GLUCOSINOLATE DEGRADATION BY BACTERIAL STRAINS ISOLATED FROM A HUMAN INTESTINAL MICROFLORA

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ABSTRACT

Glucosinolate degradation by a whole human intestinal microflora was assessed *in vitro*. Screening tests subsequently undertaken led to the isolation of twenty five strains capable of degrading glucosinolates *in vitro* in growth conditions. This strain collection represents a major tool for future investigation of glucosinolate metabolism by the digestive bacterial ecosystem.

INTRODUCTION

The various biological effects occurring in man and animals following consumption of cruciferous vegetables mainly result from glucosinolate (GSL) breakdown, either by plant myrosinase (thioglucoside glucohydrolase EC 3.2.3.1.), or by enzymatic activities of intestinal bacteria. The mechanisms by which GSL derivatives, such as isothiocyanates and nitriles, are released by plant myrosinase have been extensively investigated (Palmieri *et al.*, 1993). On the other hand, many aspects of microbial metabolism of GSL are still unclear. Gnotobiotic experiments have shown that rat, chicken and human intestinal microflora are capable of degrading GSL contained in rapeseed meal *in vivo* (Nugon-Baudon *et al.*, 1988 ; Rabot *et al.*, 1993). Furthermore single strains isolated from chicken or human microflora each lead to specific biological effects in gnotobiotic rats fed rapeseed meal (Nugon-Baudon *et al.*, 1990 ; Rabot *et al.*, 1993). Such findings confirm and extend earlier studies which demonstrated that GSL can be broken down *in vitro* by the whole digestive microflora of sheep (Lanzani *et al.*, 1974), chickens (Miguchi *et al.*, 1974) or humans (Oginsky *et al.*, 1965).

In this study, GSL degradation by a human intestinal microflora *in vitro* and isolation of GSL-degrading bacteria belonging to dominant and subdominant clusters are described. Sinigrin (SIN) was used as a model compound due to its ready availability. Gnotobiotic rats associated with a whole human intestinal microflora and fed a diet containing rapeseed meal were used as a " strain bank " . This design was devised to put the human digestive bacterial ecosystem continuously in contact with GSL, considering these compounds as potential enzymatic inducers.

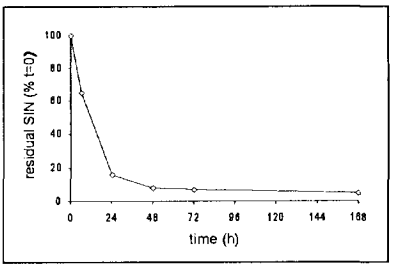
EXPERIMENTAL

GSL degradation by a whole human intestinal microflora

Faecal flora from a healthy human subject was used to inoculate 2 germ-free Fischer 344 rats fed a diet containing a myrosinase-free rapeseed meal. After a 4-week period to achieve an equilibrium between the host, the flora and the diet, faecal samples were collected and suspended in liquid brain heart infusion medium (0.01g/ml) with added SIN (12 mmol/l ; SIN-BHI). Cultures were incubated at 37°C in an anaerobic chamber and samples were withdrawn after 6, 24, 48, 72 and 168 h to measure concentrations of residual SIN. Cell-free supernatants were incubated with plant myrosinase and the glucose released following SIN hydrolysis was assayed using the hexokinase/glucose-6-phosphate dehydrogenase coupled enzyme system (Wilkinson *et al.*, 1984).

As shown in Figure 1, SIN was quickly and intensely degraded : 85% of the substrate had disappeared within 24 h and there was virtually no residual SIN at the end of incubation.

Figure 1. Kinetics of *in vitro* SIN degradation by the faecal flora of gnotobiotic rats harbouring a whole human intestinal microflora (n=2)

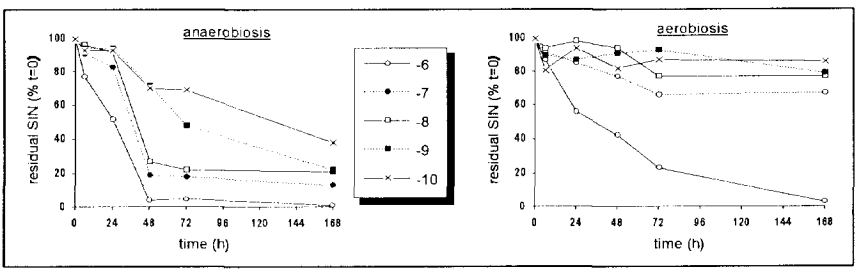


GSL degradation by dominant and subdominant clusters of human intestinal microflora

A faecal sample was collected from one gnotobiotic rat and serially diluted 10-fold in liquid SIN-BHI. 10^{-6} to 10^{-8} dilutions (subdominant and dominant clusters) and 10^{-9} and 10^{-10} dilutions (dominant clusters) were incubated at 37°C under anaerobiosis. A duplicate series of dilutions was incubated in aerobiosis to selectively investigate the GSL-degrading potential of facultative anaerobes. The concentrations of residual SIN were assayed at intervals as described above.

Bacterial growth was observed in all cultures. However the kinetics of SIN degradation differed greatly according to the dilution and the incubation conditions (Figure 2). Under anaerobiosis, SIN degradation occurred in all cultures but was proportionally greater in lower dilutions where both dominant and subdominant clusters are present. Under aerobiosis, only the 10^{-6} dilution exhibited a significant SIN degradation.

Figure 2. Kinetics of *in vitro* SIN degradation by the faecal flora of a gnotobiotic rat harbouring a whole human intestinal microflora : effects of dilution and incubation conditions.



Isolation of SIN-degrading bacteria

Samples from dilutions exhibiting SIN degradation were spread onto SIN-BHI agar plates and incubated for 2 to 7 days in the relevant conditions. Five colonies per plate were randomly picked up, subcultured into liquid SIN-BHI and maintained as stock cultures in 40% (v/v) glycerol at -20°C. Among this 150 isolate collection, 25 strains were selected as having the most potential as GSL-degrading organisms, based on the dilution from which, and the interval at which they were isolated. They were incubated in liquid SIN-BHI at 37°C under anaerobiosis or aerobiosis according to their origin. The concentrations of residual SIN were assayed at intervals as described above. Active strains were classified by their shape, motility, Gram stain and biochemical characters as determined with the API System (bioMérieux, Marcy-l'Etoile, France).

Bacterial growth was observed in all cultures and all isolates tested were able to degrade SIN to different extents (Table 1). Among them, Gram-negative bacilli belonging to the species *Escherichia coli* were predominant. However the most active strains were Gram-positive cocci belonging to the genus *Peptostreptococcus.*

Table 1. Characterisation of bacterial isolates and their ability to degrade SIN

Identity	Number of strains	Residual SIN (% t=0) after...	
		...48 h incubation	...168 h incubation
Escherichia coli	17	58 to 91	25 to 55
Enterococcus faecalis	1	67	22
Enterococcus faecium	3	55 to 60	25 to 30
Bacteroides thetaiotaomicron	1	60	30
Peptostreptococcus spp	3	45 to 70	0 to 40

DISCUSSION

Human intestinal microflora exhibits a very potent ability to degrade SIN *in vitro*. The study of bacterial clusters indicates that this enzymatic activity is present in dominant and in subdominant populations as well. However anaerobiosis seems to offer more favourable conditions to the expression of SIN-degrading potential. All 25 isolates that were tested are capable of degrading SIN *in vitro*. Previous authors reported that GSL-degrading bacteria were extremely rare when laboratory strain collections or human faecal samples were investigated (Oginsky *et al.*, 1965 ; Tani *et al.*, 1974). The great yield obtained in this study is likely to be due to the continuous feeding of gnotobiotic rats with a GSL-containing diet. Indeed Brabban and Edwards (1994) have reported that GSL-degrading micro-organisms isolated from environment could lose this ability when cultured on GSL-free media. In the future, the strain collection created in this study should help investigation of GSL metabolism in the digestive tract, with emphasis on the identification of bacterial GSL-derivatives.

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TECHNICAL SESSION C

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