RESEARCH LETTERS

Distribution of dipeptidyl peptidase (DPP) 4, DPP5, DPP7, and DPP11 in human oral microbiota – potent biomarkers indicating presence of periodontopathic bacteria

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One sentence summary: Measurement of dipeptidyl peptidase activities in subgingival dental plaque is effective for assessment of the presence of periodontopathic bacteria and disease activity.

Keywords: dental plaque; dipeptidyl peptidase; periodontopathic bacteria; *Porphyromonas gingivalis*; saliva

Topics: anaerobe, biomarker, database search, dipeptide production, oral microbiota, ortholog distribution

Issue Section: Pathogenicity and Virulence/Host Response

ABSTRACT

Dipeptidyl peptidase (DPP) 4, DPP5, DPP7, and DPP11, expressed in the periplasmic space, are crucial for energy production for *Porphyromonas gingivalis*, an asaccharolytic bacterium that causes periodontal disease. Bacterial DPP4 seems to be involved in regulation of blood glucose level via degradation of incretins. The present study aimed to identify four *dpp* orthologs in oral microbiota by database searches, and their enzymatic activities in periodontopathic and cariogenic bacteria, as well as oral specimens were determined. Search in the databases suggested that 43 species of 772 taxa possess *dpp4* and other *dpp* genes. Most species are in the genera *Bacteroides*, *Capnocytophaga*, *Porphyromonas*, *Prevotella*, and *Tannerella*, indicating a limited distribution of *dpp* orthologs in anaerobic periodontopathic rods. In accordance with those results, activities of all four DPPs were demonstrated in *P. gingivalis*, *Porphyromonas endodontalis*, and *Tannerella forsythia*, while they were negligible in *Treponema denticola*, *Fusobacterium nucleatum*, and *Aggregatibacter actinomycetemcomitans*. Furthermore, DPP activities were also detected in subgingival dental plaque at different intensities among individual specimens, while DPP4 activity presumably derived from human entity was solely predominant in saliva samples. These findings demonstrated that DPP activities in dental plaque serve as potent biomarkers to indicate the presence of periodontopathic bacteria.

INTRODUCTION

Periodontal disease is a leading cause of tooth loss in adults, resulting in a decrement in overall quality of life, particularly in elderly individuals, with 20-50% of individuals worldwide suffering from the disease (Nazir 2017). There are approximately 800 bacterial species harbored in the oral cavity (Chen *et al.* 2010) and this inflammatory disease is attributed to a complex of endogenous anaerobic rods, which colonize in subgingival pockets and form subgingival dental plaque. *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* are major periodontopathic bacteria implicated in severe forms of periodontal disease. Additionally, *Prevotella* and *Fusobacterium* species have been shown to be moderately associated with the disease (Socransky *et al.* 1998: Holt and Ebersole 2005). These anaerobes are considered to form commensal communities that mutually promote growth and survival under localized circumstances in subgingival pockets. In addition to oral disorders, the presence of periodontal disease is related to increased risk for systemic diseases, such as type 2 diabetes mellitus (Lalla and Papapanou 2011) and Alzheimer's disease (Teixeira *et al.* 2017). Therefore, methods for preterm assessment of the disease, as well as elucidation of molecular relationships between periodontal and systemic diseases are warranted.

The nutritional aspects of periodontopathic anaerobes are important for understanding the subgingival ecological niche. *P. gingivalis*, *Porphyromonas endodontalis*, and *Tan. forsythia* are asaccharolytic organisms, and thus utilize amino acids as their sole carbon and energy sources, while *Prevotella*, *Treponema*, *Aggregatibacter*, and *Fusobacterium* species ferment carbohydrates. Furthermore, *Porphyromonas* species incorporate amino acids mainly as dipeptides, whereas *Prevotella* and *Fusobacterium* efficiently utilize single amino acids (Takahashi and Sato 2002). These distinct auxotrophic features imply that these periodontopathic bacteria share a cooperative ecological niche.

For dipeptide production, *Porphyromonas* species possess a series of dipeptide-producing enzymes including dipeptidyl peptidases (DPPs), and acylpeptidyl oligopeptidase, as well as the dipeptide-producing activities of Arg- and Lys-gingipains. Those peptidases are crucial for both bacterial growth and pathogenicity (Nemoto and Ohara-Nemoto 2016). To date, four DPPs that liberate dipeptides from the N-termini of oligopeptides have been identified in *P. gingivalis*. Of those, DPP4 is specific for Pro and

weakly associated with Ala at the penultimate residue from the N-terminus (P1 residue) (Abiko *et al.* 1985; Banbula *et al.* 2000), while DPP5 (Ohara-Nemoto *et al.* 2014) and DPP7 (Banbula *et al.* 2001) show preferences for P1-position hydrophobic residues, and DPP11 is specific for P1 Asp/Glu (Ohara-Nemoto *et al.* 2011). Based on the substrate specificities, DPP activities are determined using synthetic dipeptidyl substrates with a chromogenic or fluorogenic group. DPP4 and DPP11 are determined using Gly-Pro- and Leu-Asp-*p*-nitroanilide (pNA) or 4-methylcoumaryl-7-amide (MCA), respectively. For measurement of DPP5 activity, Lys-Ala-MCA can be used, though DPP4 and DPP7 slowly hydrolyze it. The activity of DPP7 is scarcely distinguishable from that of DPP5 due to their similar hydrophobic P1-position preferences. However, a recent investigation of P2- and P1-position specificities established Phe-Met-MCA as a novel DPP7 specific substrate (Nemoto, Ohara-Nemoto, and Ono 2018). Consequently, these four different dipeptidyl substrates allow for quantitative measurements of the activities of the four known DPPs in bacterial cells as well as human specimens.

In addition to dipeptide production for bacterial nutrition, habitat segregation, and adaptation, it is noteworthy that bacterial DPPs are possibly involved in host physiological functions. We recently reported in a mouse model that DPP4 from *P. gingivalis*, *Tan. forsythia*, and *Prev. intermedia* cleaves the N-terminal dipeptide from the incretin peptide hormones GLP-1 and GIP in the same manner as human DPP4 (Ohara-Nemoto *et al*. 2017). Since incretins enhance insulin secretion from pancreatic β cells after feeding, the limited proteolysis of incretins by bacterial DPP4 caused a decrease in blood insulin levels and then elevated postprandial hyperglycemia. Those findings strongly suggested that periodontopathic bacterial DPP4 is additionally involved in glycemic control in individuals with type 2 diabetes.

In the present study, we performed a comprehensive ortholog search of the four *P. gingivalis dpp* genes in human oral microbiota to articulate the possible involvement of periodontopathic bacterial DPPs in homeostatic regulation. In addition, DPP activities were determined in representative periodontopathic and cariogenic bacterial species, as well as oral specimens using dipeptidyl fluorogenic substrates. Our findings showed that *dpp* orthologs are specifically distributed in anaerobic oral rods, suggesting conceivable relationships of periodontopathic bacterial DPPs with periodontal and systemic diseases.

MATERIALS AND METHODS

Ortholog search for *P. gingivalis dpp* genes

An ortholog search was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/kegg_ja.html). Orthologs of the *P. gingivalis dpp4* (PGN_1469), *dpp5* (PGN_0756), *dpp7* (PGN_1479), and *dpp11* (PGN_0607) genes with more than 30% identity were listed, and then bacterial species enrolled in the Expanded Human Oral Microbiome Database (eHOMD) (http://www.homd.org; Chen *et al.* 2010) were selected. BLAST and MEROPS (https://www.ebi.ac.uk/merops; Rawlings *et al.* 2018) ortholog searches were also performed for finding and ascertaining candidates.

Microorganisms and growth conditions

P. gingivalis ATCC 33277, *P. endodontalis* ATCC 35406, *Prev. intermedia* ATCC 25611, and *A. actinomycetemcomitans* ATCC 33384 were grown in Anaerobic Bacteria Culture Medium (ABCM) broth (Eiken Chemical Co., Ltd.) supplemented with 5 μg mL^{-1} hemin and 10 μg mL^{-1} menadione, as previously described (Nemoto *et al.* 2018). *Tan. forsythia* ATCC 43037 was grown in ABCM broth supplemented with 15 μg mL^{-1} N-acetylmuramic acid. For *Trep. denticola* ATCC 33520, 5% rabbit serum was further added to the broth. *F. nucleatum* ATCC 25586, *S. mutans* ATCC 25175, and *S. salivarius* ATCC 7073 were cultured in Tryptic Soy broth. All bacteria were grown under anaerobic conditions (80% N_2, 10% CO_2, 10% H_2) at 37˚C and harvested at the early stationary phase, then washed once with ice-cold phosphate buffered saline (PBS) (pH7.4), and suspended in PBS at OD_{600} of 2.0.

Oral specimens

Whole saliva and subgingival dental plaque specimens were collected from 7 laboratory-staff members aged 26 – 63 years old (mean age 46.7 years; 3 females, 4 males), as previously described (Ohara-Nemoto *et al.* 2008). The subjects were systemically healthy, and showed no or scarcely active pathogenic signs in periodontal tissues, and none had received antibiotic medication within the previous 3 months. Subgingival dental plaque was initially suspended

in 100 μL cold PBS, then a part of specimens was diluted at OD_{600} of 0.2.

Measurement of peptidase activity

Peptidase activity was measured as previously reported (Nemoto *et al.* 2018). Briefly, the reaction was performed in 200 μL of a mixture composed of 50 mM sodium phosphate (pH 7.0), 5 mM EDTA, and 20 μM aminoacyl- or dipeptidyl-MCA. To measure DPP5 activity, 0.1 M NaCl was further added to the mixture (Ohara-Nemoto *et al.* 2014). The reaction was started by addition of a suspension (5 μL) of bacterial cells or oral specimens. After 30 min at 37˚C, fluorescence intensity was measured with excitation at 380 nm and emission at 460 nm. More than three independent analyses were performed in triplicate.

RESULTS AND DISCUSSION

Ortholog distributions of *P. gingivalis dpp4*, *dpp5*, *dpp7*, and *dpp11* genes in human oral microbiota

Previous studies have shown that DPP4 and DPP5 are present in both eukaryotes and bacteria, whereas the distribution of DPP7 and DPP11 is restricted to bacterial species. Orthologs of the *P. gingivalis dpp4* (PGN_1469), *dpp5* (PGN_0756), *dpp7* (PGN_1479), and *dpp11* (PGN_0607) genes were searched using the KEGG Orthology, BLAST, MEROPS, and eHOMD databases. The eHOMD indexes 772 microbial species present in the mouth, throat, nose, sinuses, and esophagus. For bacterial species with genome data not listed in KEGG, genome or gene data present in the NCBI and MEROPS databases were used. Consequently, 43 bacterial species without strain redundancy were found to possess at least one of four *dpp* genes with a greater than 30% amino acid identity (Table 1). Thirty-two (74.4%) of 43 species categorized in the phylum *Bacteroidetes* (also termed the *Cytophaga-Flavobacterium-Bacteroidetes* group) are gram-negative bacilli growing anaerobically. Those are the six genera *Bacteroidals* (1 species), *Bacteroides* (9), *Capnocytophaga* (6), *Porphyromonas* (6), *Prevotella* (8), and *Tannerella* (2), most of which

possess all four *dpp* orthologs. Three species of the genus *Pedobactor* also classified in the phylum *Bacteroidetes* are aerobic chemoheterotrophic bacilli and scarcely isolated from the oral cavity but generally done from open water and glacier (Margesin *et al*. 2003: Muurholm *et al.* 2007). Additionally, 8 bacteria (18.6%) are aerobic gram-negative bacilli in the genera *Caulobacter* (4), *Sphingomonas* (3), and *Stenotrophomonas* (1), belonging to the phylum *Proteobacteria*. A bacterial diversity study by using 16S rRNA sequencing demonstrated the high prevalence of the phyla *Bacteroidetes*, *Proteobacteria* as well as *Firmicutes* involving the genus *Streptococcus* in human subgingival plaque from periodontally healthy and periodontitis subjects (Paster *et al.* 2001). Thus, most bacteria harboring the *dpp* genes colonize at least subgingival crevice in the oral cavity and form subgingival dental plaque. In addition, it was noticeable that no orthologs were found in other predominant oral bacteria, such as the genera *Streptococcus*, *Actinomyces*, *Neisseria*, and *Veillonella*, which are commonly isolated from buccal epithelium, tongue surface, and tooth surfaces (Aas *et al.* 2005).

Among the 43 bacterial species, all seemed to express DPP4. Furthermore, *dpp5* orthologs were also found in all species except for *Porphyromonas catoniae* and the genus *Pedobactor*, while *dpp7* and *dpp11* were observed in 32 species excluding the genera *Caulobacter* and *Sphingomonas*. Although the *dpp7* and *dpp11* orthologs were not found in three *Prevotella* species, additional genome information seems to be required, because five other *Prevotella* species were found to possess those two genes. These results indicate that *P. gingivalis*-type DPPs, especially DPP4, are generally distributed among anaerobic rods in the human oral microbiota, which are major residents that form subgingival dental plaque.

Dpp orthologs other than the *dpp4* gene are presently annotated with a variety of aliases (Table 2). This is because DPP4 (conventionally described as DPPIV; S09.003 peptidase family in MEROPS classification) has been well studied in both mammalian (Misumi and Ikehara 2013) and bacterial (Monod and Beauvais 2013) species since the 1960s. In contrast, the other DPPs have been more recently identified. Bacterial DPP5 (S09.075) was first discovered in *P. gingivalis* (Ohara-Nemoto *et al.* 2014) as an ortholog of fungal DPP5 (Beauvais *et al.* 1997). Prior to the identification of bacterial DPP5, this peptidase has been tentatively classified with various names, such as peptidase S9, prolyl oligopeptidase, WD-like β propeller containing protein, and putative peptidase.

P. gingivalis DPP7 (S46.001) prefers a hydrophobic P1 residue (Banbula *et al.* 2001) and

is distinct from mammalian DPPII/DPP7/QPP (S28.002), which is specific for Pro. We previously discovered a novel DPP specific for Asp/Glu at the P1 position, designated as DPP11 (S46.002) (Ohara-Nemoto *et al.* 2011). Since DPP11 had been categorized as an isoform of DPP7, some *dpp11* orthologs are still tentatively annotated as DPP7. Annotation of *dpp7* and *dpp11* is not readily accomplished by using the whole nucleotide sequence due to their close similarity, though is possible by using the essential amino acid residue Gly^{673} for DPP7 and Arg/Ser^{673} for DPP11 (Ohara-Nemoto *et al*. 2011; Rouf *et al.* 2013). These residues are involved in the active pocket that is responsible for substrate specificity (Rouf *et al.* 2013; Bezerra *et al.* 2017). In the present study, all candidates for *dpp7* and *dpp11* orthologs were finally verified using these essential amino acid residues, and we found that 32 bacterial species (74.4%) possessing the *dpp7* gene and that 23 species (53.4%) with the *dpp11* gene among the 43 species possessing at least one of four *dpp* genes.

DPP activities were determined in seven major periodontopathic bacteria and two cariogenic *Streptococcus* species (Fig. 1). In accordance with the ortholog search findings (Table 1), DPP4, DPP5, DPP7, and DPP11 activities were demonstrated in *P. gingivalis* and *P. endodontalis*. DPP4, DPP5, DPP7, and Leu-Asp-MCA hydrolysis corresponding to DPP11 activities were observed in *Tan. forsythia*, although *the dpp11* gene is not listed in the genome. Furthermore, *Prev. intermedia* apparently exhibited the activities of the three DPPs, whereas such activities were faint or negligible in *Trep. denticola*, *F. nucreatum*, and *A. actinomycetemcomitans*, consistent with the findings showing the absence of orthologs. Summation of DPP activities was highest in *P. gingivalis* (mean ± SD; DPP4 = 8.3 ± 0.2; DPP5 = 1.7 ± 0.05; DPP7 = 30.7 ± 0.2; DPP11 = 10.8 ± 0.2 pmol min^{-1}), followed by *P. endodontalis* (DPP4 = 3.1 ± 0.1; DPP5 = 6.4 ± 0.05; DPP7 = 19.7 ± 0.2; DPP11 = 3.1 ± 0.1 pmol min^{-1}), *Tan. forsythia* (DPP4 = 3.4 ± 0.3; DPP5 = 3.3 ± 0.1; DPP7 = 7.7 ± 0.1; DPP11 = 2.6 ± 0.2 pmol min^{-1}) and *Prev. intermedia* (DPP4 = 2.6 ± 0.2; DPP5 = 0.7 ± 0.05; DPP7 = 1.1 ± 0.1 pmol min^{-1}). These results are in agreement with the fact that dipeptide production is crucially important for *Porphyromonas* species, possibly reflecting their dipeptide incorporation system. In addition, the higher level of activities in *Tan. forsythia* may suggest that dipeptides are incorporated into this bacterium. Lower DPP activities in *Prev. intermedia* as compared to the other three bacteria is likely related to the glucose fermentation and single amino acid incorporation properties of this bacterium.

As for the cariogenic *Streptococcal* species, none of DPP5, DPP7, and DPP11 activities

were observed, while moderate Gly-Pro-MCA hydrolysis (*S. mutans*, 0.8 ± 0.1; *S. salivarius*, 2.2 ± 0.0 pmol min^{-1}) was demonstrated, in accordance with a previous study (Suido *et al.* 1986). This activity seems to be mediated by an Xaa-Pro dipeptidyl-peptidase, a member of the S15 peptidase family, which has been identified in *S. mutans* (De *et al.* 2016).

We also determined aminopeptidase activity using Arg-, Lys-, Ala-, Leu-, Met-, and Phe-MCA to compare with the DPP activity profiles (Fig. 1). Although Arg- (2.6 ± 0.1 pmol min^{-1}) and Lys-MCA (0.8 ± 0.1 pmol min^{-1}) hydrolyzing activities, mediated by Arg- and Lys-gingipains, respectively, were demonstrated in *P. gingivalis*, other aminopeptidase activities in the bacterium were basically negligible, in accord with previous reports (Suido *et al.* 1986; Ohara-Nemoto *et al.* 2014). Also, relatively high activities toward Leu-, Met-, and Arg-MCA (3.6 ± 0.2, 2.2 ± 0.0, 1.4 ± 0.0 pmol min^{-1}, respectively) were observed in *Tan. forsythia,* while the activities toward Ala-, Arg-, and Lys-MCA (3.8 ± 0.1, 2.3 ± 0.1, 1.6 ± 0.0 pmol min^{-1}, respectively) were detected in *A. actinomycetemcomitans.* Aminopeptidase activities in *Trep. denticola*, *S. mutans*, and *S. salivarius* were moderate as compared with the former organisms, and those in *Prev. intermedia* and *F. nucleatum* were subtle. Modest DPP and aminopeptidase activities in those bacteria seem to be consistent with their energy metabolism, which is dependent on carbohydrate fermentation.

Taken together, we conclude that elucidation of DPP activities is useful to estimate the presence of periodontopathic anaerobes including *P. gingivalis*, *P. endodontalis*, *Tan. forsythia*, and *Prev. intermedia*, although the Gly-Pro-MCA hydrolyzing activity considered as DPP4 may represent the prevalence of both periodontopathic and cariogenic bacteria.

The ortholog search indicated that the *dpp* genes are specifically distributed in anaerobic rods in the oral cavity, which are major residents forming subgingival dental plaque. This finding is of interest when considering the molecular mechanisms involved in relationships of periodontal-systemic diseases that feature degradation of physiologically active peptides and proteins by oral bacteria entering the blood stream via periodontopathic lesions. In fact, using a mouse model we recently demonstrated that periodontopathic bacterial DPP4 modulates blood glucose levels by degradation and inactivation of incretin hormones (Ohara-Nemoto *et al.* 2017). Since incretins are involved in not only pancreatic functions but also micro- and macro-vascular diseases, dementia, obesity, and bone fracture (Seino and Yabe 2013), degradation of incretins by bacterial DPP4 derived from subgingival dental plaque may implicate a relationship between periodontal and systemic diseases, at least a part. In addition

to the functions of DPP4, it has been reported that DPP7 from *Capnocytophaga canimorsus*, a canine oral bacterium, retards coagulation by degradation of coagulation factors (Hack *et al.* 2017). These observations provide clues for a better understanding of the molecular mechanisms of periodontopathic bacteria involved in exacerbation of systemic diseases.

To assess an effective method for monitoring periodontitis activity and investigate bacterial DPP functions, the present protocol was utilized as a first attempt to determine whether subgingival dental plaque specimens are suitable for measurements of DPP activities, while whole saliva specimens were used as a control (Fig. 2). Oral specimens were collected from seven systemically healthy subjects with no or little active pathogenic signs in periodontal tissues (46.7 ± 18.2 years old; 3 females, 4 males). Subject A alone had a subgingival depth >4 mm, but was without inflammation. The activities of four DPPs in subgingival dental plaque specimens seemed to reflect all activities derived from periodontopathic bacteria. For example, the total DPP activities were significantly high in subject A. In addition, the previous observation that the level of DPP4 activity in gingival crevicular fluid is markedly related to disease status (Cox and Eley 1992) seems to implicate the amount of periodontopathic bacteria colonizing the gingival sulcus.

In contrast, in saliva samples, the activity of DPP4 was apparently high in all of the subjects examined, while the activities of the other three DPPs were subtle. Although the level of DPP4 in saliva has been reported to be related to disease status (Elgün, Ozmeriç, and Demirtaş 2000; Aemaimanan *et al.* 2009), most of the activity is considered from salivary glands (Ogawa *et al.* 2008). Therefore, the present along with the previous observations suggest that DPP activities in subgingival dental plaque are more closely associated with the presence of periodontopathic bacteria and possibly with the disease activity as compared to those in saliva.

In conclusion, we found that DPP4, DPP5, DPP7, and DPP11 are distributed in anaerobic periodontopathic rods inhabiting the human oral microbiota, and that measurement of the activities of these peptidases provides a useful tool to assess the presence of periodontopathic bacteria and disease activity. Presently, clinical analyses on periodontopathic subjects are underway in our laboratory.

Conflicts of interest. None to declare.

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1 Table 1. Distribution and % identity of *P. gingivalis dpp* orthologs in the human oral microbiome.

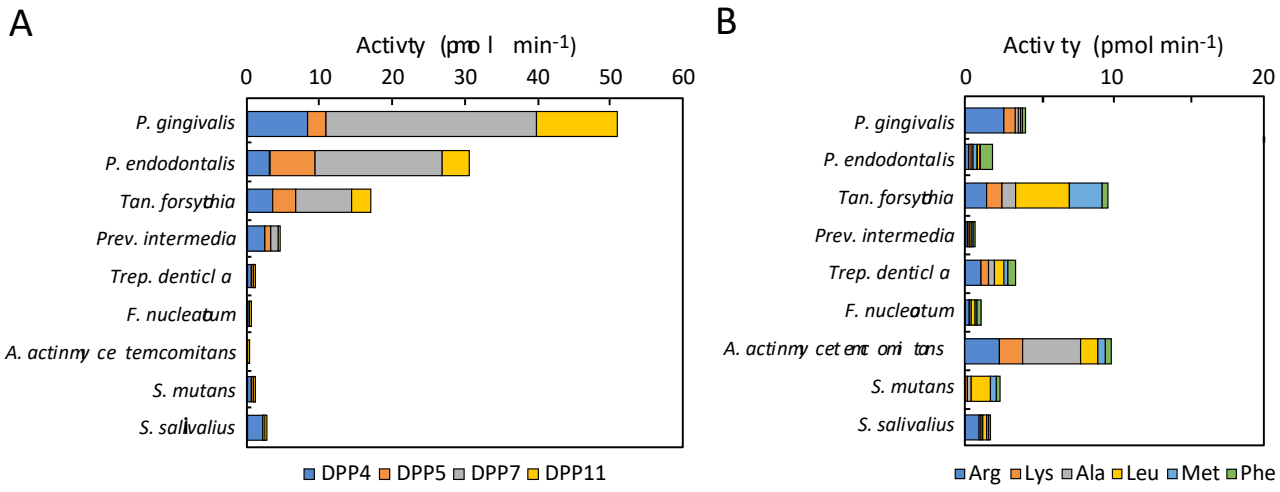
No.	Property	Species	KEGG code	DPP4	DPP5	DPP7	DPP11	Reference
1	Anaerobic	*Bacteroidales bacterium CF*	bacc	30.9 BRDCF_p911	52.8 BRDCF_p1796	44.1 BRDCF_p63	43.9 BRDCF_p63	
2		*Bacteroides cellulosilyticus*	bcel	50.1 BcellWH2_04067	58.8 BcellWH2_00568	44.9 BcellWH2_02616	46.9 BcellWH2_01041	
3		*Bacteroides dorei*	bdo	47.7 EL88_17320	55.4 EL88_10115	45.0 EL88_01065	47.4 EL88_04195	Nemoto *et al.* 2018
4		*Bacteroides fragilis*	bfb	50.9 VU15_04185	56.9 VU15_11640	45.8 VU15_00190	47 VU15_13540	Rouf *et al.* 2013; Nemoto *et al*. 2018
5		*Bacteroides helcogenes*	bhl	49.4 Bache_1336	58 Bache_2506	44.6 Bache_0623	48.2 Bache_2936	
6		*Bacteroides ovatus*	boa	49.5 Bovatus_04481	57.5 Bovatus_01140	44.5 Bovatus_03382	47.6 Bovatus_00117	Nemoto *et al.* 2018
7		*Bacteroides salanitronis*	bsa	49.2 Bacsa_1666	55 Bacsa_0556	43.2 Bacsa_3552	48.9 Bacsa_3553	
8		*Bacteroides thetaiotaomicron*	bth	50.1 Cabther_A1882	56.2 BT_0587	45.3 BT_3289	47.2 BT_0236	Nemoto *et al.* 2018
9		*Bacteroides vulgatus*	bvu	47.6 BVU_1991	54.9 BVU_3355	45.8 BVU_1292	47.4 BVU_2253	Rouf *et al.* 2013
10		*Bacteroides xylanisolvens*	bxy	49.7 BXY_33000	57.7 BXY_01510	44.9 BXY_25380	47 BXY_42300	
11		*Porphyrobacter neustonensis*	pns	30.2 A9D12_13280	30.3 A9D12_04605	30.7 A9D12_08420	-	
12		*Porphyromonas asaccharolytica*	pah	55.6 Poras_1656	61.4 Poras_0376	63.2 Poras_0497	54.3 Poras_0411	
13		*Porphyromonas catoniae*	-	60.0 MNR0996852	-	59.3 MER1065355	54.0 MER1065271	
14		*Porphyromonas endodontalis*	-	55.4 MER192286	61.5 MER0236725	65.2 MER0278904	57.9 AB610284 MER0290751	Kon 2002; Ohara-Nemoto *et al.* 2011; Nishimata *et al.* 2014; this study
15		*Porphyromonas gingivalis*	pgn	100 PGN_1469	100 PGN_0756	100 PGN_1479	100 PGN_0607	Abiko *et al.* 1985; Banbula *et al.* 2000; 2001; Ohara-Nemoto *et al.* 2011; Rouf *et al.* 2013; Ohara-Nemoto *et al.* 2014; 2017; this study
16		*Porphyromonas uenonis*	-	55 MER0192283	60.2 MER0233073	62.3 MER0275386	54.3 MER0217397	
17		*Prevotella dentalis*	pdt	42 Prede_1788	47.9 Prede_0500	-	-	
18		*Prevotella denticola*	pdn	43.4 HMPREF9137_1131	47.8 HMPREF9137_1904	35.6 HMPREF9137_0662	-	
19		*Prevotella dintalis*	pdt	42 Prede_1788	47.9 Prede_0500	-	-	
20		*Prevotella enoeca*	peo	42.3 Ccan_05140	48.5 AS203_05860	-	-	
21		*Prevotella fusca*	pfus	43.3 ADJ77_02160	47.6 ADJ77_11435	40.5 ADJ77_05700	-	
22		*Prevotella intermedia*	pit	43.1 PIN17_A1740	48.3 PIN17_A1793	42.5 PIN17_0473*	-	Ohara-Nemoto *et al.* 2017; this study
23		*Prevotella melaninogenica*	pmz	42.8 HMPREF0659_A5571	48.3 HMPREF0659_A6994	40.3 HMPREF0659_A5092	-	
24		*Prevotella ruminicola*	pru	40.7 PRU_0634	50.1 PRU_1443	40.7 PRU_2649	-	
25		*Tannerella forsythia*	tfo	61.1 BFO_1659	43.6 BFO_3080	59.1 BFO_0377	-	Ohara-Nemoto *et al.* 2017; this study
26		*Tannerella sp. oral taxon HOT-286*	toh	57.1 BCB71_01420	43.3 BCB71_01255	61.1 BCB71_09635	-	
27	Facultative anaerobic	*Capnocytophaga canimorsus*	ccm	42.3 Ccan_05140	40.5 Ccan_09440	36.3 Ccan_08540	41.7 Ccan_11580	Hack *et al.* 2017
29		*Capnocytophaga gingivalis*	cgh	42.6 CGC50_03250	44 CGC50_00410	38.5 CGC50_08370	41.5 CGC50_05115	Nemoto *et al.* 2018
28		*Capnocytophaga haemolytica*	chg	42.5 AXF12_02670	42.7 AXF12_08230	37.0 AXF12_07190	40.8 AXF12_04065	
30		*Capnocytophaga leadbetteri*	clk	42.9 CGC53_04935	41.9 CGC53_10585	37.7 CGC53_07790	41.4 CGC53_07790	
31		*Capnocytophaga ochracea*	coc	43.6 Coch_1057	41.8 Coch_1823	37.5 Coch_1768	41.0 Coch_0404	
32		*Capnocytophaga sp. oral taxon 3*	col	43.6 AM608_08390	41.9 AM608_01605	37.2 AM608_01320	41.0 AM608_05550	
33	Aerobic	*Pedobacter cryoconitis*	pcm	30.5 AY601_0660	-	-	-	
34		*Pedobacter sp. PACM 27299*	pep	30.5 AQ505_07725	-	39.2 AQ505_06390	33.4 AQ505_10325	
35		*Pedobacter steynii*	psty	31.2 BFS30_18605	-	39 BFS30_16700	32.9 BFS30_26025	
36		*Caulobacter crescentus*	ccs	30.2 CCNA_02237	31.7 CCNA_02065	-	-	
37		*Caulobacter henricii*	chq	32.6 AQ619_12080	30.9 AQ619_11240	-	-	
38		*Caulobacter segnis*	cse	32.2 Cseg_1267	31.6 Cseg_1441	-	-	
39		*Caulobacter sp. K31*	cak	31.1 CauI_3418	31.4 CauI_3102	-	-	
40		*Sphingomonas melonis*	smy	30 BJP26_07260	33.8 BJP26_09085	-	-	
41		*Sphingomonas sp. MM-1*	sphm	30.9 G432_00900	33.5 G432_03400	-	-	
42		*Sphingomonas taxi*	stax	32.7 MC45_05965	33.5 MC45_02335	-	-	
43		*Stenotrophomonas maltophilia*	sml	32.8 Smlt4503	32.8 Smlt1246	33.5 Smlt0962	-	

Human oral bacterial species, percent identity of amino acid sequence, and entry codes of orthologs for the *P. gingivalis dpp4, dpp5, dpp7*, and *dpp11* genes are listed. Genome data that have not been enrolled in KEGG were obtained from GeneBank and MEROPS. Bacterial species more than a single DPP identified in either recombinant proteins or bacterial cells are shaded. DPP activities measured using recombinant proteins are shown in blue, while those solely occurring in bacterial cells are in pink. Bacteria nos. 1-35 and 36-43 belong to the phyla *Bacteroidetes* and *Proteobacteria*, respectively. *Recombinant DPP7 of *Prev. intermedia* was expressed and its activity was confirmed (unpublished observation).

Table 2. Definitions of *P. gingivalis dpp* orthologs in the databases.

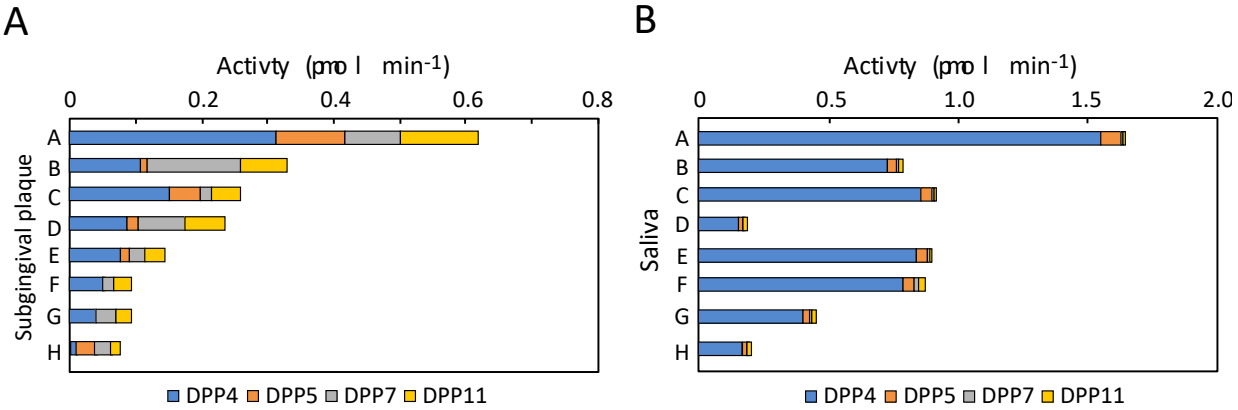
Enzyme	KEGG Orthology and GenBank
DPP4	dipeptidyl aminopeptidase IV
	peptidase S9
	peptidase S9B DPP4 domain
	putative exported DPP4
	170-kDa melanoma membrane-bound gelatinase
	prolyl tripeptidyl peptidase precur
DPP5	dipeptidyl-peptidase V
	prolyl oligopeptidase
	prolyl oligopeptidase family protein
	peptidase S9
	prolyl tripeptidyl peptidase precursor
	WD40-like beta Propeller containing protein
	dipeptidyl aminopeptidases/acylaminoacyl-peptidases
	putative exported aminopeptidase
	putative peptidase
	putative exported aminopeptidase
	putative peptidase yuxL
	WD40-like beta Propeller containing protein
	peptidase S9 prolyl oligopeptidase active site domain protein
	acylaminoacyl-peptidase
	S9A/B/C family, catalytic domain protein
	S9C (acylaminoacyl-peptidase) subfamily
	peptidase S9 prolyl oligopeptidase
	putative exported peptidase
DPP7	dipeptidyl peptidase 7
	peptidase S46
	conserved hypothetical protein
	hypothetical protein
	serine protease
	peptidase 7
	peptidase S10 [carboxypeptidase Y (*Saccharomyces cerevisiae*)]
DPP11	hypothetical protein
	conserved hypothetical protein
	peptidase S46
	dipeptidyl-peptidase 7
	serine protease
	V8-like Glu-specific endopeptidase
	hypothetical protein
	peptidase S10 [carboxypeptidase Y (*Saccharomyces cerevisiae*)]

Figure 1



DPP and aminopeptidase activity profiles for periodontopathic and cariogenic bacteria. (A) Hydrolyzing activities of DPP4, DPP5, DPP7, and DPP11 were determined. (B) Aminopeptidase activities were determined with Arg-, Lys-, Ala-, Leu-, Met, and Phe-MCA.

Figure 2



DPP activities in saliva and subgingival plaque specimens. Subgingival dental plaque and saliva samples were collected from the subjects. Hydrolyzing activities of DPP4, DPP5, DPP7, and DPP11 were determined.

Graphical Abstract