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Research Article

Primary studies on biotransformation of steroidal drug Prednisolone

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Abstract

The less biodegradable steroidal drug, Prednisolone is one of the widely used drugs in the treatment of autoimmune and infectious diseases. Transformation of prednisolone can be achieved with microbial activity. Isolation of prednisolone-resistant microorganisms was done using a sewage sample. Out of 5 isolates, one isolate was selected for further studies based on maximum tolerability to prednisolone. The isolate was identified based on MALDI-TOF. The strain was found to have a match score of 2.336 with gram-negative bacteria *Klebsiella pneumoniae* spp *pneumoniae* DSM3010 4T HAM. The isolate was found to degrade 80% prednisolone on the 5th day of fermentation. The concentration of prednisolone was determined using a colorimetric-based method and HPLC technique.

Keywords: *Klebsiella*, pollutant, prednisolone, transformation

Introduction

Pharmaceutical micropollutants concentration and persistence are increasing day by day due to the emergence of new diseases and the overuse of pharma products. During the covid 19 pandemic situation, mucormycosis cases were controlled with the use of steroidal drugs as per the RECOVERY (Randomised Evaluation of COVID-19 Therapy) trial (Tandon & Pandey, 2021). Prednisolone is a corticosteroid drug abused for doping in sports. It is a synthetic glucocorticoid drug with anti-inflammatory properties (Furman, 2019). Prednisolone being a steroid is a carbon rich, highly reduced compound, hence can be the source of carbon for microorganisms. But steroids are difficult to completely mineralize to carbon dioxide (Chiang et al., 2020). Biotransformations of steroidal drugs occur by hydroxylation, esterification, dehydrogenation/reduction, methoxylation, halogenations, and methylation (Tong & Dong, 2009).

Actinomycetes, proteobacteria, fungi, and algae have been reported to have the ability to biotransform steroid-based compounds. Microorganisms could biotransform steroidal compounds by hydroxylation reaction /Bayer-Villiger oxidation reaction or enzyme assisted mechanism. A reported list of steroid biotransforming microorganisms with the possible mechanism is given in table 1.

Table 1 Steroidal drugs and their microbial transformed metabolite.

Steroidal compound	Name of microorganism	Transformation mechanism	Biotransformed product	Reference
Diosgenin 3β-hydroxy-5-spirostene	*Streptomyces virginiae* IBL-14	Hydroxylation and Cytochrome p450 monooxygenase FcpC	Isonuatigenone	Wang et al. (2007)
	White rot fungus, *Cariolus versicolor*	Hydroxylation reaction	Polyhydroxyl metabolite	Wu et al. (2011);
	Fungal strain *Absidia coerulea*	Hydroxylation reaction	Five metabolites: (25*R*)-spirost-5-en-3β,7β,12β,25α-tetrol, (25*S*)-spirost-5-en-3β,7α,12β,25β-tetrol, (25*S*)-spirost-5-en-3β,7β,12β,25β-tetrol, (25*R*)-spirost-5-en-3β,7α,12β,25α-tetrol and (25*R*)-spirost-5-en-3β,7β,12β,24β-tetrol	Zhao et al. (2010)
Saponins	*Talaromyces stolii* CLY 6	Steroid saponin glycosidases (Rhase-TS: α-L-rhamnosidase; Gluase-TS: β-D-glucosidase)	Diosgenin	Cheng et al. (2021)

Dehydroepiandrosterone (DHEA)	*Isaria farinosa*	Hydroxylation	7α- and 7β-hydroxy-DHEA	Kozłowska et al. (2018);
Cholesterol	*Arthrobacter, Bacillus, Brevibacterium, Corynebacterium, Microbacterium, Mycobacterium, Nocardia, Protaminobacter, Serratia,* and *Streptomyces*	Cholesterol oxidase	Cholestenone, cholesta-1,4-dien-3-one	Arima et al. (1996)
Mibolerone	*Cunninghamella blakesleeana*, *C. echinulata*, and *Macrophomina phaseolina*	Hydroxylation	Seven metabolites, 10*β*,17*β*-dihydroxy-7*a*,17*a*-dimethylestr-4-en-3-one, 6*β*,17*β*-dihydroxy-7*a*,17*a*-dimethylestr-4-en-3-one, 6*β*,10*β*,17*β*-trihydroxy-7*a*,17*a*-dimethylestr-4-en-3-one, 11*β*,17*β*-dihydroxy-(20-hydroxymethyl)-7*a*,17*a*-dimethylestr-4-en-3-one, 1*a*,17*β*-dihydroxy-7*a*,17*a*-dimethylestr-4-en-3-one, 1*a*,11*β*,17*β*-trihydroxy-7*a*,17*a*-dimethylestr-4-en-3-one , and 11*β*,17*β*-dihydroxy-7*a*,17*a*-dimethylestr-4-en-3-one	Siddiqui et al. (2017)
Estrogens	Algae: *Chlorella vulgaris*	Biotransformation and bioconcentration	Estrone	Lai et al. (2002)
Cortisone	*Rhodococcus rhodnii*	Hydroxylation/ bayer-Villiger oxidation	Two steroids, 1,9β,17,21-tetrahydoxy-4-methyl-19-nor-9β-pregna-1,3,5(10)-trien-11,20-dione and 1,9β,17,20β,21-pentahydoxy-4-methyl-19-nor-9β-pregna-1,3,5(10)-trien-11-one	Zappaterra et al. (2021)
Progesterone	*Aspergillus wentii*	Hydroxylation	11 α-hydroxyprogesterone	Yildirim et al. (2010)
Beclomethasone dipropionate	*Aspergillus niger*	-	(i) beclomethasone 17-monopropionate, (ii) beclomethasone 21-monopropionate, (iii) beclomethasone, and (iv) 9beta,11beta-epoxy-17,21-dihydroxy-16beta-methylpregna-1,4-diene-3,20-dione 21-propionate	Ahmad et al. (2014)

Materials and Methods

Isolation and identification of prednisolone transformers

Sewage sample was collected from the wastewater treatment plant and stored in the refrigerator till their use. The sample was serially diluted and was used for the isolation of prednisolone transforming microorganisms. Modified minimal mineral salt medium (MMSM) ($FeSO_4 \cdot 7H_2O$ (0.023 g/L), $CaCl_2 \cdot 2H_2O$ (0.13 g/L), $MgSO_4 \cdot 7H_2O$ (0.025 g/L), Na_2HPO_4 (7.5 g/L), KH_2PO_4 (5 g/L), ammonium sulphate (5 g/L), pH 7.0) was used. Prednisolone (AR Grade) was dissolved in methanol and added to the medium in the concentration of 10-20 mg%. MMSM plates were incubated at room temperature for 48 hours. Isolates that showed good growth were selected for the fermentation studies. Identification of the isolate was done using MALDI-TOF (Haag et al., 1998).

Fermentation studies

Isolates that tolerate 20 mg % concentration of prednisolone, were grown in MMSM broth for 5 days at room temperature. Reduction in the concentration of prednisolone was evaluated based on the colorimetric method (Kwan & Schott, 1984). Microbial biomass was also evaluated as per the conventional method.

Reverse-phase HPLC based analysis

Analysis of prednisolone and its bacterial transformed product was determined with a slight modification of the method reported by Ghosh et al. in 2011. The elution of

prednisolone was done using reverse phase HPLC with isocratic mode, a hypersil ODS C18 (250 × 4.6 mm, packed with 5 microns). Methanol:water (58:42) solvent system was used as mobile phase with 1 ml/min flow rate and monitored at 254 nm. For the determination of biotransformation of prednisolone, the cell-free fermented broth was mixed with chloroform in 1:1 proportion. The organic phase was concentrated and was redissolved in methanol for reverse phase HPLC analysis.

Results and Discussion

Isolation and identification of prednisolone transformers:

Corticosteroid drug, prednisolone has been prescribed to treat health issues like skin diseases, infections, certain cancers, allergies, and immunosuppressive drugs after organ transplantation. Prednisolone has also been reported to use in the treatment of autoimmune illnesses like rheumatoid arthritis. Prednisolone drug is available as Flo-Pred, Pediapred, Orapred, and Orapred ODT brand name. Both solid and liquid formulation of prednisolone drug is available in the market.

The presence of contaminants like heavy metals, pesticides, steroidal and nonsteroidal drugs pollute water which can be treated biologically. Both bacteria and fungi have been reported to have a role in bioremediation. *Klebsiella* sp. shown extracellular polymeric substances (EPS) secretion and auto-aggregation for the remediation of nitrogen polluted water (Padhi et al., 2013). *Klebsiella* sp. WAH1 has been reported to have a diclofenac degradation pathway highly developed reactions of hydroxylation, decarboxylation, and dechlorination (Sharma et al., 2021). Researchers are trying for more bioactive prednisolone derivatives with microbial interference. Transformation of prednisolone to 20β-hydroxy prednisolone compound by *Streptomyces roseochromogenes* TS79 has been reported (Zhang et al., 2011).

A sewage sample was used for screening of prednisolone (10 mg%) transforming microorganisms. Total five bacterial strains and one fungal strain were isolated but the strain showing maximum tolerance (20 mg%) was identified as *Klebsiella pneumoniae* spp *pneumoniae* DSM3010 4T HAM based on the MALDI TOF method based on a 2.336 match score. MALDI TOF MS spectra of the isolate are given in Fig. 1

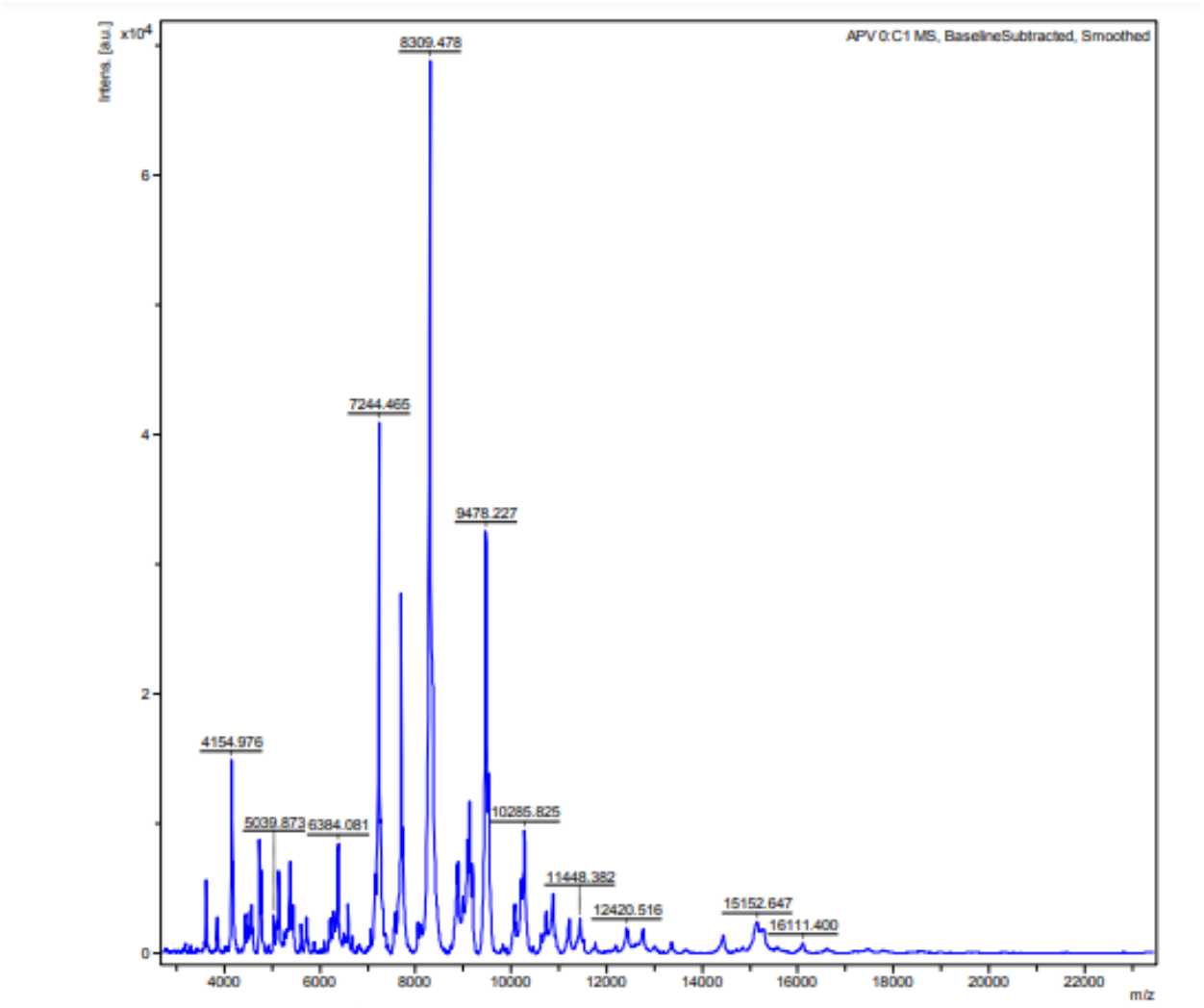

Figure 1 MALDI -TOF MS Spectra of bacterial strain indicating the protein profile (2-20Kda).

Fermentation studies

The isolate was grown in a minimal medium containing Prednisolone (20 mg%) concentration, pH was maintained at 7. Incubation was done at room temperature for 5 days to determine time course effect with the relationship between prednisolone reduction as per colorimetric-based method and biomass, as per Fig. 2. An experiment was done in triplicates for the statistical analysis.

Reverse-phase HPLC based analysis

Reverse-phase HPLC was done for standard prednisolone and transformed metabolite of prednisolone in *Klebsiella* sp. fermented broth.

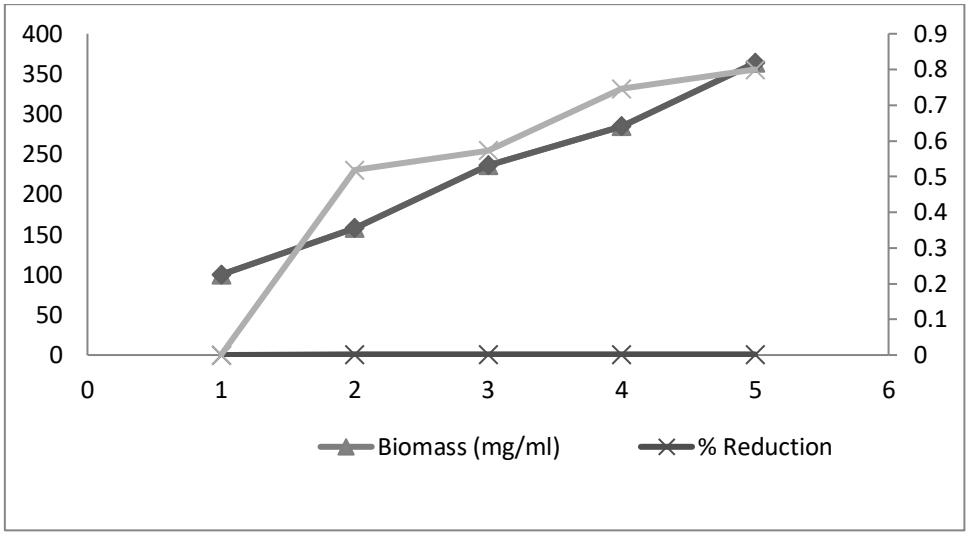

Figure 2 Time course effect of biomass of *Klebsiella* sp. on prednisolone reduction.

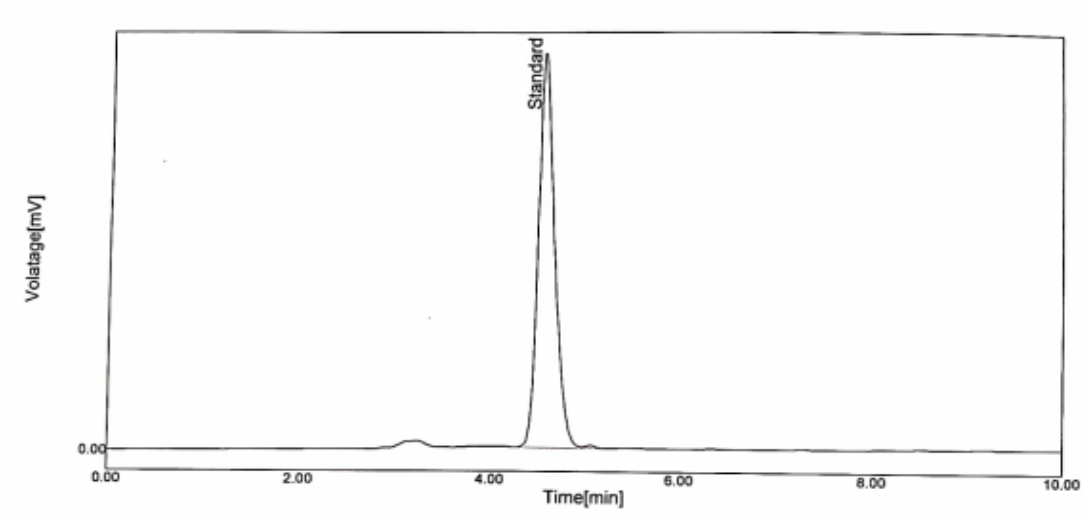

Figure 3 Chromatogram of standard prednisolone.

Prednisolone (10 mg) was eluted at retention time around 4.5667 min with symmetric peak shape, Fig. 3. In HPLC chromatogram of fermented broth, besides prednisolone five unknown metabolites were found with a retention time of 3.2667, 3.9167, 4.1833, 5.2667, and 8.8167 min/ml as per Fig. 4. Streptomyces roseochromogenes TS79 has been reported for 95.1% conversion of prednisolone (7.5 mg/ml) to 20β-hydroxy prednisolone. Analysis of transformed product was done using ^{1}H NMR (Zhang et al., 2011). As far as our knowledge of authors is concerned, very few reports are available on the microbial biotransformation of prednisolone for comparative analysis. Further research is required for the optimization of fermentation conditions for the transformation of prednisolone and structural analysis of prednisolone transformed metabolite.

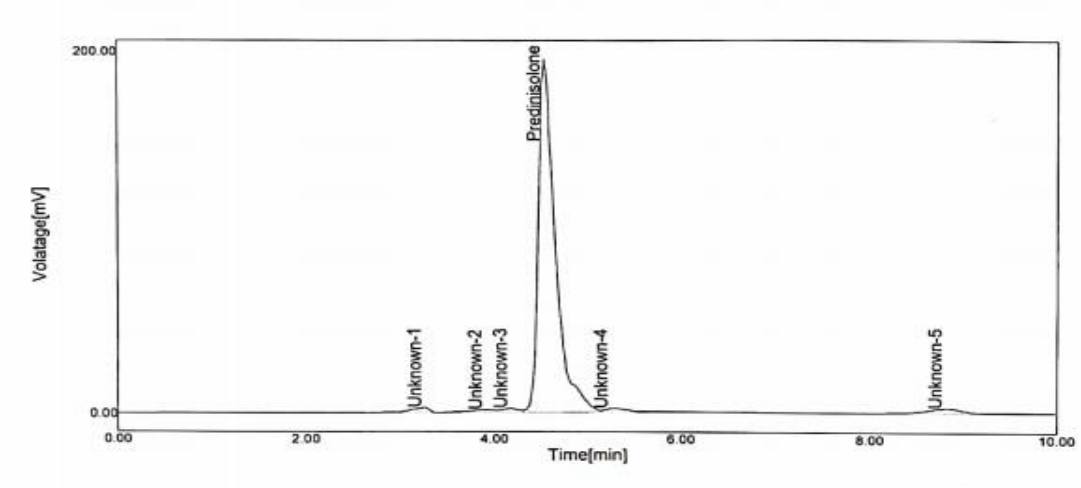

Figure 4 Chromatogram of prednisolone transformed metabolite.

Conclusions

Pharmaceutical pollutants are causing an environmental problem due to their ecotoxicological effects when mixed in water and soil. Biodegradation of non-steroidal drugs has been reported, but steroidal drugs due to their structure, are difficult to have ring cleavage action. Biotransformation of prednisolone was carried out using sewage isolate *Klebsiella* sp. The isolate was found to transform 80 % of prednisolone to some hydroxylated products. Further confirmation can be done by structural analysis based on the data of GC-MS and NMR spectroscopy of the metabolite isolated from *Klebsiella* sp. fermented broth. It has been reported that intermediates or the end product of pharmaceutical drugs are more toxic than the parent compound. The selection of the strain for bioremediation is dependent on the degradative pathway with the less toxic product. This study can be extended further by testing the ecotoxicological screening of intermediates and end product of prednisolone.

Author Contributions: The experiments and data analysis were performed by Vrishali Bankar, Ankita Gaikwad, Priti Gaikwad and Neha Patil. The experiment was supervised by Meghmala Waghmode. The manuscript was drafted by Meghmala Waghmode and revised by all the co-authors.

Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: The data that support the finding of this study are available from the corresponding author, upon reasonable request.

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