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.

1. Avgustin G, Wallace RJ, Flint HJ (1997) *Int J Syst Bacteriol* 47, 284-288
2. Wallace RJ (1996) *J Nutr* 126, 1326-1334

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

Escherichia coli oppE, met, trp, his, pro mutant (strain SS3240), defective in tripeptide uptake and resistant to triornithine (orn_3), was transformed with a plasmid library of strain B_14 chromosomal DNA, and plated out on minimal medium supplemented with methionine, proline, histidine, and the tripeptide L-lysyl-tryptophanyl-lysine. Colonies which appeared on the selective plates were then tested for orn_3 sensitivity and tryptophan prototrophy, to confirm that the recombinant DNA clones carried gene(s) involved with oligopeptide transport. Plasmid DNA was extracted from four transformants possessing the appropriate phenotype (i.e. orn_3-sensitivity and tryptophan auxotrophy) and used to retransform SS3240. All plasmid DNA preparations transformed SS3240 to an *oppE*$^+$ phenotype, and one of the plasmid clones (pANS1000) was selected for further examination. A 4.0 kilobase *Bam*HI-*Pst*I fragment from pANS1000 was subcloned in pBluescript SK$^+$ to generate pANS1001, and *E. coli* SS3240/pANS1001 transformants were shown to possess an *oppE*$^+$ phenotype. Tri-alanine (Ala_3) uptake rates were measured using procedures described by Payne and Bell [1] and determined to be 51.4, 7.2, 29.2 and 54.1 nmol Ala_3 min^{-1} (mg protein)$^{-1}$ for cultures of *E. coli* SS320 (*oppE*$^+$), SS3240 (*oppE*), SS3240/pANS1000 (*oppE*$^+$), and SS3240/pANS1001 (*oppE*$^+$), respectively. Nucleotide sequence analysis of pANS1001 identified an open reading frame which possesses considerable homology (~59% identity) with the RprX protein of *Bacteroides fragilis*, which belongs to a family of histidine protein kinase receptors (e.g. EnvZ and PhoA) found in a number of eubacteria. The RprX protein modulates the expression of genes encoding outer membrane porins such as OmpF and OmpC [2], which are known to influence peptide uptake in *E. coli* [3]. Our findings to date suggest that *P. ruminicola* possesses a gene or genes with functions analogous to the *oppE* locus in *E. coli*, and that OppE-like gene products might be involved in coordinating porin expression.

1. Payne JW, Bell G (1979) *J Bacteriol* 137, 447-455
2. Rasmussen, BA, Kuvacs, E (1993) *Mol Microbiol* 7, 765-776
3. Andrews JC, Short SA (1985) *J Bacteriol* 161, 484-492

HYDROGEN TRANSFER

Alternative hydrogen sink pathways in hindgut fermentation.

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Besides methanogenesis (M), non assimilatory sulphate reduction and/or reductive acetogenesis (RA) have been identified as major pathways of metabolic hydrogen disposal in hindgut fermentation for a number of animals, based mainly on stoichiometry of metabolic hydrogen recovery [1]. These alternative hydrogen sinks to methanogenesis do not function in the rumen, although the bacteria capable of using them have been isolated.

This report summarizes experiments in our laboratory over the past ten years re-