Ain Shams University
Faculty of Pharmacy
Microbiology & Immunology dept.

Microbial biotransformation of vitamin D_3 into biologically active 1 α, 25-dihyroxyvitamin D_3

A Thesis

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Abbreviation	Definition
VDBP	Vitamin D-binding protein
25(OH) D_3	25-hydroxyvitamin D_3
1α, 25$(OH)_2$ D_3	1α, 25-dihydroxyvitamin D_3
Vdh	Vitamin D_3 hydroxylase
P450/ CYP	Cytochrome P450 enzyme
VDR	Vitamin D receptor
CD14	Cluster of differentiation 14
1α(OH) D_3	1α-hydroxyvitamin D_3
A. autotrophica	*Amycolata autotrophica*
A. saturnea	*Amycolata saturnea*
A. hrydrocarbonoxydans	*Amycolata hrydrocarbonoxydans*
A. alni	*Amycolata alni*
YMG	Yeast extract malt extract glucose
TLC	Thin layer chromatography
MS	Mass spectrometry
R_f	Retention factor
HPLC	High performance liquid chromatography
cfu	Colony-forming unit

Abstract

One hundred and eighty bacterial isolates were recovered from different soil collected samples using paraffin baiting technique. Grown bacterial colonies, appearing as white chalky particles around the solidified paraffin wax globules, were collected and screened for vitamin D_3 biotransformation activity. Vitamin D_3 dissolved in ethanol was added to 2 days old main culture and incubation conditions were 28°C and 200 rpm. Extraction of vitamin D_3 and its metabolites was carried out by a modified Bligh and Dyer method using methanol and methylene chloride.

Preliminary analysis, using TLC, showed that five isolates (A11-2, A13-4, A8-4, A26-7 and A26-8) could transform vitamin D_3 into 1α, 25-dihydroxyvitamin D_3 (calcitriol) with 25-hydroxyvitamin D_3 (calcidiol) as an intermediate. Such results were further confirmed using mass spectrometric analyses. The relative intensities of the produced calcidiol and calcitriol by the five positive isolates (A11-2, A13-4, A8-4, A26-7 and A26-8), as analyzed by MS/MS, were compared and it was found that the isolate A11-2 exhibited the highest product intensity for either calcitriol or calcidiol.

The test isolate A11-2 was identified using microscopical, culture and biochemical characteristics as well as Biolog microbial identification system assay. Microscopical, culture and some biochemical characteristics showed great similarity between the test isolate A11-2 and the standard strain, *A. autotrophica* NRRL B-11275. However, by conducting the Biolog microbial