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BIOTRANSFORMATION OF 5α–ANDROSTANE-3,17-DIONE BY MICROALGAL CULTURES.

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Abstract: Twenty- four different strains of unicellular algae have been used in the bioconversion of 5α-androstane-3,17-dione.

Biotransformations of exogenous substrates by plant cell cultures have been extensively reported[1-5]. Generally, cell suspensions or immobilized cell cultures of various plant species have been used as bioreactors, whereas microalgae have been occasionally employed[6,7].

In a systematic approach to the utilization of these microrganisms in the bioconversions, we report the action of twenty- four different freshwater algal strains, belonging to different genera of Chlorophyceae and Rhodophyceae, on 5α androstane-3,17-dione.

All the algae, with the exception of N 002 *Galdieria sulphuraria*, N 182 *Cyanidium caldarium*, N 229 *Dunaliella acidophila*, and Go 183.80 *Dunaliella primolecta*, were cultured in Bold Basal Medium (BBM) in 100 ml Erlenmeyer flasks at 24°C. *G. sulphuraria*, *C. caldarium*, and *D. acidophila* were cultured in Allen medium, *D. primolecta* in Dunaliella medium. For analytical purposes axenic cultures of the strains were grown at 24°C in 400 ml of medium in 1000 ml Erlenmeyer flasks rocked on a plexiglas shaker apparatus, with a photoperiod of 16hr light : 8hr dark, with a total irradiation of 150 $\mu E.m^{-2}.s^{-1}$ provided by daylight fluorescent lamps (Philips TLD 36 W/54) below the apparatus. For the semipreparative cultures the algae were grown at the same conditions in 2000 ml of medium in cylinders with magnetic stirrers operating at 120 rpm and were bubbled with air enriched with 3% CO_2

5α androstane-3,17-dione (**1**) was added to the cultures (10mg/100ml) during the exponential phase of the algal growth. After seven days the algae were filtered off on Whatman paper filters (diameter of pores=1,6 μm) and the medium was extracted with ethyl acetate.

All the bioproducts were isolated by preparative TLC or HPLC and identified on the basis of their IR, UV MS and ^{1}H-NMR features. The yields of bioconversion were calculated by HPLC and are reported in Table 1. Control experiments using the sterilized media showed no transformation.

As can be seen most algal strains induced bioconversion of 5 α-androstane-3,17-dione and the activity seems to be species-specific. Eleven strains died after two or three days from the sterol addition; however, also in these cases the substrate was partly bioconverted and it is probable that the death of the algae was caused by some products of biotransformation. Two strains methabolized **1** completely and after seven days no steroidic material was detected, confirming that some strains could be able to utilyze sterols as carbon or energy sources.

The main bioreactions were the reduction of the carbonyl groups, the hydroxylation or the introduction of double bonds. Most algae induced selective reduction of 5α-androstane-3,17-dione to 5α-androstan-3β-ol-17–one (**2**), 5α-androstan-3α–ol-17-one (**3**), and 5α–androstan- 17β-ol-3-one (**4**), whereas the completely reduced 5α-androstane-3β,17β-diol (**5**) and 5α-androstane-3α,17β-diol (**6**) were generally obtained in small amounts.

Few strains induced oxidative processes leading to more unsaturated or hydroxylated products. C 211-8b *Chlorella emersonii* and T32 *Chlorella zopfingiensis* gave hydroxylations leading to 5α-androstane-3β,11α-diol-17-one (**7**), 5α-androstane-3β,9α-diol-17-one (**8**), 5α-androstane-3β,6α-diol-17-one (**9**) and 5α-androstane 3β,7α-diol-17-one (**10**), whereas the strain C379-1C *Stichococcus bacillaris* was able to oxidize **1** into androst-4-ene-3,17-dione (**11**), androsta-4,6-diene-3,17-dione (**12**), androst-4-en-17β-ol-3-one (**13**), and androsta-4,6-dien-17β-ol-3-one (**14**).

The unicellular algae may be considered useful bioreactors for biotransformations. In fact, these microrganisms present undoubted advantages over the cell cultures of higher plants, as they have a high degree of genetic diversity and very often do not require organic media. Moreover, many strains may withstand extreme culture conditions, such as low pH, high temperature and high salinity, which can help to ensure axenic culture conditions.

Table 1. Biotransformation induced by the algal strains.

STRAIN	SPECIES	BIOPRODUCT YIELDS (%)													
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
N 002	*Galdieria sulphuraria*	-	-	-	-	33	67	-	-	-	-	-	-	-	-
N 182	*Cyanidium caldarium*	64	6	19	4	3	4	-	-	-	-	-	-	-	-
Go 183.80	*Dunaliella primolecta**	72	19	-	9	-	-	-	-	-	-	-	-	-	-
N 229	*Dunaliella acidophila**	77	5	-	14	-	-	-	-	-	-	4	-	-	-
T 293	*Chlamydomonas sphagnophila**	79	10	-	9	2	-	-	-	-	-	-	-	-	-
C 11-36a	*Chlamydomonas dysosmos*	40	39	11	2	1	1	-	-	-	-	-	-	-	-
C 11-96	*Chlamydomonas acidophila**	63	30	-	5	2	-	-	-	-	-	-	-	-	-
T 287	*Oocystis marsonii*	100	-	-	-	-	-	-	-	-	-	-	-	-	-
C 211-8b	*Chlorella emersonii*	12	22	-	17	13	-	11	4	11	11	-	-	-	-
C 211-8k	*Chlorella sorokiniana*	17	27	-	55	1	-	-	-	-	-	-	-	-	-
C 211-9a Gö	*Chlorella saccharophila*	88	6	-	6	-	-	-	-	-	-	-	-	-	-
C 211-11b	*Chlorella vulgaris**	73	20	3	3	1	-	-	-	-	-	-	-	-	-
C 211-11g	*Chlorella kessleri*	90	10	-	-	-	-	-	-	-	-	-	-	-	-
T 32	*Chlorella zopfingensis**	-	5	37	-	1	17	13	3	14	10	-	-	-	-
T 1804	*Chlorella nocturna**	76	12	-	12	-	-	-	-	-	-	-	-	-	-
C 249-1	*Muriella aurantiaca* *	28	28	-	15	29	-	-	-	-	-	-	-	-	-
N 035	*Viridiella fridericiana*	18	6	-	59	17	-	-	-	-	-	-	-	-	-
N 188	*Pseudococcomyxa simplex**	72	5	18	-	1	-	-	-	-	-	-	-	-	-
C 277-1	*Scotiella oocystiformis*	89	7	-	4	-	-	-	-	-	-	-	-	-	-
T 76	*Scenedesmus quadricauda**	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T 749	*Ankistrodesmus falcatus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C 379-1c	*Stichococcus bacillaris*	30	-	-	-	-	-	-	-	-	-	52	15	2	1
N 217	*Stichococcus sp.*	77	2	21	-	-	-	-	-	-	-	-	-	-	-
T 1178	*Ulotrix acuminata**	17	34	49	-	-	-	-	-	-	-	-	-	-	-

T= UTEX- the Culture Collection of Algae at the University of Texas at Austin
C = CCAP Culture Center of Algae and Protozoa, Ambleside, Cumbria, England.
GO = Sammlung von Algenkulturen, Gottingen, Germany
N = Collezione di alghe del Dipartimento di Biologia Vegetale di Napoli, Italia
* died after 2 - 3 days

1 5α-androstane-3,17-dione, **2** 5α-androstan-3 β-ol-17-one; **3** 5α-androstan-3β-ol-17-one; **4** 5α-androstan-17β-ol-3one, **5** 5α-androstane- 3β,17β-diol; **6** 5α-androstane- 3α,17β-diol, **7** 5α-androstane- 3β,11α-diol-17-one, **8** 5α-androstane -3β, 9α-diol-17-one, **9** 5α-androstane- 3β,6α-diol-17-one; **10** 5α-androstane-3β,7α-diol-17-one; **11** androst-4-ene-3,17-dione, **12** androsta-4,6-diene-3,17-dione; **13** androst-4en-17β-ol- 3-one, **14** androsta-4,6-dien - 17β-ol-3-one

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