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Bacterial detoxification of saponins in the crop of the avian foregut fermenter *Opisthocomus hoazin*

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ABSTRACT

The hoatzin is a folivorous bird with microbial fermentation in the crop that consumes plants that contain saponins. We investigated detoxification activity of saponins by bacteria from the hoatzin crop, and how this activity was affected in the presence of methanogens. Strains and mixed cultures were grown in presence of 200 $\mu g \cdot ml^{-1}$ *Quillaja* saponins. Detoxification was determined as loss of haemolytic activity. The methanogenesis was inhibited added bromoethanesulphonic acid (BES). Mixed cultures showed higher rate of saponin detoxification than strains and it was reduced by BES. The hoatzin bacteria detoxify saponins, and methanogenic Archaea might act as a hydrogen sink.

KEYWORDS: bacteria, saponins, detoxification, methanogens, hoatzin

INTRODUCTION

The toxic effects of saponins are due to their ability to form complexes with membrane sterols (Bangham and Horne, 1962). In ruminants, they inhibit rumen fermentation and may cause ruminant bloat (Sen et al., 1998), apparently through the inhibition of some rumen bacteria and protozoa (Wang et al., 2000; Wina et al., 2006). Microbial degradation of saponins produce the presumably less toxic

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sapogenins, with the consequent decrease of their cytolytic activity (Pillion et al., 1996).

The hoatzin diet comprises plants that contain saponins such as *Acacia* sp. and *Pithecellobium ligustrinum* (Domínguez-Bello et al., 1994). The aim of this study was to determine if bacterial cultures from the hoatzin crop could detoxify saponins and how this activity was affected in the presence of methanogens.

MATERIAL AND METHODS

To determine the effect of inoculum concentration on saponin detoxification, three dilutions of crop contents (52, 13 and 0.65 mg·ml^{-1}) were homogenized and grown anaerobically at 37ºC in broth M10 (Ogimoto and Imai, 1981) containing 200 µg.ml^{-1} *Quillaja* saponins. Detection of methanogenic bacteria was performed by PCR amplification of a 0.76 kb region of the methyl coenzyme M reductase gene. The *16S rRNA* gene sequence of one clone from crop contents and another from cultures were sequenced GenBank (AF506286 and AY089708).

Saponin detoxification was determined by the loss of haemolytic activity of culture supernatants on rabbit red blood cells. Rabbit blood was diluted 1:2 in citrate buffer (%: dextrose 2, sodium citrate 0.8, citric acid 0.055 and NaCl 0.42), and centrifuged (2700 *g* per 5 min in a Sorvall T6000B). The pellet with the red blood cells was washed and resuspended in 5 volumes of 0.8% NaCl. Supernatants (50 µl) of cultures were added to a 96-well plate followed by addition of 50 µl of the red blood cell suspension. After 1 h, plates were centrifuged and supernatants were measured absorbance at 540 nm.

To investigate the importance of methanogens on saponin detoxification, compared saponin detoxification by crop contents of 52 mg·ml^{-1} grown with and without the methanogenesis inhibitor 2-bromoethanesulphonic acid (BES; 60 mM).

RESULTS

Mixed cultures of three crop dilutions (52, 13 and 0.65 mg·ml^{-1}) were able to reduce haemolytic activity of *Quillaja* saponins by 80%, in few hours (Table 1). The rate of saponin detoxification was a function of inoculum concentration, decreasing when the inoculum was diluted ($P<0.05$). Isolated strains of *Streptococcus* and a *Bacillus* species from saponin-containing cultures showed slower (days instead of hours) saponin detoxification activity than mixed cultures.

Table 1. Effect of bacterial inoculum concentration (52 , 13 and 0.65 mg of crop content per ml of culture medium) on bacterial count, rate of saponin detoxification and the time to reach detoxification of 80% of saponin concentration by mixed crop cultures in two hoatzin crops

Bacterial inoculum mg ml^{-1}	Hoatzin crop	Bacteria/g crop	Rate of detoxification ($\mu g.ml^{-1}$ saponins) h^{-1} and regression coefficient*	Time for 80% saponin detoxification, h
52	B12	$4.97 \pm 2.75 \times 10^{12}$	27.86 ± 1.59 (R^2:0.98)^a	4
	B13	$1.33 \pm 0.27 \times 10^{12}$	23.99 ± 2.84 (R^2:0.90)^a	4.5
13	B12	$3.75 \pm 0.96 \times 10^{9}$	5.87 ± 0.44 (R^2:0.99)^b	24
	B13	$2.25 \pm 0.96 \times 10^{9}$	5.60 ± 0.70 (R^2:0.94)^b	24
0.65	B12	nd	1.31 ± 0.03 (R^2:0.90)^c	100
	B13	nd	1.09 ± 0.06 (R^2:0.91)^c	140

values of bacterial counts and saponin detoxification rates are expressed as mean ± SD
values with different superscript are significantly different ($P<0.05$); nd - non determined

Methanogens were detected by PCR amplification of the methyl coenzyme M reductase gene in both crop contents and in saponin-detoxifying cultures. The *16SrRNA* sequence matched *Methanobrevibacter* sp. (Genbank AY196672) and *Methanosphaera stadtmanae* (AY196684).

Inhibition of methanogens with BES caused a significant decrease in the rate of saponin detoxification ($P<0.05$; Table 2).

Table 2. Effect of BES (60 mM) on the rate of saponin detoxification by mixed crop cultures with an inoculum of 52 mg of crop content per ml of culture medium

Animal	Bacterial count per g of crop	Rate of detoxification ($\mu g\ ml^{-1}$ saponins) h^{-1} and regression coefficient[1]	Time for 80% saponin detoxification, h
B12 Control	$4.97 \pm 2.75 \times 10^{12}$	27.86 ± 1.59 (R^2: 0.98)^a	4
B12 + BES	$1.52 \pm 0.53 \times 10^{12}$	18.32 ± 1.85 (R^2: 0.95)^b	5.5
B13 Control	$1.33 \pm 0.27 \times 10^{12}$	23.99 ± 2.84 (R^2: 0.90)^a	4.5
B13 + BES	$5.71 \pm 0.27 \times 10^{11}$	12.72 ± 1.22 (R^2: 0.92)^c	9.5

values of bacterial count and saponin detoxification rate are expressed as mean ± SD
[1] values with different superscript are significantly different ($P<0.05$)

DISCUSSION

Crop cultures from the hoatzin were able to substantially reduce the haemolytic activity of *Quillaja* saponins, suggesting the crop capability to microbial detoxification of dietary saponins, as has been shown in rumen bacteria that degrade *Quillaja* saponins to quillaic acid, a triterpenoid molecule which does not produce haemolysis (Pillion et al., 1996; Makkar and Becker, 1997). The reduction of saponin detoxification by BES suggests a synergistic action of

archaeal methanogens and eubacterial degraders. Consortia with methanogens has been described in the bacterial degradation of aromatic compounds in the cow rumen (Bisaillon et al., 1993), where methanogenics are a sink for H_2.

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