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The Cultivable Human Oral Gluten-Degrading Microbiome and its Potential Implications in Celiac Disease and Gluten Sensitivity

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Abstract

Celiac disease is characterized by intestinal inflammation caused by gluten, proteins which are widely contained in the Western diet. Mammalian digestive enzymes are only partly capable of cleaving gluten, and fragments remain that induce toxic responses in celiac patients. We found that the oral microbiome is a novel and rich source of gluten degrading enzymes. Here we report on the isolation and characterization of the cultivable resident oral microbes that are capable of cleaving gluten, with special emphasis on its immunogenic domains. Bacteria were obtained by a selective culturing approach and enzyme activities were characterised by: 1) Hydrolysis of paranitroanilide-derivatised gliadin-derived tripeptide substrates; 2) Gliadin degradation in-gel (gliadin zymography); 3) Gliadin degradation in solution; 4) Proteolysis of the highly immunogenic α -gliadin-derived 33-mer. For select strains pH activity profiles were determined. The culturing strategy yielded 87 aerobic and 63 anaerobic strains. Species with activity in at least two of the four assays were typed as: *Rothia mucilaginosa* HOT-681, *Rothia aeria* HOT-188, *Actinomyces odontolyticus* HOT-701, *Streptococcus mitis* HOT-677, *Streptococcus sp.* HOT-071, *Neisseria mucosa* HOT-682 and *Capnocytophaga sputigena* HOT-775, with *Rothia* species being active in all four assays. Cleavage specificities and substrate preferences differed among the strains identified. The approximate molecular weights of the enzymes were ~75 kD (*Rothia* spp.), ~60 kD (*A. odontolyticus*) and ~150 kD (*Streptococcus* spp.). In conclusion, this study identified new gluten-degrading microorganisms in the upper gastro-intestinal tract. A cocktail of the most active oral bacteria, or their isolated enzymes, may offer promising new treatment modalities for celiac disease.

Keywords

celiac disease; gliadin; oral bacteria; proteases; degradation

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Introduction

Celiac disease (CD) is an inflammatory disorder with auto-immune features and a primary manifestation in the human small intestine. The disease is triggered upon ingestion of wheat gluten or similar proteins found in barley and rye (mainly hordeins and secalins, respectively). Gluten, comprising glutenins and gliadins, contain a relatively high concentration of glutamine and proline residues within their structures [1, 2], endowing certain gluten-derived domains with high resistance to degradation by human gastrointestinal proteases. None of the major human gastrointestinal proteases, such as pepsin, trypsin, chymotrypsin, carboxypeptidases A and B, elastases and brush-border membrane enzymes of the small intestine contain the necessary proteolytic capabilities to effectively cleave certain immunogenic gluten peptides, due to a lack of post-proline cleavage-site specificity. An example of a gliadin-derived, human intestinal protease resistant peptide is the 33-mer peptide derived from α -gliadins (LQLQPFQPQLPYPQPQLPYPQPQLPYPQPQPF) [3]. Apart from its resistance to proteolysis, it contains multiple, in part overlapping, immunogenic epitopes which stimulate CD4+ T-cells in the lamina propria of HLA-DQ2-positive CD patients [4].

In most patients adherence to a gluten-free diet reverses the immune-mediated small intestinal damage and fully restores absorptive functions, whereas reintroduction of dietary gluten leads to renewed disease [5]. However, to completely exclude even traces gluten from the daily diet is a difficult task, since gluten is an abundant constituent of most refined foods. In addition, there are other complicating factors such as cross-contamination of foods and inadequate food labeling. Finally, the necessity for leading a strict gluten-free life (as opposed to a diet that only excludes obvious and major sources of gluten such as bread, pizza or pasta) means a socio-economic burden to the patient. Therefore, alternate or adjunctive treatments beyond the strict gluten free diet are considered an area of therapeutic need [6].

To achieve a more complete digestion of gluten, and specifically its immunogenic domains, proline and glutamine-specific endopeptidases from bacteria, fungi and barley are currently being explored, and such therapies are in phase I–II clinical studies [7–13]. Notably, research in our laboratory has recently shown that gluten-degrading proteases are naturally present in the upper human gastro-intestinal tract. These enzymes are not from human origin, but produced by the wealth of bacteria that colonise the oral cavity and duodenum [14, 15]. Here we performed a large scale screening of the cultivable portion of the human oral microbiome for bacteria with proteolytic enzymes cleaving any of five selected gliadin-derived synthetic tripeptide enzyme substrates, intact gliadins, and the immunodominant α -gliadin 33-mer peptide. Such bacteria could possibly fulfill physiological roles in the digestion of gluten *in vivo*. Moreover, they may serve as a safe source of enzyme(s) with clinical potential in the treatment of gluten sensitivity and CD.

Materials and Methods

Collection of dental plaque and whole saliva

Protocols for the collection of human dental plaque and whole saliva (WS) were approved by the Institutional Review Board at Boston University. Written informed consent was obtained from the participating subject prior to sample collection. Samples were collected after the donor refrained from oral hygiene for 24 hr. Plaque was collected using a sterile dental scaler and suspended in a buffer mimicking the ion composition of saliva, containing 50 mM KCl, 1.5 mM potassium phosphate, 1 mM CaCl₂ and 0.1 mM MgCl₂, pH 7.0. Bacterial aggregates were resuspended with a pipet. Whole saliva (WS) was collected under conditions of masticatory stimulation as described [15].

Culturing of bacteria

Dental plaque and whole saliva suspensions were diluted 10^3 , 10^4 and 10^5 times in saliva ion buffer. Aliquots of 50 μ l were plated either on Brucella agar (BA; Hardy Diagnostics, Santa Maria, CA) or on gluten-limited agar (GA) which was prepared in-house [15]. Plates were incubated aerobically or anaerobically in a jar using GasPak pouches (BD Diagnostics, Sparks, MD) at 37°C for 24–72 hr. Individual colonies were sub-cultured on segmented gluten agar or Brucella agar, respectively, with up to 20 strains per plate. Colonies were then subcultured repeatedly on Brucella agar (1 strain per plate) to obtain macroscopically pure cultures. The final pure strains were plated on gluten agar to confirm their growth on this selective medium. The bacterial strains were maintained frozen in a mixture of BHI/glycerol (80/20% v/v).

16S rDNA identification of bacteria

DNA extraction from colonies grown on Brucella agar was performed using the UltraClean Microbial DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA) followed by sequencing using an ABI prism cycle-sequencing kit (BigDye® Terminator Cycle Sequencing kit) as previously described [16].

Hydrolysis of gliadin-derived enzyme substrates

Cells were harvested with a sterile cotton swab from Brucella agar plates and resuspended in saliva ion buffer to an OD_{620} 1.2. Enzymatic activity was examined towards five gliadin-derived synthetic enzyme substrates: Z-YPQ-pNA, Z-QQP-pNA, Z-PPF-pNA, Z-PFP-pNA and Z-LPY-pNA. The tripeptide substrates represent sequences that are common in the immunogenic gliadin domains. Substrates were mixed with 200 μ l bacterial suspensions (OD_{620} 1.2) to a final concentration of 200 μ M and incubated at 37°C. For initial screening of bacteria for substrate hydrolysis, data were interpreted macroscopically over a 48 hr time interval with assessment time points of 1 hr, 16 hr, 24 hr, 40 hr and 48 hr. Kinetic enzyme assays were carried out for selected strains, measuring substrate hydrolysis at 405 nm using a Genios microtiter plate reader (Tecan, Männedorf, Switzerland).

Degradation of gliadin in-gel (gliadin zymography)

Cells were harvested with a cotton swab from Brucella agar plates and suspended in saliva ion buffer to an OD_{620} 5.0. Cells contained in a 150 μ l aliquot were centrifuged and suspended in zymogram sample buffer containing 0.25 M Tris/HCl, 10% glycerol, 2% SDS and 0.0025% bromophenol blue. Gliadin zymography (6%) was carried out as described previously [15]. Gels were processed in renaturing and developing buffers (InVitrogen) according to the manufacturer's instructions. After 48 hr incubation at 37°C, gels were stained in 0.2% Coomassie Brilliant blue dissolved in 40% methanol and destained without the dye.

Degradation of gliadin in solution

A mixture of gliadins obtained from Sigma (St. Louis, MO) was dissolved to 5 mg/ml in 60% (v/v) ethanol. The solution was diluted in bacterial cell suspensions in saliva ion buffer (OD_{620} 1.2) to 250 μ g/ml. After incubation time points of 0, 2 and 5 hr at 37°C, 100 μ l aliquots were removed and boiled for 5 minutes to inactivate enzyme activity. For some strains different time points and cell densities were used (noted in the Figure legends). To each sample aliquot EDTA was added to a final concentration of 2.5 mM to complex calcium ions in the saliva ion buffer, and samples were dried using a Speedvac. Pellets were suspended in 1x SDS sample buffer and analyzed on pre-cast 12% Bis-Tris-PAGE (InVitrogen, Carlsbad, CA).

cleavage but no evidence for activity in the gliadin zymogram, and group V (n=5): cleavage of YPQ↓, QQP↓, PPF↓, PFP↓ and LPY↓ and no band in the gliadin zymogram gel (Table 1).

16S rDNA sequencing results

The 21 strains of interest were speciated by 16S rDNA analysis. As expected, in some cases, strains within one group represented the same species. A clear picture emerged as to the differences in species in the five groups. The taxa identified in group I were: *Rothia mucilaginosa* HOT-681 and *Rothia aeria* HOT-188; in group II: *Actinomyces odontolyticus* HOT-701; in group III: *Streptococcus mitis* HOT-677 and *Streptococcus* sp. HOT-071; in group IV: *Neisseria mucosa* HOT-682; and in group V: *Capnocytophaga sputigena* HOT-775 (Table 1). A representative zymogram with a selection of strains from each group is shown in Figure 1.

Degradation of gliadin in Solution

A subset of strains was selected to study their capacity to degrade gliadin in solution. These were three strains from Group I (WSA-2B, WSA-21B, WSA-26), one strain from group II (PA-20b), four strains from group III (PA-12, PA-21, PA-3b, and PA-19b), two strains from group IV (PA-1b, PA-2b), and two strains from group V (PA-33R, PA-90R). Results obtained with *Rothia aeria* (strain WSA-8) were reported previously [15]. The final OD₆₂₀ and incubation times for strains in groups I, II and III were OD₆₂₀ 1.2 and 0, 2 hr and 5 hr, respectively, and for strains in group IV and V the OD₆₂₀ was 0.2 and the incubation times were 0, 4 hr and 8 hr, respectively. The OD and time adjustment were made based on the apparent high protein content in these strains (see e.g. Figure 1). Only bacteria in group I cleaved gliadin in solution (Supplemental Figure 1). Results for strains WSA-2B (group I), PA-20b (group II), and PA-33R (group V) are shown in Figure 2, left panels.

Degradation of the 33-mer Gliadin Peptide

The same 12 strains were also investigated for degrading the gliadin-derived 33-mer peptide. The cell densities and incubation times used were the same as for the gliadin degradation experiments. Supplemental Figures 2A, B, C show the chromatograms obtained. Strains in group I (*R. mucilaginosa* HOT-681) cleaved the 33-mer partly in the 5 hr time span and buffer conditions examined. The strain in group II (*A. odontolyticus* HOT-701) cleaved the 33-mer completely within a 2 hr time interval, with proteolysis already noticeable in the t=0 sample (taken immediately after mixing of the 33-mer peptide with PA-20b suspension). The degradation patterns at t=2 hr and t= 5 hr were comparable, and remained stable over a 24 hr time span (not shown). Strains in groups III (*S. mitis* HOT-677 and *Streptococcus* HOT-071) and IV (*N. mucosa* HOT-682) were unable to cleave the 33-mer peptide. In contrast, group V bacteria (*C. sputigena* HOT-775) cleaved the 33-mer rapidly without any visible evidence for surviving major degradation fragments. Results for strains WSA-2B, PA-20b and PA-90R are shown in Figure 2 (right panels). Table 1 summarizes all the results obtained.

pH activity profiles

Three strains with prominent activities were selected for pH activity determinations: WSA-21B (*R. mucilaginosa* HOT-681), PA-20b (*A. odontolyticus* HOT-701) and PA-90R (*C. sputigena* HOT-775). Enzymatic activities for all three strains were optimal at neutral to basic pH conditions. At pH 5.0, rates of hydrolysis were reduced for all strains and at pH 3.0 none of the strains were active over the 8 hr time interval examined (Figure 3). The latter would suggest that the strains are not active at the pH conditions encountered in the fasting stomach. To investigate if the strains could withstand low pH conditions for a time interval

consistent with gastric passage, we performed an experiment in which a mixture of the three strains were incubated at pH 3.0 for 1.5 hr, after which the pH was adjusted to pH 7.0. Enzyme activity assessment confirmed activity of the bacterial mixture at pH 7.0 and inactivity at pH 3.0. Importantly, bacteria preincubated at pH 3.0 were not irreversibly inactivated, since their activity could be fully restored when the pH was adjusted to pH 7.0.

Discussion

This study was aimed at the discovery and broad characterisation of natural resident microbes in the most upper part of the gastro-intestinal tract that can degrade immunogenic gliadins implicated in celiac disease. The approach consisted of a culture-based initial enrichment step for such bacteria from the mixed bacterial oral samples. Enzyme activities associated with the isolates were determined in four independent assays. Bacteria could be separated into five groups after analyzing degradation patterns and bacterial IDs. The most active microbes were tested at various pH, showing that their enzymes were not active at acidic pH, but that a temporary exposure to a low pH consistent with gastric transfer is permissible. Additional information on the identified strains' rank of abundance in the oral cavity, and the number of known and putative peptidases, are given in Table 2.

Bacteria in Group I comprised *Rothia mucilaginosa* HOT-681 and *R. aeria* HOT-188. Both strains were active in all four assays and therefore are of high interest. *Rothia* species are harmless residents of the oral cavity and the oral pharynx [16], and have also been identified in duodenal biopsies [17]. There are case reports of infections caused by *R. mucilaginosa* in immunocompromised patients, but overall it is considered a harmless coloniser of the oral cavity [18] They hydrolyzed the Z-YPQ-pNA and Z-LPY-pNA substrates, and cleaved the highly immunogenic α -gliadin 33-mer peptide after QPQ↓ and LPY↓ [14]. The LPY↓ specificity is unique and adds to the post-proline and post-glutamine activities currently being the focus of luminal enzyme therapy [19].

The bacterial species identified in Group II was *A. odontolyticus* HOT-701. Its enzymes also cleaved the substrates Z-YPQ-pNA and Z-LPY-pNA, mixed gliadins and the 33-mer peptide. The molecular weight of the enzyme was distinctly lower than that of the *Rothia* bacteria. *A. odontolyticus* HOT-701 is typically isolated from patients with advanced dental caries [20], and has rarely been implicated in bacteremia [21]. It is described as an anaerobic microorganism, but we could culture it both under aerobic and under anaerobic conditions. The typing of *A. odontolyticus* was made based on 16S rDNA analysis. Since the level of homology in the 490 bp sequence after removal of any ambiguous base calls was 98%, it cannot be excluded that strain PA-20b represents a closely related *Actinomyces* subspecies. Therefore we obtained ATCC strain 17929 (*A. odontolyticus* Batty) for validation of the results with isolate PA-20b. The zymogram results of the ATCC strain were indistinguishable from PA-20b, confirming the gluten-degrading capacity of *A. odontolyticus* (data not shown). Notably, strain PA-20b degraded gliadin in-gel but not in solution, suggesting that substrate conformation is an important determinant for recognition. PA-20b quickly degraded the 33-mer, faster than the *Rothia* bacteria. However, the degradation fragments were stable and of substantial length, given their high retention times in the HPLC chromatograms. Therefore, PA-20b enzymes would work optimally in combination with other enzymes to achieve the full fragmentation of immunogenic gliadin peptides.

The bacteria in group III comprised *Streptococcus mitis* HOT-677 and *Streptococcus sp.* HOT-071. *Streptococcus mitis* HOT-677 is the second most abundant oral microorganism. It has been detected on the buccal epithelium, maxillary anterior vestibule, tongue dorsum, hard and palate, tonsils, tooth surfaces and subgingival plaque [22]. The selection of *S. mitis*

following the plating approach might have been due to its high abundance, rather than it being a truly gliadin-degrading organism. Indeed, it exhibited low gliadin-degrading activities in gel, and negligible or undetectable activities in solution. Neither of the streptococcal species cleaved the 33-mer peptide. Nevertheless, because of its high abundance, *S. mitis* could still contribute substantially to the overall gliadin-degrading capacity in the human mouth.

The bacterium in Group IV was typed as *N. mucosa* HOT-682. This is a gram-negative aerobic bacterium that is a nonpathogenic resident of the oropharynx of humans. As for the other strains identified, rare cases of systemic infections with *Neisseria mucosa* have been reported [23, 24]. Interestingly, *Neisseria mucosa* strains hydrolyzed three of the five gliadin-related tripeptide substrates, but not any of the larger substrates (33-mer, or intact gliadin, either in gel or in solution). Given this limited activity, *Neisseria mucosa* is deemed of less interest for enzyme isolation and potential exploitation in CD.

Lastly, group V comprised the bacterium *C. sputigena* HOT-775. This species stood out from the other identified microorganisms in being able to cleave all five gliadin-based tripeptide substrates. This result demonstrates that a wide variety of protease specificities are contained within this single microorganism. *C. sputigena* HOT-775 is a gram negative oral coloniser. It has been implicated in a number of infectious diseases, e.g. septicaemia, osteomyelitis, abscesses and keratitis [25], perhaps, owing to its high and diverse protease activities (Table 2). *C. sputigena* HOT-775, like *A. odontolyticus* HOT-701, rapidly cleaved the immunogenic 33-mer gliadin peptide, grouping this bacterium automatically into the category of high interest. In contrast to *A. odontolyticus* HOT-701, no larger protease-resistant peptides were observed, indicating extensive fragmentation of the 33-mer peptide by *C. sputigena* HOT-775. It can be assumed, but needs further proof, that immunogenic epitopes would thus be neutralised. *N. mucosa* HOT-682 and *C. sputigena* HOT-775 did not cleave intact gliadin, neither in a partly denatured state in the gel matrix, nor in a native conformation in solution, suggesting that larger substrates are not recognised by their proteases.

While ingested gluten is a necessary trigger for CD development, gluten reactivity alone cannot explain the absence of disease in most subjects with HLA-DQ2 and -DQ8 phenotypes. A prominent role for the gastrointestinal microbiome in the switch from tolerance to an inflammatory immune response to gluten is becoming evident [26]. Recently, this concept was further supported by studies showing a difference between the developing gut microbiome in HLA-DQ2+/DQ8+ children of patients with biopsy-proven CD [27] and children from healthy mothers [28]. Besides modulating the immune response, our study provides evidence that the microbiota of the (upper) GI tract may also play a more direct role by degrading toxic gluten peptides, and perhaps aiding to gluten detoxification in vivo.

How far the gluten-degrading microorganisms contribute to gluten digestion in vivo remains to be established. Apart from their potential physiological effects as natural colonizers of the upper GI tract, the bacteria - and their enzymes- could also be exploited therapeutically as digestive aids. Thus gluten-degrading enzymes from other sources are already being pursued as adjunctive therapeutics for celiac disease [7–13]. Similarly, the gluten-degrading enzymes from the oral bacteria could be isolated and further pursued as a pharmaceutical drug, or dietary supplement. The advantage is that the sources of these enzymes are natural human body-associated microbes, and most oral bacteria are indeed harmless residents. Given their tolerated natural colonization of the upper GI tract an alternative approach would be to develop the gluten-degrading bacteria themselves, as probiotic agents, to achieve gluten digestion. Successful application would require the probiotic to be effective along the

gastrointestinal canal and to be safe when consumed in larger quantities, aspects that are currently being addressed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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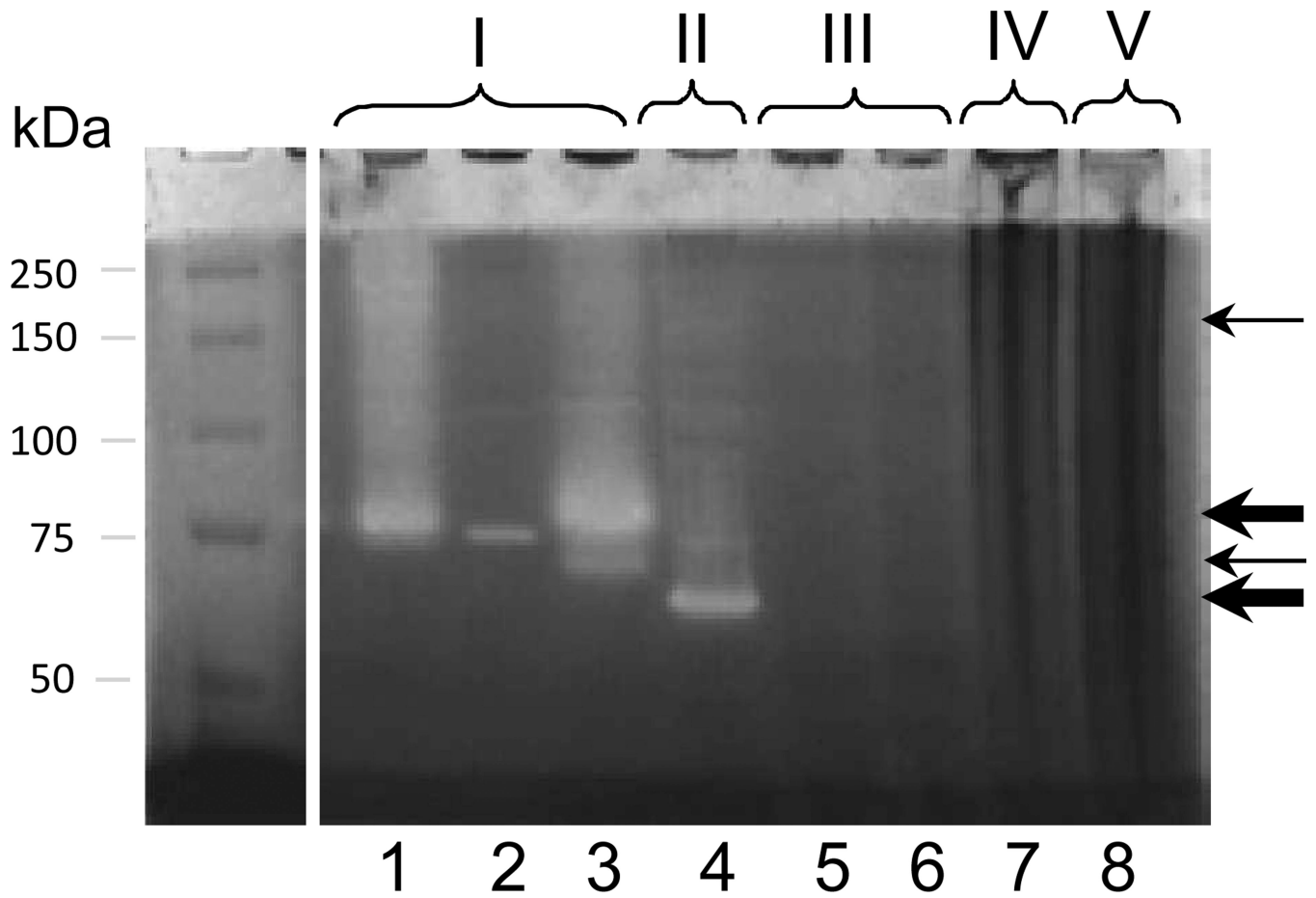


Figure 1.

Gliadin zymography of selected oral strains. An aliquot of 150 μ l cells (OD_{620} 5.0) was applied per lane. Lane 1, WSA (whole saliva)-2B (*R. mucilaginosa*); lanes 2: WSA-8 (*R. aeria*), lane 3: WSA-21B (*R. mucilaginosa*); lane 4: PA (dental plaque)-20b (*A. odontolyticus*); lane 5: PA-12 (*Streptococcus mitis*); lane 6: PA-21 (*Streptococcus sp.* HOT-071); lane 7: PA-2b (*Neisseria mucosa*); lane 8: PA-90R (*Capnocytophaga sputigena*).

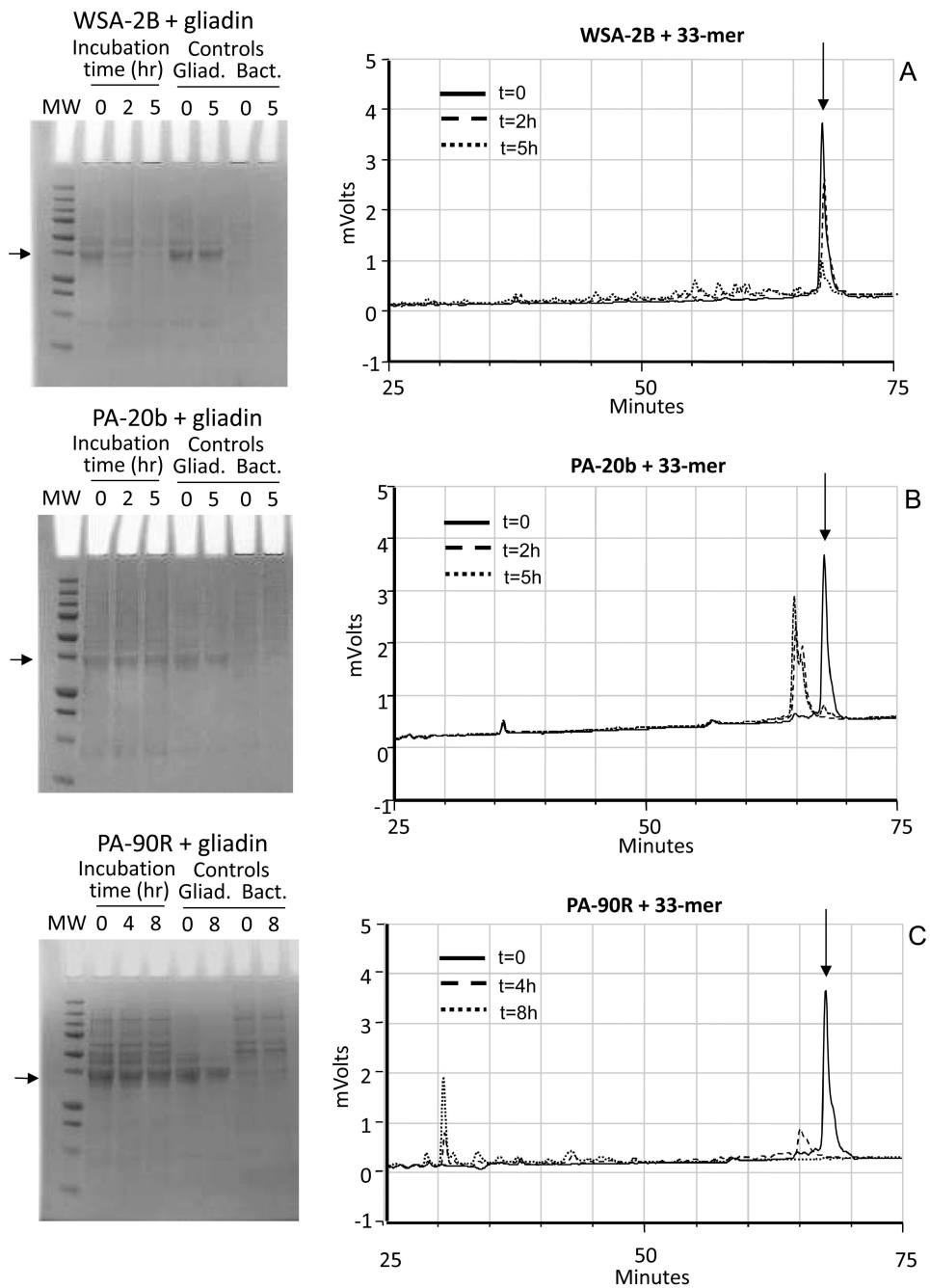


Figure 2. Kinetics of gliadin degradation and 33-mer degradation by WSA-2B, PA-20b and PA-90R. Arrows point to the major protein component in the gliadin preparation at ~37 kD (SDS gels, left panels) and the 33-mer (RP-HPLC chromatograms, right panels). The cell densities used for the incubations were OD₆₂₀ 1.2 for WSA-2B and PA-20b and OD₆₂₀ 0.2 for PA-90R. The final concentration of mixed gliadins and 33-mer peptide were 250 µg/ml. Degradation mixtures were analyzed at t=0, 2h and 5h (strains WSA-2B and PA-20b) and t=0, 4h and 8h (strain PA90R).

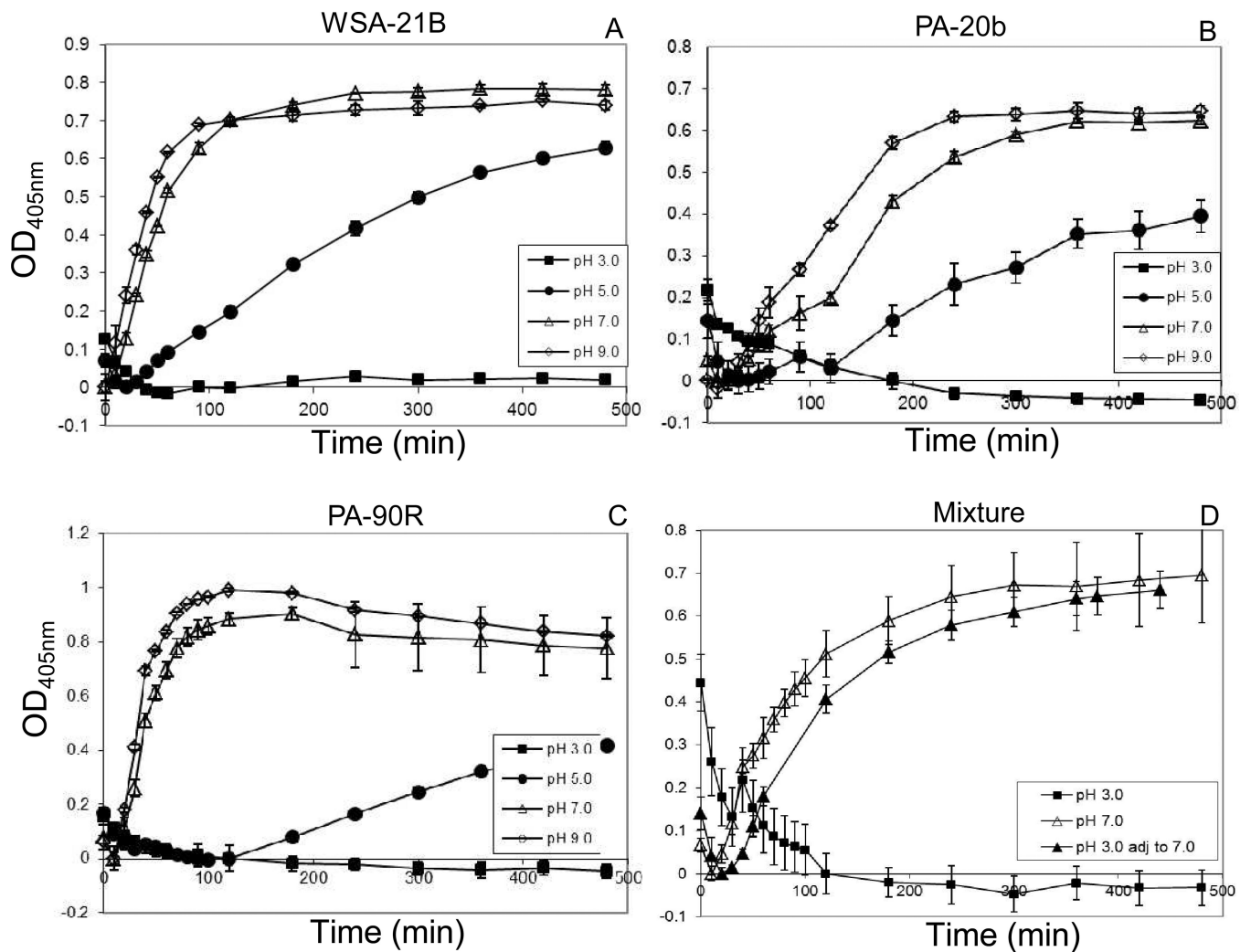


Figure 3.

pH gluten peptide degrading activity profiles of WSA-21B, PA-20b and PA-90R. Strains were suspended to a final OD₆₂₀ 1.2 in Tris solutions with pH values adjusted to pH 3.0, 5.0, 7.0 and 9.0, and hydrolysis of Z-YPQ-pNA (final concentration 200 μ M) was monitored spectrophotometrically at 405 nm over a 0–480 min time interval. A, WSA-21B; B, PA-20b; C, PA-90R. D, equal volumes of the three strains were mixed at pH 3.0, 7.0, or at pH 3.0 followed by adjustment of the pH after 1.5 h to 7.0. Z-YPQ-pNA hydrolysis in the three suspensions is shown.

Table 1

Summary Evaluation of Aerobic Strains^a

Strain name	Strain ID#	YPQ	QQP	PPF	PPF	LPY	Gliadin zymo band (MW)	Gliadin degr. in solution	33-mer degradation	pNA-derivatized tripeptide substrates	
										GROUP I	GROUP II
<i>Rothia mucilaginos</i> a HOT-681	WSA-2B	+ 1 hr	-	-	-	+ 16 hrs	~70/75 kD	Yes	Yes	Yes	GROUP I
<i>Rothia sp.</i> HOT-188 (<i>Rothia aeria</i>)	WSA-8	+ 1 hr	-	-	-	+ 16 hrs	~75 kD	Yes	Yes	Yes	GROUP I
<i>Rothia mucilaginos</i> a HOT-681	WSA-21B	+ 1 hr	-	-	-	+ 16 hrs	~70/75 kD	Yes	+/-	+/-	GROUP I
<i>Rothia mucilaginos</i> a HOT-681	WSA-26	+ 1 hr	-	-	-	+ 16 hrs	~70/75 kD	Yes	Yes	Yes	GROUP I
<i>Actinomyces odontolyticus</i> HOT-701	PA-20b	+ 1 hr	-	-	-	+ 16 hrs	~60 kD	No	Yes	Yes	GROUP II
<i>Streptococcus mitis</i> HOT-677	WSA-7A	+ 16 hrs	-	-	-	-	~150 kD	n.d.	n.d.	n.d.	GROUP III
<i>Streptococcus mitis</i> HOT-677	PA-8	+ 16 hrs	-	-	-	-	~150 kD	n.d.	n.d.	n.d.	GROUP III
<i>Streptococcus sp.</i> HOT-071	PA-9	+ 16 hrs	-	-	-	-	~150 kD	n.d.	n.d.	n.d.	GROUP III
<i>Streptococcus mitis</i> HOT-677	PA-12	+ 16 hrs	-	-	-	-	~150 kD	No	No	No	GROUP III
<i>Streptococcus sp.</i> HOT-071	PA-21	+ 24 hrs	-	-	-	-	~150 kD	No	No	No	GROUP III
<i>Streptococcus sp.</i> HOT-071	PA-3b	+ 16 hrs	-	-	-	-	~150 kD	No	No	No	GROUP III
<i>Streptococcus sp.</i> HOT-071	PA-4b	+ 16 hrs	-	-	-	-	~150 kD	n.d.	n.d.	n.d.	GROUP III
<i>Streptococcus mitis</i> HOT-677	PA-19b	+ 24 hrs	-	-	-	-	~150 kD	No	No	No	GROUP III
<i>Streptococcus sp.</i> HOT-071	PA-23b	+ 24 hrs	-	-	-	-	~150 kD	n.d.	n.d.	n.d.	GROUP III
<i>Neisseria mucosa</i> HOT-682	PA-1b	+ 1 hr	+/- 24hrs	-	-	+ 16 hrs	-	n.d.	n.d.	n.d.	GROUP IV
<i>Neisseria mucosa</i> HOT-682	PA-2b	+ 1 hr	+/- 24hrs	-	-	+ 16 hrs	-	No	No	No	GROUP IV
<i>Capnocytophaga sputigena</i> HOT-775	PA-33B	+ 1 hr	+ 1 hr	+ 1 hr	+ 1 hr	+ 1 hr	-	n.d.	n.d.	n.d.	GROUP V
<i>Capnocytophaga sputigena</i> HOT-775	PA-33R	+ 1 hr	+ 1 hr	+ 1 hr	+ 1 hr	+ 1 hr	-	No	Yes	Yes	GROUP V
<i>Capnocytophaga sputigena</i> HOT-775	PA-90B	+ 1 hr	+ 1 hr	+ 1 hr	+ 1 hr	+ 1 hr	-	n.d.	n.d.	n.d.	GROUP V
<i>Capnocytophaga sputigena</i> HOT-775	PA-90R	+ 1 hr	+ 1 hr	+ 1 hr	+ 1 hr	+ 1 hr	-	No	Yes	Yes	GROUP V
<i>Capnocytophaga sputigena</i> HOT-775	PA-90	+ 1 hr	+ 1 hr	+ 1 hr	+ 1 hr	+ 1 hr	-	n.d.	n.d.	n.d.	GROUP V

^aTotal number of aerobic bacteria screened: 87; total number of bacteria cleaving one or more of the five tripeptide-pNA substrates: 78 +color change due to substrate hydrolysis, occurring after the indicated incubation time (macroscopic assessment) Gliadin and 33-mer degradation in solution: 250 µg/ml protein, proteolysis assessed by SDS PAGE and RP-HPLC, resp

Table 2

Oral abundance and number of proteases in selected species

Strain name	Group	Approximate Rank abundance in the oral cavity (#) ^a	Number of known and putative peptidases ^b
<i>Rothia mucilaginosa</i> HOT-681	I	170	13
<i>Rothia aeria</i> HOT-188	I	221	unavailable
<i>Actinomyces odontolyticus</i> HOT-701	II	326	41
<i>Streptococcus mitis</i> HOT-677	III	2	19
<i>Streptococcus sp.</i> HOT-071	III	37	unavailable
<i>Neisseria mucosa</i> HOT-682	IV	64	8
<i>Capnocytophaga sputigena</i> HOT-775	V	166	22

^aInformation from Dewhirst et al 2010.^bInformation from www.merops.ac.uk