quirement for peptides, whereas *Prevotella ruminicola*, which requires peptides for optimal growth, is one of the few rumen bacteria to ferment xylose rapidly.

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Peptide hydrolysis by human colonic bacteria is also a biphasic process carried out by *Bacteroides*-like organisms. N McKain, RJ Wallace (*Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK)*

Peptide breakdown in the rumen occurs via a two-stage process. Firstly, oligopeptides are cleaved by dipeptidyl peptidases and then the resultant di- and tripeptides are metabolised by separate di- and tripeptidases. The latter enzymes are present in several species of bacteria and in protozoa, while dipeptidyl peptidases occur predominantly in bacteria formerly known as *Prevotella ruminicola* and now reclassified as *P. albensis*, *P. brevis*, *P. bryantii* and *P. ruminicola* [1]. The present study was undertaken to determine if a similar pattern occurs in human colonic bacteria. Aminopeptidase activities of mixed faecal suspensions from four human donors and of 14 of the most numerous species of human colonic bacteria were measured using alanine oligopeptides and various dipeptidyl- and amino acyl-arylamidase substrates. The pattern of hydrolysis of Ala_4 and Ala_5 in faecal suspensions suggested that the main mechanism of peptide hydrolysis was by cleavage of dipeptides. However, a significant quantity of single amino acid cleavage also occurred, which was much greater than that found in the rumen. Dipeptidyl *p*-nitroanilides and 4-methoxynaphthylamides were broken down more rapidly than amino acyl derivatives, consistent with a greater activity of dipeptidyl peptidases. The predominant *Bacteroides* spp. of the intestine, including *B. fragilis*, *B. distasonis*, *B. thetaiotaomicron* and *B. vulgatus*, also had greater dipeptidyl peptidase activity than amino acyl aminopeptidase activity, while *Bifidobacterium*, *Clostridium* and *Enterococcus* spp. had a more variable pattern of peptidase activities. Thus peptide hydrolysis in the human intestine, as in the rumen, appears to be a two-stage process which is initiated by dipeptidyl peptidases present in the most numerous *Bacteroides* spp. Presumably this mechanism confers an advantage to these bacteria, although the nature of that advantage is not yet known.

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Isolation and characterisation of peptidase and peptide permease mutants of *Prevotella albensis*. N Walker, RJ Wallace (*Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK)*

Bacteria in the group formerly known as *Prevotella ruminicola*, now reclassified as *P. albensis*, *P. brevis*, *P. bryantii* and *P. ruminicola* [1], play a significant role in peptide breakdown in the rumen. Specifically, they possess dipeptidyl peptidase activities which are typical of mixed rumen

microorganisms but which do not occur in other bacterial species [2]. The aim of the present work was to obtain mutants of *P. albensis* M384 which were defective in certain aspects of peptide metabolism, in order to characterise the role of different peptidases and peptide permeases in the wasteful uptake and hydrolysis of peptides by *P. albensis.* Several 4-methoxynaphthylamide (MNA) substrates for dipeptidyl peptidases, including GlyArg-MNA, GlyPro-MNA, LeuVal-MNA and LysAla-MNA, as well as Leu-MNA, were toxic to *P. albensis* and were used to select mutants. Washed cells of *P. albensis* were treated with the mutagen ethyl methanesulfonate (EMS) by incubating cells in 2% EMS for 30min at 39°C. Cells were allowed to recover in defined minimal medium overnight, and plated on defined minimal medium to which was added a sterile disc of filter paper. Ten µl of MNA-containing substrate or MNA were added to the disc. Growth of the wild-type occurred almost up to the edge of the disc with MNA, indicating minor toxicity, but clear zones 25mm, 22mm, 30mm, 23mm and 30mm in radius were observed with GlyArg-MNA, Gly-Pro-MNA, LeuVal-MNA, LysAla-MNA and Leu-MNA respectively, indicating that these substrates were more toxic than MNA. It also implied that the substrates were taken up by *P. albensis* and the released MNA accumulated intracellularly. When mutagenised *P. albensis* was plated in the same way, several colonies grew in the clear zone with each substrate, indicating resistance. The Leu-MNA mutants were unstable and were lost. The others did not include any that appeared to have single peptidase or peptide permease lesions: they seemed to be regulatory mutants in which groups of dipeptidyl peptidase activitities were lost or decreased. None of the mutants had significantly different activities in whole and sonicated cells, indicating that none were permease mutants. All had decreased rates of ammonia production from Trypticase. The mutants all grew well in basal medium containing volatile fatty acids, methionine and ammonium sulfate. The growth rate of some continued to be stimulated by adding Trypticase to the medium, but others were not. This new method for raising peptidase mutants has therefore yielded regulatory mutants of *P. albensis* which indicate that the loss of peptidase activity need not affect the competitiveness of the organism in terms of the maximum growth rate which can be achieved.

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Characterization of a gene from *Prevotella ruminicola* strain B_14 which complements an *E. coli* mutant defective in peptide transport. L Peng[1], M Morrison[1,2] ([1]*Department of Animal Science;* [2]*School of Biological Sciences, University of Nebraska, Lincoln, NE68583-0908, USA)*

Ruminal bacteria currently classified as *Prevotella ruminicola* are characterized by their unusual selectivity towards the amino-nitrogen sources they use for growth. The bacteria grow well with oligo- and polypeptides, but individual amino acids do not support growth. To better understand how *P. ruminicola* utilizes oligopeptides for growth, we have undertaken to clone and isolate genes from strain B_14 that encode for proteins involved with oligopeptide transport. An