PCR probes for ammonia hyper- producing bacteria in the rumen of New Zealand ruminants

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Abstract

Competitive PCR (cPCR) primers were developed to detect and enumerate 5 hyper ammonia-producing (HAP) bacteria previously isolated from New Zealand ruminants, and 3 previously described HAP bacteria, *Clostridium aminophilum, C. sticklandii* and *Peptostreptococcus anaerobius*. Primers were designed by aligning 16S ribosomal RNA gene sequences and identifying unique site for each bacterium. Primers were matched as closely as possible in terms of length, G+C content and T_m to either the universal eubacterial forward (fd1*) or reverse (rd1*) primers to anchor the PCR at either the 5' or 3' end of the 16S rRNA gene. Primer specificity was tested in amplification reactions with DNA extracted from 35 bacterial isolates, mostly from the rumen. The primers designed for isolates C2 and D5 produced amplified PCR products only with their respective target DNAs. Primers developed for isolates S1, D4 and *P. anaerobius* also amplified DNA from closely related species, *P. asaccharolyticus*, *Fusobacterium necrophorum* and isolate D1, respectively, in addition to their respective target DNAs. Internal controls were developed for each of the chosen primers by creating deletions in the amplified target DNA using restriction endonuclease digestions and religating the terminal fragments. The deleted internal control fragments were reamplified and cloned into the PCR cloning vector pGEM-T. Cloned internal control DNAs were coamplified with known amounts of their respective target DNAs to generate standard curves so that unknown samples could be quantitated. DNAs extracted from rumen samples from sheep fed a diet of chaffed lucerne and infused with either monensin or buffer were probed for HAP bacteria using the cPCR probes. The results showed that isolates C2, D5, S1 and *C. sticklandii* and *C. aminophilum* were below the detectable limits of the cPCR technique and their population could not be enumerated. The absence of any PCR amplifiable DNA of these organisms in the rumen

samples was confirmed by conventional PCR in the absence of internal control DNAs, by additional purification of rumen DNAs followed by reamplification, and by preamplifying rumen DNA with the universal eubacterial primers fd1* and rd1* prior to PCR with primers specific to each organism. However the D4/*F. necrophorum* and D1/*P. anaerobius* probes showed detectable populations in the samples. *In vivo* the D1/*P.anaerobius* population in the rumen ranged from 3 to $7x10^8$ cells ml^{-1}. Monensin showed no inhibitory effect on the D1/*P.anaerobius* population, which maintained steady levels throughout the sampling period. D4/*F.necrophorum* populations ranged from $3x10^8$ to $1.4x10^9$ bacteria ml^{-1}. Monensin had little effect over the first 48hr compared to control sheep but after 72hr D4/*F.necrophorum* populations increased and finally reached $1.4 x10^9$ bacteria ml^{-1} at 96 hrs.

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