Distribution of urease producing bacteria in the rabbit caecum

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The distribution of urease-producing bacteria was examined in lumenal and mucosal-associated populations of the rabbit caecum. The total number of bacteria recovered on a non-specific, semi-defined medium were 1,7 × 10^9 per g from lumen contents and 2,0 × 10^5 per g of washed, homogenized caecal wall. Urease activity was shown by 5,3% of strains isolated from lumen contents and 21,7% mucosal-associated strains. A higher proportion of bacteria associated with the caecal wall (63%) were facultative compared with 20% from the caecal content.

Die verspreiding van bakterieë wat urease produseer is ondersoek in die buisholte en slymvlies geassosieerde populasies van die konyn se sakderm. Die totale aantal bakterieë wat herwin is op 'n nie-spesifieke, semi-gedefinieerde medium was 1,7 × 10^9 per g van die buisholte inhoud en 2,0 × 10^5 per g van gewas en gehomogeniseerde sakdermwand. Urease aktiwiteit is aangetoon vir 5,3% van die stamme geïsoleer uit die buisholte inhoud en 21,7% van die slymvlies geassosieerde stamme. 'n Groter gedeelte van die bakterieë wat geassosieer word met die sakdermwand (63%) was fakulatief in vergelyking met die 20% van die sakderm inhoud.

Keywords: Urease, rabbit caecum, anaerobic bacteria, mucosal-associated flora

Introduction

The complex anaerobic flora responsible for fermentation in the caecum of monogastric herbivores has been poorly characterized in comparison with that of the rumen (Fonty, 1979). Although each animal appears to have its own unique population of bacteria, the sparse evidence presented to date indicates that the chemical processes involved may be very similar. As in the ruminant, nitrogen is of vital importance to the maintenance of intestinal micro-organisms of all animals, including man. In addition to urinary excretion of urea, as much as 30% of this source of nitrogen may be recycled via the gut, where it is converted to ammonia by ureolytic enzymes of intestinal bacteria, (Brown, Hill & Richards, 1971). There are indications that urease activity may be concentrated at the point of diffusion of urea across the epithelium, as is shown with blocks of rumen wall, (McCowan, Cheng & Costerton, 1980) and from diffusion studies on the human colon (Wolpert, Phillips & Summerskill, 1971). This study attempts to confirm these observations by comparing the distribution of urease producing bacteria in lumenal and mucosal-associated populations of the rabbit caecum.

Materials and Methods

Bacterial Cultures: Adult New Zealand White rabbits, exsanguinated under anaesthesia for serum collection, were used in this study. Ligated portions of the caecum were transferred to an anaerobic glove box where 1 g of lumen contents was serially diluted in prereduced anaerobic diluting fluid. Caecal wall, in 1 g portions, was washed until free of particulate matter, homogenized, and diluted as above. Culture media were prepared according to the Anaerobe Laboratory Manual (Holdeman, Cato & Moore, 1977) with primary isolation on M10 plate medium, isolates maintained on RGCA slants, and unrease assay performed in PY broth with 250 mM urea as the only nitrogen source.
Urease Activity: Ammonia production in the urea medium was detected by the method of Wozny, Bryant, Holdeman & Moore (1977) by adding Nessler's reagent to four-day broth cultures in spot plate wells.

Results

A total of 154 strains isolated from three rabbits (approximately 50 from each) were examined for urease production. The total number of bacteria recovered on M10 semi-defined medium were 1,7 ± 2,2 × 10^9/g from lumen contents and 2,0 ± 1,1 × 10^5/g from homogenized washed caecal wall. Whereas the lumen cultures at high dilutions showed a diversity of colonial and morphological types, mucosal-associated cultures were limited to only three colony forms.

Urease activity detected by ammonia production was shown by four out of 76 strains (5,3%) isolated from lumen contents. In contrast, 17 of 78 mucosal-associated strains (21,7%) were positive for urease. Although these cultures have not been identified for purposes of this study, it is interesting to note that there was a far higher component of facultative organisms associated with the caecal wall (63%) whereas only 20% of the strains cultured from caecal contents were facultative. Ureolytic activity was unevenly distributed between facultative and obligate anaerobes.

Discussion

The results reported here indicate that ureolytic activity in the rabbit caecum is localized predominantly in micro-organisms closely associated with the mucosal lining of the caecal wall. Unlike other primary producers such as the cellulolytic bacteria of the rumen which are limited to a narrow range of species, urease production appears not to be

the prerogative of any particular group, but rather spread over representatives of both facultative and obligately anaerobic genera. Wozny *et al* (1977) reported 5,8% urease producers in bovine rumen contents and 14% in human faeces. These figures did not take into consideration the significant contribution to total ureolytic activity made by the micro-organisms that colonize the epithelial surface of these ecosystems.

Although the rabbit has been discounted as a laboratory model for rumen fermentation, studies of the caecal ecosystem can still yield valuable information on herbivore nutrition. Of even more importance is the role that the domestic rabbit can and does play as a supplement or even replacement of conventional animal protein. Until more is known about digestion of plant foods in the monogastric herbivore, manipulations such as the use of urea as a dietary supplement can be of limited empirical value.

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