

Original Article

BIOTRANSFORMATION OF PROGESTERONE TO 17 α -HYDROXYPROGESTERONE BY USING PLANT CELL SUSPENSION CULTURE OF *CATHARANTHUS ROSEUS*

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ABSTRACT

Objective: The present investigation was aimed to perform biotransformation potential of *Catharanthus roseus*.

Methods: Cell suspension culture of *C. roseus* were prepared by using Murashige and Skoog medium. Biotransformation of an important bioactive progesterone (4-pregnane-3, 20-dione) was confirmed by cell suspension culture of *Catharanthus roseus*.

Results: Progesterone, which was used as precursor in cell suspension culture, was found to be bio transformed within three days into a new product through a region selective hydroxylation process. The results confirmed that most of the progesterone added, was hydroxylated at its 17 α -position leading to 17 α -hydroxyprogesterone as a major biotransformation product after twelve days of incubation with 20% v/v cell density. The optimum biotransformation was achieved with the concentration of 100 μ g/ml progesterone at pH 5.5. Similarly, glucose was found as the most suitable source of carbon. The optimum incubation time was observed to be 12 days at 20% v/v cell density. Also higher progesterone biotransformation was observed using un immobilized cells as compared to immobilized cells.

Conclusion: The results of present work suggest that *C. roseus* can successfully biotransformed to the product similar to an original organic substrate, which is entirely synthetic. Such a biotechnological process could be of great interest for production of new chemical compounds like cortisone, hydrocortisone and other important steroids.

Keywords: *Catharanthus roseus*, Apocynaceae, Cell suspension culture, Biotransformation, Progesterone, 17 α -hydroxyprogesterone.

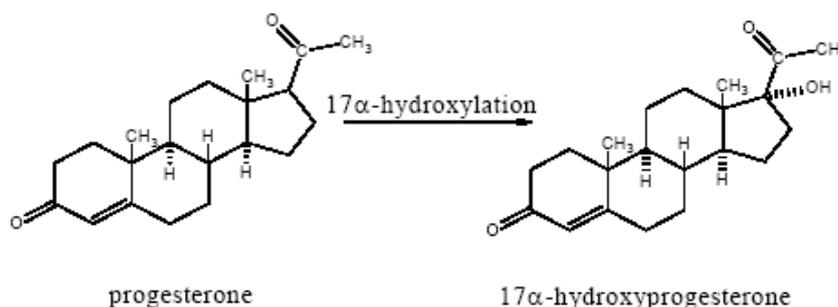
INTRODUCTION

The biotransformation of readily available inexpensive natural products to a more valuable and useful substances through incubation with biological systems has attracted much attention as this approach allows the functionalisation of inactivated carbon atoms, and is still a promising field for the practical application of plant cell culture [1]. In the recent years, much attention has been paid to the ability of cultured plant cells to transfer enantio selectively not only secondary metabolites, but also organic exogenous substrates into biologically active compounds to achieve specific biotransformation [2-4]. Cell suspension cultures of plants have also been efficiently employed for the biotransformation of organic compounds, e. g., steroids [5-7], terpenes [8-10], alkaloids [11] and flavonoids [12]. However, there have been few examples of biotransformation of organic foreign substrates. Many investigators have studied biotransformation of organic compounds by plant cell suspension culture, reporting selective and specific conversion ability including enantio selective oxidation, stereo selective reduction, region selective hydroxylation, enantioselective glucosylation, esterification, methylation and isomerization [13, 10, 14].

Steroidal drugs, a large category of compounds with steroid nucleus, are mainly used to render anti-inflammatory response, treat cancer

and antiretroviral activity for HIV infected therapy clinically [15]. Biological transformation using plant cell suspensions culture allows structural modification on exogenously supplied steroid to provide useful substances [16]. Biotransformation has been used for the production of steroidal drugs universally. Metabolites obtained may be pharmacologically active or can be used in the commercial synthesis of other useful steroid compounds [17, 18, 7]. Some kind of these important biotransformation consisted in 11 β , 17 α , 16 α and 21 hydroxylations of progesterone producing corticosteroids. Hydroxysteroids have been implicated in many physiological conditions. 17 α -Hydroxysteroid is an important precursor for medroxyprogesterone acetate production and economically important in the pharmaceutical industry [19-22]. It can be synthesized from progesterone by 17 α -Hydroxylation using biotransformation scheme 1.

It has been observed that chemical synthesis is the most common approach despite associated drawbacks. Chemical synthesis of progesterone from various precursors such as stigma sterol, diosgenin and cholesterol involves several critical steps and yield of the final product is also very less. Purification of the product obtained from chemical synthesis is another major issue.



Scheme 1: Production of 17 α -hydroxyprogesterone from progesterone by biotransformation

Introduction of hydroxyl group into the steroid nucleus involves the activation of chemically unreactive carbon centers. Chemical hydroxylation at these centers demand several reaction conditions and suffer from lack of stereo selectivity, unless complex routes are employed [23]. Further, introduction of hydroxyl group at 17 α -position into steroidal nucleus is a complicated and tedious process because 17 α -hydroxy progesterone can get readily converted into hydroxycortisone, however there is no specific process available for the production of this important intermediate that could bridge the gap between demand and supply [24]. In contrary, biological hydroxylation can specifically use plant cells that can be run under comfortable controlled conditions to resolve this issue.

In the present study shortcomings are envisaged to overcome by implementing biotransformation approach. Biotransformation bears greater advantages than the chemical process in terms of specificity, percentage yield and environmental friendly. This technique utilizes the biocatalyst of microorganism, plant and animal cells. Biocatalyst favor mild reaction conditions and the problems concerning racemization, epimerization, rearrangement and straight decomposition can be minimized substantially [25]. However, there are two important reasons behind choosing plant enzymes for biotransformation purpose. Firstly, these enzymes are able to catalyze reaction stereo-specifically and secondly, plant enzyme can perform regio-specific modifications that are not easily carried out by chemical synthesis or by microorganisms [26]. We are interested in the feasibility of using plant cell cultures for the biotransformation of foreign substrates.

Numerous plant cell cultures are reported to metabolize progesterone. The biotransformation of progesterone by plant cell culture, using the variety of plant species that differed somewhat in their transformation capacity has been reported earlier [27]. In general, the yield of product was low ranging from 2 to 10%. Only the culture of *Parthenocissus species* transformed progesterone to pregn-4-en-20 α -ol-3-one was reported for 65% yields. In a different biotransformation process, 5 α -pregnan-3, 20-dione and then 5 α -pregnan-3 β -ol-20-one is a reaction product obtained from the cell cultures of *Solanun tubersum*, *Digitalis purpurea*, *Nicotiana rustica*, *Herdera helix* and *Atropa bellodona*. Culture of *Digitalis lanata* only transformed progesterone to 5 α -pregnan-3, 20-dione [28].

Two pathways have been reported for transformation of progesterone using cultures of *N. tabacum* and *Rosa species*. The *Rosa* culture converted progesterone to 4 α -pregnen-20 β -ol, 3-one and 5 α -pregnan-3 β -ol-20-one, whereas the *N. tabacum* culture transformed progesterone into 4 α -pregnen-20 α -ol-3-one and 5 α -pregnan-3 β -ol-20-one [27]. The biotransformation of progesterone by cultures of *D. deltoidea* and *Cherirathus cheiri* has reported the need to reduce the double bond [29]. 5 α -pregnan-3 β -ol-20-one palmitate with *Sophara*, 5 α -pregnan-3 β -ol, 20-one and 5 α -pragnen-3 β , 20 β -diol with *Dioscorea deltoidea* and 5 α -pregnan-3, 20-dione, 5 α -pregnan-3 β -ol, 20-one and its glucosides, 5 α -pregnan-3 β , 20 α -diol and its glucosides, 5 α -pregnan-20 α -ol, 3-one and its glucosides, 5 α -pregnan-20 β -ol, 3-one and its glucosides with *D. purpurea* were obtained as the transformation products of progesterone biotransformation [30-32].

The regioselective hydroxylation of 4S(-)-perillyl alcohol and 3S(-)-citronellol by cultured suspension cells of *C. roseus* have been reported earlier [13]. The previous reports reveal that cell suspension culture of *C. roseus* [strains C₂₀] is able to convert a synthetic product possessing phenylsulphoxide group, which is very useful in organic chemistry [33]. Due to our interest on plant cell hydroxylation of steroid, attempts were made to investigate the role of substrate structure on hydroxylation by *C. roseus*. Since C₂₁ steroids are potential anti-inflammatory agents, closely related steroids, i.e. progesterone, was chosen for this study. In the present studies, we observed the biotransformation potential of *C. roseus* for progesterone biotransformation. We developed callus as well as suspension cultures of *C. roseus* (L) for biotransformation studies in the present investigation. Effect of various parameters such as pH, precursor concentrations, and incubation time and carbon sources were studied for transformation of progesterone to 17 α -hydroxyprogesterone. The comparative effect of immobilized cells and un immobilized cells on biotransformation was also studied.

MATERIALS AND METHODS

Materials

Progesterone was obtained as a gift sample from Aarge Drug Pvt. Ltd., Parwanoo, India. Plant growth hormones kinetin (kn) and 2, 4-dichlorophenoxy acetic acid (2, 4-D) and Murashige and Skoog's (MS) medium were purchased from Himedia Mumbai, India. 17 α -hydroxyprogesterone was obtained as a gift sample from Belco Pharma, Rohtak, Haryana, India. HPLC grade solvents (Methanol, Acetonitrile and Water) were purchased from Sigma Aldrich, St. Louis, U. S. A. Ethyl acetate; chloroform and hexane for TLC were purchased from Sigma Aldrich, U. S. A.

Callus initiation

The callus cultures were prepared by transferring the explants in MS media as reported previously with slight modifications [34, 32]. The young leaf buds of *C. roseus* (L.) were collected from Pharmacognosy garden of Department of Pharmaceutical sciences, Dr. Hari Singh Gour Vishwavidyalya, Sagar (M. P.) India. These leaf buds were washed with tap water followed by mild detergent solution and distilled water respectively. After washings, the buds were sterilized with 90% ethanol (EtOH) for 30 seconds followed by HgCl₂ (0.1% of w/v) for 90 seconds. Thereafter, these leaf buds were rinsed with sterile distilled water 2-3 times and cut into 2 mm x 2 mm explants. These explants were transformed on solidified Murashige and Skoog's (MS) medium containing 3 mg/l 2,4-dichlorophenoxyacetic acid, 0.3 mg/l kinetin, 3% w/v sucrose and 0.9%w/v agar (pH 5.8). The cultures were then incubated in dark at 25 \pm 1 $^{\circ}$ C in B. O. D. incubator (Toshniwal, India). After callus initiation, culture tubes were subjected to illumination (for 16 h light and 8 h dark) at 1500-2000 lux by fluorescent lamp and kept at 25 \pm 1 $^{\circ}$ C. The callus cultures were maintained by sub-culturing in the same medium for the duration of four weeks as reported previously [33].

The suspension cultures were prepared by the method reported previously with slight modifications [35]. After subculturing for several generations, the callus tissue was transferred to freshly prepared media (50 ml) supplemented with 3 mg/ml of 2,4-D and 0.3 mg/ml of kinetin and 3% w/v sucrose in 250 ml Erlenmeyer's flask to prepare suspension cultures of *C. roseus*. Then grown with continuous shaking at 120 rpm on a rotatory shaker (Remi, India) at 25 \pm 1 $^{\circ}$ C under 16 h photoperiod (1500-2000 lux) for one week. They were maintained in same medium and sub-cultured every seven days into 250 ml flask [5 ml of cells suspension into 45 ml of fresh MS medium] under the similar culture conditions.

Preparation of immobilized *Catharanthus roseus* cells

Immobilized *C. roseus* cells in alginate beads were prepared by the method reported previously with slight modification [3]. Suspension of 30%v/v cells in 5% w/v of sodium alginate was prepared. This slurry of sodium alginate cells was dropped through a 1 mm orifice in 50 mM CaCl₂ solution to obtain spherical beads. The resulting beads were allowed to stand for 1 h and washed with distilled water. These beads were added to freshly prepared MS medium (50 ml/flask) containing 1 mg/l 2,4-D and 0.1 mg/l kinetin and 3%w/v sucrose. Thereafter, the flasks were shaken on rotatory shaker at 120 rpm in the dark at 25 \pm 1 $^{\circ}$ C.

Extraction and identification

After filtration of cell suspension cultures, cells were crushed with fine sand in a mortar and extracted in twice the volume of chloroform. Also, the culture medium was extracted three times by agitator with CHCl₃ twice. Combined organic layer was concentrated under vacuum. Then after the residue was analyzed by thin layer chromatography as reported previously [36] (silica gel 60F-254, Merck Art 5717; solvent systems, EtOAc: CHCl₃ 10:90 and EtOAc: Hexane 20:80). Spots were detected by 5% vanillin solution (ethanol: H₂SO₄:: 7:4) or UV light.

High performance liquid chromatography analysis

The chloroform extracts of cells, culture medium or both were analyzed for progesterone and its metabolites using HPLC Shimadzu, (SPD-10MA system, Japan) using reverse phase separating C-18

column (Phoenix 25 x 0.25 cm). 20 μ l of the extract was injected with a Hamilton syringe. The acetonitrile: methanol: water (25:25:50) was used as mobile phase and a flow rate of 0.1 ml/min. Detection was done at absorption maxima 254 nm by photodiode array detector [37].

Biotransformation experiments

The biotransformation studies have been performed according to method as reported earlier [36]. The biotransformation experiments were carried out in 250 ml Erlenmeyer's flask containing 60 ml of freshly prepared MS medium containing 1 mg/ml of 2,4-D and 0.1 mg/ml of kinetin and 3% w/v sucrose with unimmobilized cells as well as immobilized cells of *C. roseus*. Then incubated for one week at 25 \pm 1 $^{\circ}$ C on rotatory shaker (120rpm). 20% v/v initial packed cell volume was used for biotransformation purpose. To these pre cultured cells, progesterone in concentration of 100 μ g/ml was aseptically added using membrane filter (0.22 μ m) followed by incubation of cell suspension cultures under similar conditions as mentioned previously in the original culture. At regular intervals, the samples were withdrawn and the cells were removed under sterile conditions for subsequent extraction with chloroform. The extracts were concentrated under the vacuum and thereafter analyzed using TLC and HPLC to identify and quantify products obtained by biotransformation.

Effect of various parameters on biotransformation

Incubation time

In order to investigate the effect of incubation time on biotransformation of progesterone, un immobilized cells and immobilized cells were pre cultured in freshly prepared MS medium containing (1 mg/l 2, 4-D and 0.1 mg/l kinetin and 3% w/v sucrose) for 1 week at 25 \pm 1 $^{\circ}$ C under similar conditions as mentioned previously. Thereafter, 100 μ g/ml of progesterone was added to the flask and incubated for 21 days. At a regular interval every 3 days, cells and medium were collected, extracted and analyzed for progesterone and its metabolites.

Substrate concentration

The initial experiment was performed to determine the optimum concentration of progesterone that can be fed to give maximal biotransformation yield. In order to investigate the effect of fed precursor (progesterone) concentration on biotransformation, cell suspension culture (20 %v/v) was incubated into MS medium containing various concentration viz. 50 μ g/ml, 100 μ g/ml, 150 μ g/ml, 200 μ g/ml and 250 μ g/ml of progesterone and after 12 days of incubation under similar conditions mentioned earlier, samples were extracted and analyzed.

pH

The effect of pH on progesterone biotransformation was determined using immobilized and un immobilized cells with fixed concentration of progesterone. For this purpose pH range of 3.5 to 8.5 was selected and 0.1N HCl or 1N NaOH were used to achieve the desirable pH. In each case, after 12 days of incubation the media was extracted and analyzed as mentioned previously.

Carbon source

Various carbon sources glucose, sucrose, fructose, maltose and sodium acetate were used with fixed concentration of progesterone (100 μ g/ml) in MS medium to study the effect of different source of carbon on biotransformation. After 12 days incubation period under similar conditions mentioned earlier, samples were extracted and analyzed.

RESULTS AND DISCUSSION

Induction of callus

After two to four weeks of leaf bud explants transplantation into static MS medium, which was maintained at 25 \pm 1 $^{\circ}$ C in dark, cream colored friable callus was induced. The callus was maintained in the same medium and subcultured under the similar conditions as described earlier after every fourteen days, which was used for further biotransformation investigations (fig. 1A and B).

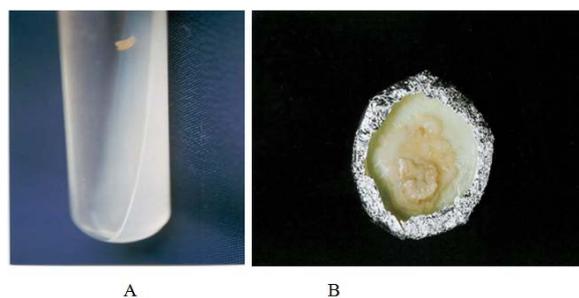


Fig. 1 A: Explant of *C. roseus* B: Four week old callus culture

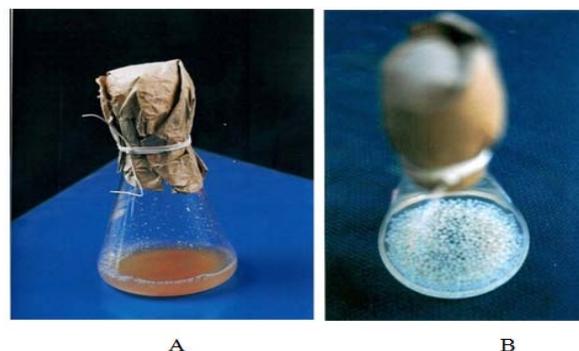


Fig. 2 A: One week old suspension culture, B: Suspension culture cells entrapped in sodium alginate beads

After two to three weeks of transferring the callus tissue into MS medium at 25 \pm 1 $^{\circ}$ C in the light, the light cream-colored suspension culture was established (fig. 2A). 6.5%v/v and 91.5%, packed cell volume and cell viability respectively were recorded after three weeks.

Biotransformation

In an attempt to study the aptitude of cells to metabolize progesterone, several experiments were simultaneously carried out to optimize the cultures; cell suspension culture of *C. roseus* with progesterone dissolved in ethanol, cell suspension culture of *C. roseus* without progesterone (control culture), liquid medium with progesterone in ethanol without cells and cell suspension culture of *C. roseus* with ethanol without progesterone. After incubation for seven days, cells and liquid medium were analyzed separately by TLC. TLC studies revealed that progesterone remains unchanged after seven days of incubation, when it was added into the culture medium without cells. However, it was partially metabolized when added to cell suspension culture. More than one spot was observed in TLC from both cells as well as culture medium which corresponds to the standard samples of progesterone and 17 α -hydroxyprogesterone. This type of transformation from progesterone to 17 α -hydroxyprogesterone using plant cell cultures is critical as well as bear utility, which has also not been reported in earlier literature to the best of our knowledge. This indicated that progesterone was absorbed by cells and converted into various products.

Biotransformation of steroid has been extensively reported. However, the studies on 17 α -hydroxylation are rare and the nature of 17 α -hydroxylation in microorganism is unknown. In the higher eukaryotes, progesterone 17 α -hydroxylase is catalyzed by a cytochrome P450. Cytochrome P450 was expressed in *Saccharomyces cerevisiae* and converted progesterone to 17 α -hydroxyprogesterone with the yield of 31.4%. Progesterone is readily metabolized by *Digitalis purpurea* tissue cultures to 5 α -pregnan-3, 20-dione and 5 α -pregnan-3 β -ol-20-one [38-40]. Furthermore, *Rosa* species tissue cultures were reported to form Δ^4 -pregnene-20 β -ol-3-one and Δ^4 -pregnene-20 α -ol-3-one while *C. roseus* and *L. esculentum* tissue culture form Δ^4 -pregnene-14 α -ol-3, 20-dione [27, 41]. In the present investigation, culture of *C. roseus*

was able to carry out hydroxylation at the 17 α -position to yield Δ^4 -pregnene-17 α -ol-3, 20-dione. This stereo specific *de novo* hydroxylation at 17 α -position is a common hydroxylation carried out by many strains of *Curvularia lunata*, *Cunninghamella blakesleeana*, *Sacchaomyces cerevisiae* and *Nocardia* DSM, but has not been previously reported as occurring in plant cell cultures [42-44]. These products resulting from hydroxylation in the 14 α , 6 β and 17 α -positions indicate that certain plant cell cultures are capable of introducing a hydroxyl group apparently without the need for the steroid to be conjugated to a sugar moiety.

The stereo specific hydroxylation of non activated positions, as in the case of 14 α , 11 α and 17 α , performed by the plant cell cultures (as well as by microorganisms) cannot, however, be achieved easily by chemical synthesis.

Although until recently only reductions of unglycosidated steroids have been reported, apparently some cell cultures can carry out oxidative reactions, namely hydroxylations, on unglycosidated steroids. In addition, reductive and oxidative reactions can be performed by the same plant cell culture. The introduction of a hydroxyl group into one or more positions of progesterone by means of the cultures of *C. roseus* and *L. esculentum* was stereospecific. Cultures of *L. esculentum* yielded hydroxylations in the 6 β , 11 α , and 14 α positions, whereas *C. roseus* cultures carried out hydroxylation in the 14 α position [41]. In the present study new metabolite was obtained as Δ^4 -pregnene 17 α -ol-3, 20-dione by using cell suspension culture of *C. roseus* upon incubation of progesterone. Thus, if a new center of asymmetry was formed by hydroxylation, only one of the possible epimers arises. This is similar to the situation found with microorganisms [42-44].

To attain the maximum biotransformation yield, we observed the effects of various parameters viz. precursor (progesterone) concentration, incubation time, pH of the medium and carbon

sources on biotransformation of progesterone, which impact the production of metabolites.

Effect of fed progesterone concentration on biotransformation

The initial experiment was performed to determine the optimum concentration of precursor i.e. progesterone that showed maximum biotransformation yield. In order to investigate the effect of fed progesterone concentration on biotransformation, cell suspension culture of *C. roseus* (L) with various concentrations of progesterone; 50 μ g/ml, 100 μ g/ml, 150 μ g/ml, 200 μ g/ml and 250 μ g/ml were used in the culture and incubated for 12 days (Table1). Fig. 3 revealed that the biotransformation product of progesterone i.e. 17 α -hydroxyprogesterone was dependent on the precursor concentration. As the fed precursor concentration was increased the magnitude of biotransformation initially increased and reached upto optimum level and thereafter decreased. The concentration of 17 α -hydroxyprogesterone reached to a maximum value and then decreased. After the optimum level of precursor concentration, negative effect of progesterone content on biotransformation is evident when more than 100 μ g/ml had been added to culture medium. This may be attributed to decline in percent viability of cells. This result was confirmed by microscopic examination of cells by comparison with suspension cultures without progesterone. The dead cell ratio did not increase, except for cultures containing more than 100 μ g/ml of progesterone. The maximum biotransformation yield of 17 α -hydroxyprogesterone of 22.33%w/w was observed with optimum fed concentration of progesterone (100 μ g/ml) over higher concentration in an immobilized cell suspension media. At higher concentration, the greater part of fed precursor was accumulated into the cells, which can lead to the lethal effect on the cells. The data suggests an optimal concentration of substrate is required beyond which the cell gradually lose their transformation capacity due to the lethal effect of fed substrate on cells of culture as shown previously [45].

Table 1: Effect of substrate (Progesterone) concentration on biotransformation by *C. roseus* after 12 days incubation period

Substrate concentrations [μ g/ml]	Progesterone percent [%]	17 α -Hydroxy progesterone percent [%]
50	20.25 \pm 0.81	15.15 \pm 0.92
100	33.23 \pm 0.72	22.33 \pm 0.64
150	38.82 \pm 1.0	18.34 \pm 1.0
200	45.13 \pm 1.2	11.78 \pm 0.82
250	42.11 \pm 0.62	9.18 \pm 0.68

n=3

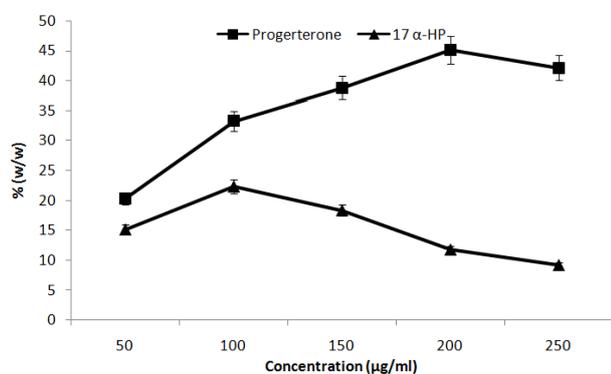


Fig. 3: Biotransformation yield by *C. roseus* cell suspension culture at different concentration of progesterone added to MS media after 12 days incubation

Effect of incubation time on progesterone biotransformation

To investigate the effect of incubation time on biotransformation of progesterone, the cell suspension cultures of *C. roseus* containing 100 μ g/ml progesterone was incubated for 21 days and at regular interval every 3 days, samples were analyzed for progesterone and

its metabolite. The time course study of progesterone, indicated that biotransformation product i.e. 17 α -hydroxyprogesterone, was detected from 3rd day of the experiment in culture medium (fig. 4). Its yield increased gradually from 3rd to 6th day followed by rapid increase from 9th to 12th day. The yield of 17 α -hydroxyprogesterone was found to be 13.92 μ g/ml and 12.70 μ g/ml at 9th and 12th day respectively. Beyond that almost stationary biotransformation were recorded until 21 days.

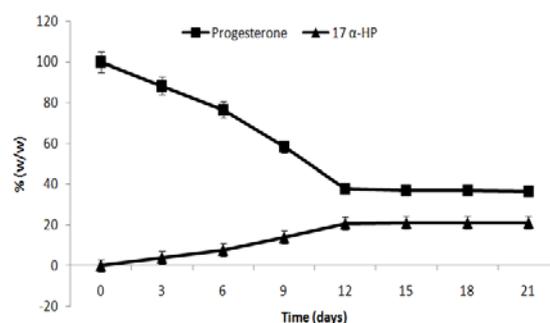


Fig. 4: Biotransformation yield by *C. roseus* cell suspension culture at different time period with 100 μ g/ml progesterone was used

Effect of pH on progesterone biotransformation

Fig. 5 showed that at pH below 5.0 the biotransformation yield was very poor and negligible amount of 17 α -hydroxyprogesterone was obtained. However, maximum biotransformation was observed between pH 5.5 to 6.0 with 20.70 %w/w and 18.89 %w/w yield of 17 α -hydroxyprogesterone, respectively. Beyond pH 6.0 no noticeable change was observed. There was a steady but slow decline in the rate of progesterone transformation over the range pH 5.5 to 6.0. The

marked drop in hydroxylation activity at pH 7.5 could be correlated with the loss of cell viability at this pH. The poor biotransformation rate below pH 5.0 and beyond pH 7.5 might be attributed to enzymes inactivation at these pH ranges.

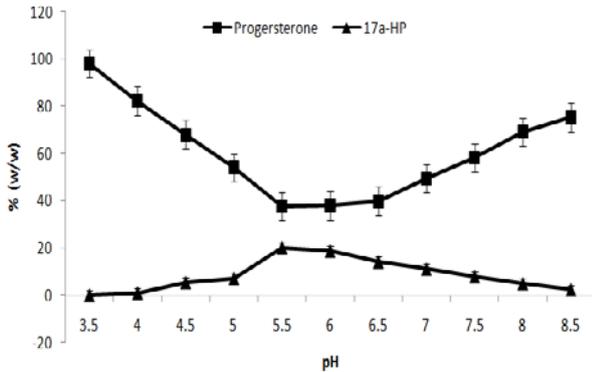


Fig. 5: Effect of pH on biotransformation of progesterone. After 12 days incubation with 100 μ g/ml progesterone, samples were analyzed

Effect of carbon source on progesterone biotransformation

In order to investigate the effect of carbon source on biotransformation of progesterone, various additives such as glucose, sucrose, fructose, maltose and sodium acetate were used in the culture medium. After constant incubation period (12 days), highest biotransformation yield of 17 α -hydroxyprogesterone was recorded with glucose as carbon source. The yield of 17 α -hydroxyprogesterone was found to be 20.70 μ g/ml, 18.71 μ g/ml, 16.9 μ g/ml and 15.18 μ g/ml with sucrose, fructose, maltose and sodium acetate respectively. Carbon sources are invariably present in the tissue culture media especially for its energy values; however, the type of such source is of paramount importance. Replacement of glucose with sucrose, fructose, maltose, and sodium acetate lead to

considerable decrease in biotransformation (Fig.6). Although, sucrose is most commonly used in tissue culture work, the present study indicates that glucose is favorable carbon source for progesterone biotransformation. Substitution of fructose, maltose and sodium acetate brought about a decrease in biotransformation rate in the following order i.e. fructose > maltose > sodium acetate.

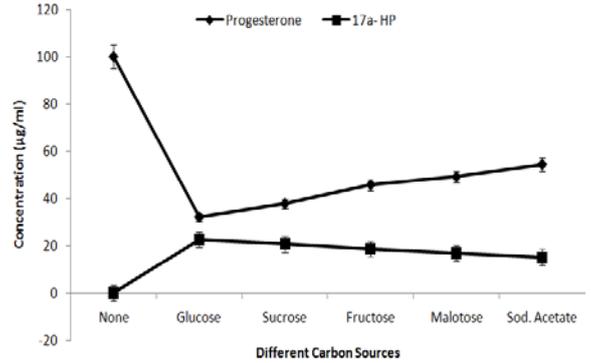


Fig. 6: Effect of various Carbon source on biotransformation. 12 days after addition of progesterone (100 μ g/ml), the pattern of 17 α -hydroxyprogesterone were determined

Biotransformation by immobilized cells and unimmobilized cells

To investigate the effect of immobilization, separate set of studies were performed. As evident from fig. 7 un immobilized cell suspension culture of *C. roseus* showing lesser progesterone biotransformation observed from 3rd to 6th day, suddenly increased progesterone biotransformation from 9th to 12th day. After 12 days of incubation, there was almost stationary biotransformation observed until days 21st. In case of immobilized cells system, (fig. 2B and 7) slower biotransformation rate was observed following the same pattern then after stationary biotransformation recorded post 18th day instead of 12th day. Both type of suspension cultures, immobilized cells and un immobilized cells, retained the ability to hydroxylate progesterone over this period and remained constant in each case. In these preparations, the difference indicates that the immobilized cells show slow biotransformation for prolonged period of time. However, the biotransformation rate was greater with un immobilized cells suspension. The yield was found to be 20.70% w/w and 11.40% w/w after 12 days in case of un immobilized cells and immobilized cells respectively. These might be due to a retarded uptake of progesterone and slow release of biotransformation products by entrapped cells.

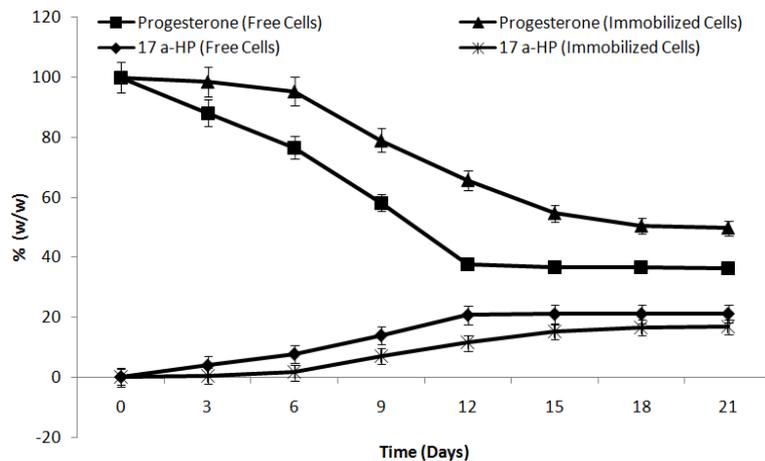


Fig. 7: Biotransformation yield by freely suspended cells and immobilized cells of *C. roseus* at 20%v/v cell density (free or Calcium alginate beads) incubated in 50 ml MS media with 100 μ g/ml progesterone

The results demonstrate the ability of freely suspended cells and calcium alginate entrapped cells of *C. roseus* to yield the biotransformation product i.e. 17 α -hydroxyprogesterone under a variety of culture conditions. In all cases, the ability to hydroxylated progesterone could be correlated with viability of both types of cells. The difference between the rates of progesterone transformation by free as well as immobilized cells is probably due to a diffusion barrier.

CONCLUSION

In the present work, a new metabolites is produced which is a chemical intermediate in the biosynthesis of other steroidal hormones, including corticosteroids and the androgens and estrogens. A suitable regioselective hydroxylation process was investigated for efficient biotransformation of progesterone to 17 α -hydroxyprogesterone by using *C. roseus* cell suspension culture. The study unfolds the technology for potential utilization of plant cell culture in enzymatic conversion to optically active forms, or for specific biotransformation of natural compounds to produce rare or unusual steroids, which are difficult to obtain from other natural or synthetic sources.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest

ABBREVIATION

2,4-D-2,4-dichlorophenoxyacetic acid, EtOH-Ethanol, EtOAc-Ethyl acetate, HP-hydroxyl progesterone, Kn – kinetin, MS medium-Murashige and Skoog's medium, TLC-Thin layer chromatography

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