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Development of a real-time PCR method for quantification of *Prevotella histicola* from the gut

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ABSTRACT

We designed species-specific primers and developed a qPCR method for enumerating *P. histicola* from intestinal samples. The two designed primer sets showed specificity for the target 16S rRNA gene of *P. histicola*. The absolute qPCR method was sensitive to quantify as few as 10^3 colony-forming units (CFU) in the gut.

Members of genus *Prevotella* are part of the oral microbial community [1,2]. In some cases, they are also present in human intestine [1,3–5]. *P. histicola* is a gram-negative obligate anaerobe first isolated from human oral mucosal tissue [6]. Recently, we isolated *P. histicola* MCI001 from the human intestine, and showed that this isolate has the ability to regulate intestinal and systemic immune responses. Using a humanized mouse model of arthritis that mimics human rheumatoid arthritis (RA), we showed that *P. histicola* MCI001 suppresses arthritis [4]. Interestingly, while *P. histicola* regulated the host's adaptive immune response, it did not affect the host's innate immunity [4]. Recently, the human gut microbiome has received a lot of attention and many studies have indicated an increase or decrease of particular bacterial species in various disease conditions [4,7,8]. These reports indicate the use of selected species as potential biomarkers or for treating diseases. Though microbiome research has grown over the past decade, there is still a lack of advanced techniques for analysis of targeted bacterial species except by sequencing. Because of the unculturable nature of most intestinal bacteria and the complexity of bacterial diversity, *in vivo* sampling leads to inaccuracy in quantification. Using conventional methods of PCR with genus-specific primers, it is difficult to detect gut-colonized *P. histicola* [4]. This generated a need for an appropriate method to quantify *P. histicola* from fecal and gut samples of *P. histicola*-treated mice. In the present study, we report a PCR-based quantitative method for enumerating *P. histicola* in gut samples by using real-time polymerase chain reaction (RT-PCR) with species-specific primers targeting part of 16S ribosomal RNA (rRNA) gene (Table 1). Primers were designed using Primer-BLAST software [9]. For designing the primers, 16S rRNA gene sequences of the *P. histicola* type strain were obtained from NCBI (GenBank accession: EU126661). Selected *P. histicola* sequence were aligned with 30 type strains of closely related *Prevotella* species using ClustalW program to define the selected target sequence for *P. histicola* (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The selected primer sequences were subjected to BLAST search to check similarity and specificity and only those matching with *P. histicola* were included into the study. Two primer sets that were specific for *P. histicola* in the *in silico* studies (Table 1) were synthesized (Integrated DNA Technologies).

The type strain of *P. histicola* DSM-19854 was obtained from

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Table 1
Primers Used in this study.

Primer Set	Designation	Sequence (5′–3′)	Position (by *E. coli* Numbering)	Amplicon size (bp)	Reference
Genus Specific Primer	g-Prevo-F	CACRGTAAACGATGGATGCC	–	527–529	Matsuki et al. [10]
	g-Prevo-R	GGTCGGGTTGCAGACC	–		
Primer Set 1	PhisF	TCACTGACGGCATCAGATGTG	162–183	289	Present Study
	Phis1R	CAATCACACGTGACTGACT	450–433		
Primer Set 2	PhisF	TCACTGACGGCATCAGATGTG	162–183	213	Present Study
	Phis2R	GGCTGGTTCAGGCTCTCGC	374–456		

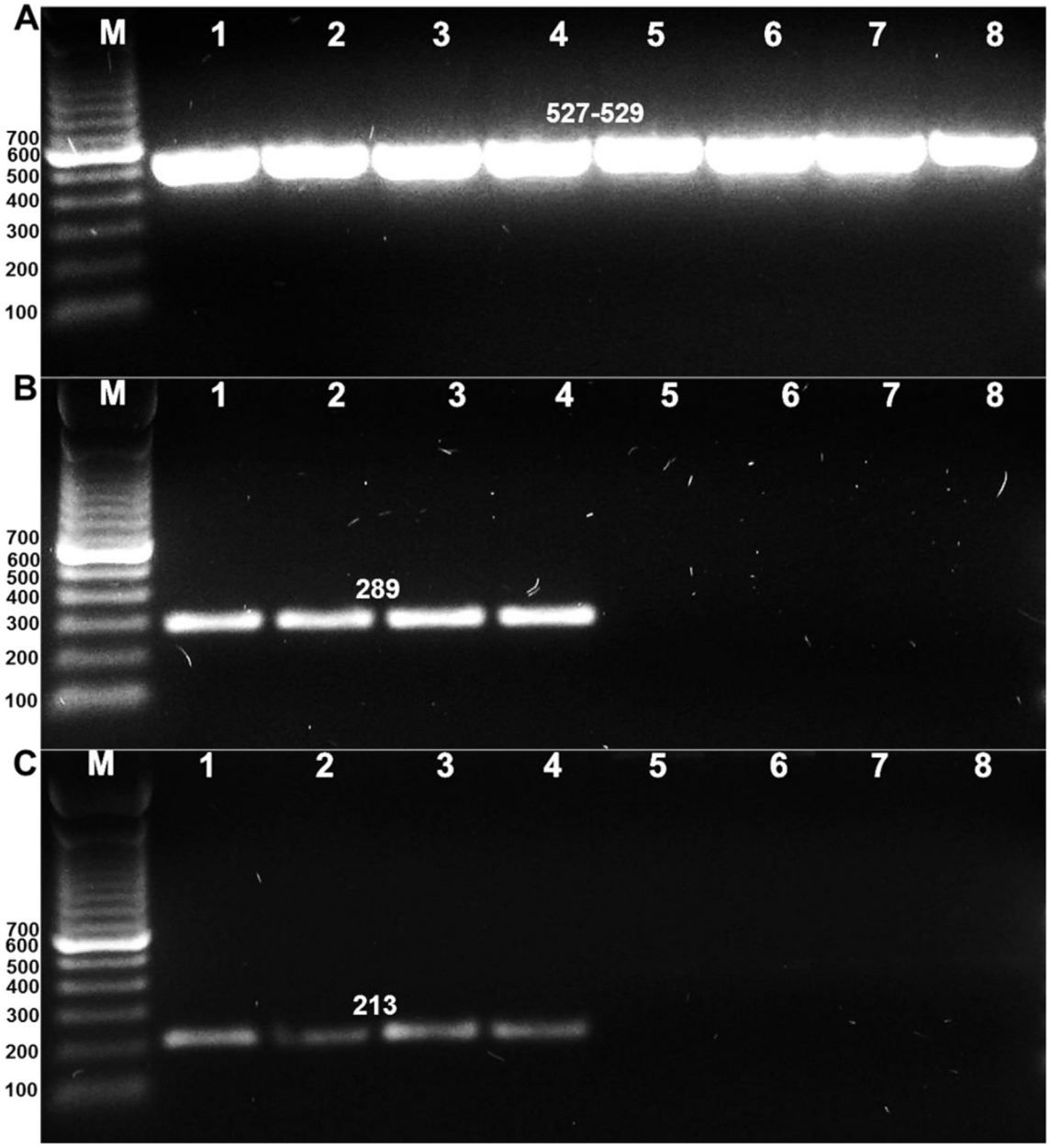

Fig. 1. Specificity of *P. histicola* primer sets. Lanes 1 to 4 show bands of PCR amplified DNA from *P. histicola* DSM-19854 (1 and 2) and *P. histicola* MCI001 (3 and 4). Lanes 5 to 8 show the bands of PCR amplified DNA from *P. melaninogenica* DSM-7089 using, A; *Prevotella* genus specific primer set, B; Primer set 1, and C; Primer set 2. Results are representative of three individual experiments.

DSMZ, Germany and *P. melaninogenica* ATCC 25845 was acquired from ATCC, USA. *P. histicola* MCI001 and *P. melaninogenica* isolates used in the study were recovered in our laboratory from gut tissue as published [4]. As recommended by Zhou et al. [11], to avoid cross-reactivity of the designed primers in PCR reaction, we have validated the specificity of the *P. histicola* primer sets with