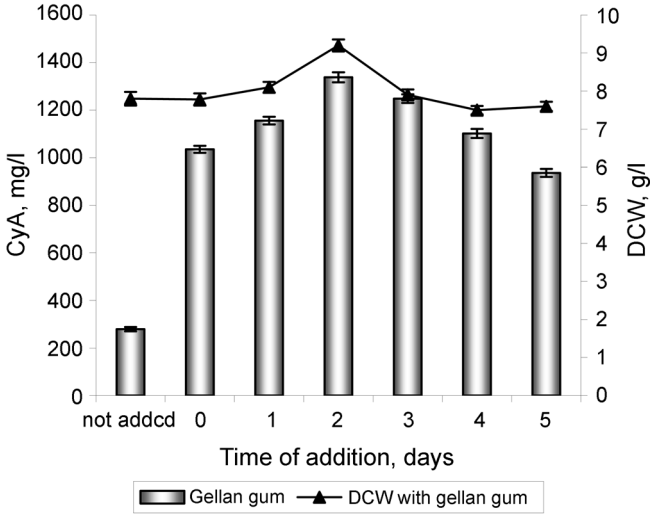

Fig. 4. Effect of time of addition of amino acid combination (6 g/l L-valine and 5 g/l L-leucine) on production of CyA.
Values were found to be significantly different, as measured by Duncan's multiple-comparison test.

Table 1. Effect of repetitive use of immobilized mycelia beads for production of CyA.

Run	Bead diameter (mm)[A]	DCW (g/l)[A]	CyA (mg/l)[A]
1	4.8±0.1[a]	2.8±0.1[a]	546±23[a]
2	7.3±0.2[b]	4.2±0.1[b]	897±20[b]
3	10.2±0.2[c]	6.6±0.2[c]	768±15[c]
4	12.5±0.3[d]	9.8±0.3[d]	680±10[d] (1,559±24)[B]

Values in the same column with different letters are significantly different (p=0.05), as measured by Duncan's multiple-comparison test.
[A] values are mean ± SD of three determinations.
[B] values in parenthesis report the total CyA yields after disruption of the beads.

disrupted and the maximum CyA production of 1,559 mg/l was obtained. The DCW was found to increase from 2.8 g/l after the first cycle to 6.6 g/l after the third cycle. The increase in the DCW after each cycle indicated the leakage of cells with each transfer, which may have been due to the increase in the size of the beads, making the environment crowded in the fermentation flask.

In conclusion, the present study established that gellan gum can be used as a whole-cell immobilization material for *T. inflatum* for the production of CyA. The study also demonstrated the reusability of gellan for the semi-continuous production of CyA. The immobilized beads could also be packed in a column to study the continuous production of CyA.

Acknowledgments

The authors are thankful to the Department of Biotechnology, Government of India for funding this project. The gift of CyA standard from RPG Life Sciences Ltd, Mumbai, India is gratefully acknowledged.

References

1. Abdel-fattah, Y. R., H. El Enshasy, M. Anwar, H. Omar, E. Abolmagd, and R. A. Zahra. 2007. Application of factorial experimental designs for optimization of cyclosporin A production by *Tolypocladium inflatum* in submerged culture *J. Microbiol. Biotechnol.* **17:** 1930–1936.
2. Agathos, S. N., J. W. Marshall, C. Maraiti, R. Parekh, and C. Moshosing. 1986. Physiological and genetic factors for process development of cyclosporin A fermentation. *J. Ind. Microbiol.* **1:** 39–48.
3. Anisha, G. S. and P. Prema. 2008. Cell immobilization technique for the enhanced production of a-galactosidase by *Streptomyces griseoloalbus. Bioresour. Technol.* **99:** 3325–3330.
4. Bajaj, I. B., S. A. Survase, P. S. Saudagar, and R. S. Singhal. 2007. Gellan gum: Fermentative production, downstream processing and applications. *Food Technol. Biotechnol.* **45:** 341–354.
5. Baker, E. E., R. J. Prevoznak, S. W. Dew, and B. C. Buckland. 1984. Thienamycin production by *Streptomyces catteleya* cells immobilized in celite beads. *Dev. Ind. Microbiol*. **24:** 467–474.
6. Balakrishnan, K. and A. Pandey. 1996. Influence of amino acids on the biosynthesis of cyclosporin A by *Tolypocladium inflatum. Appl. Microbiol. Biotechnol.* **45:** 800–803.
7. Bihari, V., P. Gosivani, S. Rizvi, S. Base, and V. Voratt. 1984. Studies on immobilized fungal spores for microbial transformation of steroids. 11aα-Hydroxylation of progesterone with immobilized spores of *A. ochraceus* G8 on polyacrylamide gel and other matrix. *Biotechnol. Bioeng.* **25:** 1403–1408.
8. Chun, G. T. and S. N. Agathos. 1989. Immobilization of *Tolypocladium inflatum* spores into porous celite beads for cyclosporin A production. *J. Biotechnol.* **9:** 237–254.
9. Chun, G. T. and S. N. Agathos. 1991. Comparative studies of physiological and environmental effects on the production of cyclosporin A in suspended and immobilized cells of *T. inflatum*. *Biotechnol. Bioeng.* **37:** 256–265.
10. Cinquin, C., G. Le Blay, I. Fliss, and C. Lacroix. 2004. Immobilization of infant fecal microbiota and utilization in an *in vitro* colonic fermentation model. *Microb. Ecol.* **48:** 128–138.
11. Dreyfuss, M., E. Härri, H. Hofmann, H. Kobel, W. Pache, and H. Tscherter. 1976. Cyclosporin A and C. New metabolites from *Trichoderma polysporum*. *Eur. J. Appl. Microbiol.* **3:** 125–133.
12. Ferrance, J. P. 2007. Gellan beads as a transparent media for protein immobilization and affinity capture. *J. Chromatogr. A* **1165:** 86–92.
13. Gbewonyo, K. and D. Wang. 1983. Confining mycelial growth to porous micro beads: A novel technique to alter the morphology of non-Newtonian culture. *Biotechnol. Bioeng.* **25:** 967–983.
14. Gilleta, F., C. Roisin, M. A. Fliniaux, A. Jacquin-Dubreuil, J. N. Barbotin, and J. E. Nava-Saucedo. 2000. Immobilization of *Nicotiana tabacum* plant cell suspensions within calcium alginate gel beads for the production of enhanced amounts of scopolin. *Enz. Microb. Technol.* **26:** 229–234.
15. Jal, P. K., S. Patel, and B. K. Mishra. 2004. Chemical modification of silica surface by immobilization of functional groups for extractive concentration of metal ions. *Talanta* **62:** 1005–1028.
16. Jones, A., D. N. Wood, T. Razniewska, G. M. Gaucher, and L. A. Behie. 1986. Continuous production of penicillin G by *Penicillium chrysogenum* cells immobilized on celite biocatalyst support particles. *Can. J. Chem. Eng.* **64:** 547–552.
17. Kahan, B. D. (ed.). 1984. *Cyclosporin: Biological Activity and Clinical Applications*. Crune & Straton Inc., Orlando.
18. Kennedy, J. F. and J. M. S. Cabral. 1983. Immobilized living cells and their applications, pp. 189–280. *In* Chibata, I. and L. B. Wingard Jr. (eds.). *Applied Biochemistry and Bioengineering.* Academic Press, New York.
19. Kubo, W., S. Miyazaki, and D. Attwood. 2003. Oral sustained delivery of paracetamol from *in situ*-gelling gellan and sodium alginate formulations. *Int. J. Pharm.* **258:** 55–64.
20. Lee, J. and S. Agathos. 1989. Effect of amino acids on the production of cyclosporin A by *T. inflatum*. *Biotechnol. Lett.* **11:** 77–82.
21. Lee, T. H., G. T. Chun, and Y. K. Chang. 1997. Development of sporulation/immobilization method and its application for the

continuous production of cyclosporin A by *Tolypocladium inflatum. Biotechnol. Prog.* **13:** 546–550.
22. Miyazaki, S., N. Kawasaki, W. Kubo, K. Endo, and D. Attwood. 2001. Comparison of *in situ* gelling formulations for the oral delivery of cimetidine. *Int. J. Pharm.* **220:** 161–168.
23. Nisha, A. K., S. Meinnanalakshmi, and K. Ramasamy. 2008. Comparative effect of amino acids in the production of cyclosporin by solid and submerged fermentations. *Biotechnology* **7:** 205–208.
24. Potumarthi, R., Ch. Subhakar, A. Pavani, and A. Jetty. 2008. Evaluation of various parameters of calcium-alginate immobilization method for enhanced alkaline protease production by *Bacillus licheniformis* NCIM-2042 using statistical methods *Bioresour. Technol.* **99:** 1776–1786.
25. Sallam, L. A. R., A. H. El-Refai, A. A. Hamdi, A. H. El-Minofi, and S. I. Abd-Elsalam. 2005. Studies on the application of immobilization technique for the production of cyclosporin A by a local strain of *Aspergillus terreus. J. Gen. Appl. Microbiol.* **51:** 143–149.
26. Schlosser, D., S. Irrgang, and H. Schmander. 1993. Steroid hydroxylation with free and immobilized cells of *Penicillium raistricki* in the presence of B, cyclodextrin. *Appl. Microbiol. Biotechnol.* **39:** 16–20.
27. Schmuader, H., D. Shlosser, T. Gunther, A. Hattenbach, J. Sauerstien, F. Jungnickel, and M. Augesten. 1991. Application of immobilized cells on biotransformation of steroids. *J. Basic Microbiol.* **31:** 453–477.
28. Sekar, C. and K. Balaraman. 1998. Immobilization of the fungus *Tolypocladium* sp. for the production of cyclosporin A. *Bioprocess Eng.* **19:** 281–283.
29. Sun, W. and M. W. Griffiths. 2000. Survival of *Bifidobacteria* in yogurt and simulated gastric juice following immobilization in gellan-xanthan beads. *Int. J. Food. Microbiol.* **61:** 17–25.
30. Survase, S. A., N. S. Shaligram, R. C. Pansuriya, U. S. Annapure, and R. S. Singhal. 2009. A novel medium for the enhanced production of cyclosporin A by *Tolypocladium inflatum* MTCC 557 using solid state fermentation. *J. Microbiol. Biotechnol.* **19:** 462–467.
31. Survase, S. A., U. S. Annapure, and R. S. Singhal. 2009. Statistical optimization of cyclosporin A production on a semi-synthetic medium using Tolypocladium inflatum MTCC 557, *Global J. Biotechnol. Biochem*. **4:** 184–192.
32. Wang, X., Z. Gai, B. Yu, J. Feng, C. Xu, Y. Yuan, Z. Lin, and P. Xu. 2007. Degradation of carbazole by microbial cells immobilized in magnetic gellan gum gel beads. *Appl. Environ. Microbiol.* **73:** 6421–6428.