NOTES

GLUCOSYLATION OF MYCOPHENOLIC ACID BY *STREPTOMYCES AUREOFACIENS*

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Mycophenolic acid, a metabolite of several *Penicillium* species,[1] has antibacterial,[2] antifungal,[2] antiviral,[3] and antitumor properties.[3] It has been tested clinically as an anticancer agent[4] and as an inhibitor of the proliferative skin disease psoriasis.[5] The diverse biological activities of the compound have led to studies on its chemical[6] and microbiological modification.[7] One series of chemically prepared derivatives with good antitumor activity contains a sugar linked to the phenolic hydroxyl group of the molecule.[6] These glycosides are made by a relatively expensive multi-step synthesis. Microorganisms catalyze various glycosylation (particularly glucosylation) reactions[8~11] and thus may afford an alternative route to the preparation of these compounds.

JONES *et al.*[7] conducted an extensive microbial transformation study with mycophenolic acid. Over 500 cultures were screened, but glycosides were not among the 16 transformation products identified. Our study was directed toward glycosylation by screening cultures known to catalyze this type of reaction. Six cultures were tested (Table 1), and two of these, *Streptomyces candidus* NRRL 5449 and *Streptomyces aureofaciens* NRRL 2209, produced a compound with the same Rf value (Silica gel G F_{254}, CH_3CN - H_2O, 90: 10) as authentic 7-O-(β-D-glucopyranosyl)-mycophenolic acid. The glucoside was isolated from the *S. aureofaciens* culture because this microorganism appeared to produce it in larger amounts.

Production of Mycophenolic Acid Glucoside

A loop inoculum of *S. aureofaciens* from a modified BENNETT'S[12] agar slant culture was transferred to 50 ml of CSS XII medium in a 250-ml Erlenmeyer flask. The flask was incubated for 48 hours at 30°C on a rotary shaker operating at 250 rpm ($2\frac{1}{2}''$ stroke). Five-ml portions of this culture were used to inoculate 100 ml of CSS XII medium in 500-ml Erlenmeyer flasks. These cultures were incubated for 48 hours and then 10 ml of water containing 50 mg of mycophenolic acid (pH adjusted to 7.0) was added to each flask together with 4 ml of water containing 250 mg/ml of filtered sterilized glucose. The flasks were incubated an additional 72 hours, their contents then pooled, adjusted to pH 4.0 with HCl, and filtered through Eaton-Dikeman #623 paper coated with Hyflo filter aid. The mycelium/Hyflo pad was extracted with methanol. The methanol was removed *in vacuo* and the remaining aqueous portion was added to the culture filtrate. The filtrate was extracted with an equal volume of chloroform to remove impurities and any remaining mycophenolic acid. The glucoside was recovered from the extracted broth by chromatography over an XAD-2 resin (Rohm and Haas Company, Philadelphia, PA). One liter of extracted broth was passed through a 750 mm × 40 mm OD column packed with 700 ml of XAD-2 resin. The column was washed with 4 liters of deionized water and then eluted with a methanol - water gradient. The glucoside eluted with a 50% methanol - water mixture and fractions containing the product were pooled and

CSS XII Medium

Dextrose	15 g
Nutrisoy grits	15 g
Corn steep liquor	10 g
Stadex starch 2	20 g
$CaCO_3$	4 g
KCl	0.2 g
$MgSO_4 \cdot 7H_2O$	0.2 g
$FeSO_4 \cdot 7H_2O$	2 mg
Deionized water	1 liter

pH adjusted to 7.0 with 5 N NaOH.

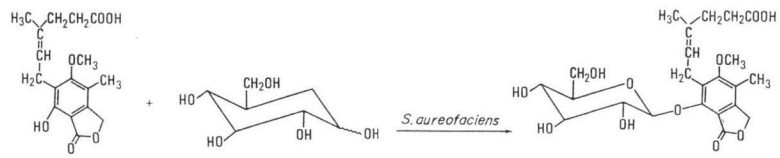
Table 1. Microorganisms tested for their ability to glycosylate mycophenolic acid.

Culture accession number[1]	Culture	Substrate glycosylated	Ref.	Glycosylation of mycophenolic acid
QM 1999	*Aspergillus niger*	Pyridoxine	13	—
QM 7397	*Aspergillus awamori*	Maltose	14	—
QM 380	*Aspergillus flavus*	Sucrose	15	—
QM 941	*Penicillium chrysogenum*	Lactose	16	—
NRRL 5449	*Streptomyces candidus*	Monensin	17	+[2]
NRRL 2209	*Streptomyces aureofaciens*	Alizarin Anthraflavin	18	++++

[1] Fermentation Laboratory, Northern Regional Research Laboratory, 1815 North University Street, Peoria, IL 61604.

[2] Not confirmed by isolation and structure determination.

Fig. 1. Glucosylation of mycophenolic acid by *Streptomyces aureofaciens*.



Mycophenolic acid Glucose 7-O-(β-D-Glucopyranosyl)-mycophenolic acid

concentrated *in vacuo*. Final purification was achieved by high performance liquid chromatography (HPLC) using reverse-phase silica gel. The HPLC column (280 mm long and 20 mm OD) was operated at 100 psi with a flow rate of 7 ml/minute. The column effluent was monitored by UV absorption at 250 nm and the glucoside was eluted with an isocratic solvent mixture of 65% water - 35% methanol. Fractions containing the product were pooled and concentrated *in vacuo* to obtain purified mycophenolic acid glucoside. The overall yield of product based on the weight of mycophenolic acid added to the culture was 77% (51% on a molar basis). The molecular weight of the product, as determined by field desorption mass spectroscopy, was 483. The same molecular weight was found for the chemically prepared mycophenolic acid glucoside. Nuclear magnetic resonance spectroscopy (100 MHz) indicated that microbiologically-prepared and chemically-prepared mycophenolic acid glucosides are identical (Fig. 2). The spectra also show that the anomeric proton (1H, 6.05 ppm, d, J=8.0 Hz)* of both compounds is in the axial position indicating that the glucosides are β-D linked.

* The chemical shift was concentration dependent.

These results demonstrate that mycophenolic acid glucoside can be prepared in high yield by microbial transformation. In addition, larger amounts of the glucoside can be obtained by periodically adding additional mycophenolic acid to the culture and extending the incubation time (HORTON and ABBOTT, unpublished data). Further improvements might be made by optimizing cultivation conditions (temperature, pH, *etc.*) or by enzyme or cell immobilization. Even in the absence of the latter improvements, the microbiological process appears to be more economical than chemical synthesis.

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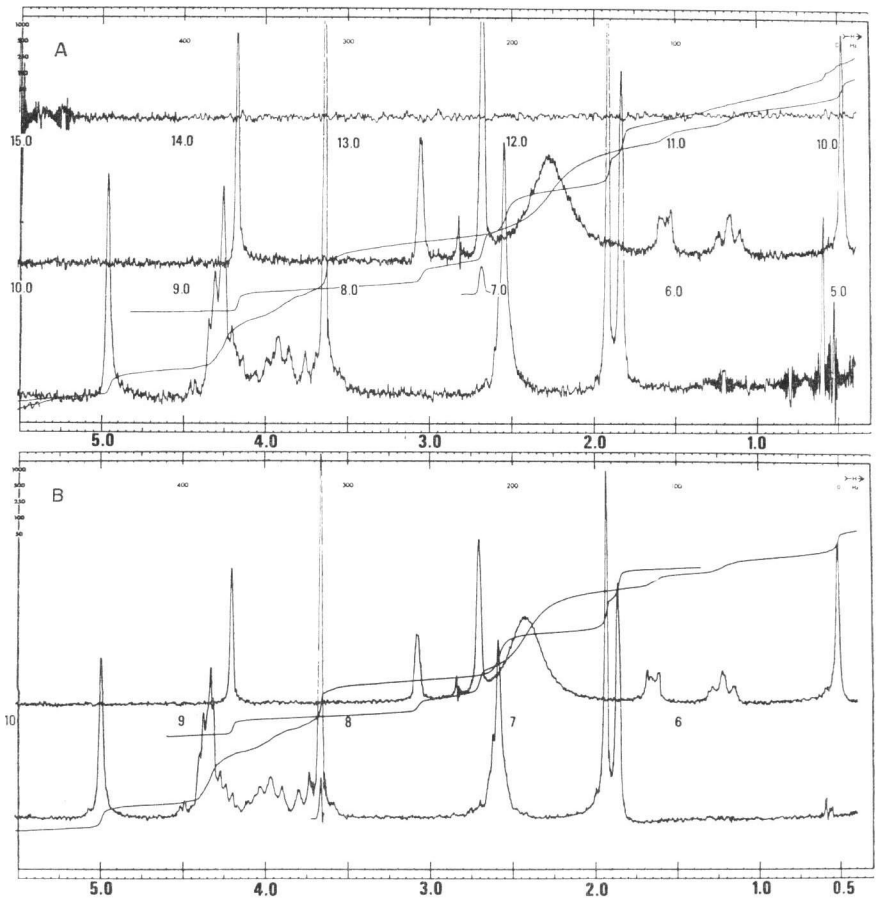
Fig. 2. 100 MHz proton magnetic resonance spectra of mycophenolic acid glucose prepared (A) microbiologically and (B) chemically.

The compounds were dissolved in deutero pyridine and the resonances are expressed as ppm downfield from tetramethylsilane.



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