Measurement of induced β-galactosidase activity in intact cells of *Streptomyces* species

J. Sánchez, M. E. Arias and C. Hardisson

Departamento de Microbiología, Universidad de Oviedo, Oviedo (Spain), 4 November 1980

Summary. β-Galactosidase activity in intact cells of 21 species of *Streptomyces* was measured using ONPG hydrolysis, without addition of a permeabilizing agent. Differences in the induction efficiency of ONPG-hydrolytic activity by lactose or galactose, which could have taxonomic implications, were observed among the species.

The measurement of β-galactosidase activity in intact cells has been systematically applied to the study and taxonomy of the Enterobacteriaceae[1,2], *Mycobacterium*[3] and anaerobes[4]. In general, the method used consisted of adding an o-nitrophenyl-β-D-galactopyranoside (ONPG) solution to a bacterial suspension cultivated on lactose-inductive medium, and subsequent permeabilization of the cells with toluene before the enzyme assay. Qualitative readings are made by observing the colour intensity produced by o-nitrophenol liberated after incubation.

No systematic study of β-galactosidase has been made within the genus *Streptomyces*, a differentiated filamentous prokaryote, although some authors have investigated the inductive responses in *S. griseus*[5] and *S. violaceus*[6], using cellular extracts to measure the activity. In the last species lactose induces 2 electrophoretically different ONPG-hydrolytic bands, one of which is coincident with that induced in galactose medium[7]. We present in this work the results of screening for the presence of induced ONPG-hydrolytic activity in intact cells of 21 strains representing different species of *Streptomyces*.

The following method was used: 100 ml flasks, containing 20 ml of medium (glycerol, 10 g; asparagine, 1 g; yeast extract, 0.25 g; $(NH_4)_2HPO_4$, 1 g; KH_2PO_4, 0.68 g; $Na_2HPO \cdot 12\,H_2O$, 1.8 g; $MgSO_4 \cdot 7\,H_2O$, 0.5 g; $CaCl_2 \cdot 2\,H_2O$, 0.2 g; distilled water, 1000 ml), were inoculated with 1 ml of spore suspension obtained by adding sterile medium to well sporulated colonies grown on GAE solid medium[8]. The flasks were then incubated during 24 h at 28 °C in a Gallenkamp orbital incubator at 200 rpm. In this medium the majority of the strains grew well. 3 ml of this medium were used as inoculum for the same medium, but without yeast extract. Glycerol, D-galactose or lactose at 0.11 M concentration were used as carbon sources. Lactose or galactose were applied as inducers since these compounds are the most effective in this genus[5,6]. Cells were grown for 24 h at 28 °C; longer or shorter incubation times did not essentially alter the results. Addition of glycerol did not affect the inducer capacity of lactose or galactose (unpublished results). After incubation, cells were washed, resuspended in 20 ml of 0.1 M sodium phosphate buffer, pH 7.2, and incubated at 28 °C with shaking. 2 ml of ONPG (Koch-Light Labs., Colnbrook, Bucks. England) solution were added after 15 min of incubation, giving a final concentration of 3 mM. The results of the hydrolysis were observed visually after 1 h, using the glycerolgrown cells as control (basal level of activity). Values for growth (dry weight measurements) ranged from 10 to 54 mg/ml of cells in all the strains tested. In cases where a marked difference in growth on lactose and galactose existed, the colour intensity of the cell-free supernatants was measured in a spectrophotometer at 420 nm[9], and the absorption values corrected for the cellular dry weight.

The ONPG-hydrolytic activity was induced by galactose, lactose or both, in the 21 species assayed (table). In 10 strains (about 48%) activity was induced by galactose, in 6 by lactose (28%) and in 5 by both sugars (24%). Of the latter, 3 exhibited higher activity in galactose than in lactose containing medium, 1 similar activity in both, and the other more activity in lactose than in galactose medium. Thus, galactose was a more effective inducer of ONPG-hydrolytic activity than lactose in *Streptomyces*. However, a situation similar to that encountered in *S. violaceus*, where different activities appear to be induced by galactose and lactose, may well occur in the strains studied. It was not possible to find a definitive relationship between dry weight and ONPG-hydrolytic activity induced by lactose and galactose. It thus seems that it would be interesting to pursue additional investigations of the physiological role and the taxonomic implications of both enzymatic activities in this genus, measuring the galactose and lactose induced activity (ies) in different strains of the same species.

Hydrolysis of ONPG by intact cells of *Streptomyces* species grown on galactose or lactose

Activity on galactose medium high[a]		Activity on lactose medium high[a]		Significant activity on both media	
S. flaveolus	ATCC 3319	*S. bambergiensis*	ATCC 13879	*S. ambofaciens*	ATCC 23877[b]
S. violaceus	CECT 3196	*S. viridochromogenes*	ATCC 14290	*S. aureofaciens*	ATCC 10762[b]
S. coelicolor	CECT 3080	*S. venezuelae*	ATCC 10595	*S. ederensis*	ATCC 15304[b]
S. scabies	CECT 3352	*S. lavendulae*	CECT 3148	*S. griseus*	CECT 3102[c]
S. glaucescens	ATCC 23622	*S. erythraeus*	ATCC 11635	*S. flavovirens*	CECT 3208[d]
S. acrimycini	ATCC 19885	*S. fluorescens*	ATCC 15860		
S. violascens	ATCC 23968				
S. fradiae	ATCC 10745				
S. albus	CMI 52766				
S. antibioticus	ATCC 1189				

[a] With little or no activity if grown on the other sugar; [b] greater activity on galactose medium; [c] greater activity on lactose medium; [d] similar activity on both media. Abbreviations: ATCC, American Type Culture Collection; CECT, Colección Española de Cultivos Tipo; CMI, Commonwealth Mycological Institute.

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