

Supplementary Information

Protease resistance of porcine acidic mammalian chitinase under gastrointestinal conditions implies that chitin-containing organisms can be sustainable dietary resources

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(a)

AMCase	GAPDH	β -actin	Pep A	Pep C	H ⁺ /K ⁺ -ATPase	Gastrin	Gif	Mucin	Chit1
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(b)

TGACTTCACAGGCACTTCTGCAACCAGGGCAAGTCCCCCTGATCAACACCCCTGAAGGATGCT
CTTGGCCTGAATAGCACAAAGTTGCACCGACCTCCACTACATGGTCTACATTGTTCCAGTATGATT
CCACCCCACGGCAAGTCCACGGCACAGTCAGGCGGAGAACGGGAAGCTTGTCATCTTCTAGGC
GGACTGTTAGTTGCATTCCACCTTTCTTGACAAAACCTA**ACTTGCGCAGAAAAT**GAGATGAG
ATTGGCATGGCTGGATCCGGCGGCATCGATTCTTCTTACTACACAGGAAGCCTGA**ACTGGTGC**
CTGTTCCGTGAGGGTTACTGGCAGATTACCTGGACAGCATCACCGAGTCCTCAAGACCCA
CAAGTACGACCCCGCCAGAGGTACCGCTTCGGCGACTTCAGCGTGGCCTGGAGGCCATGGCC
TA**CTTGAAGCTGCC**TACTTGCCAGATCAGC**ATC**CTGGGTT**CACTGACTACTTCAC**GGCCAT
GGCCCAGGAGGGCTGGTCCC**ACTGCTGTGT**GGGGCTGCGGCCACAGTGGGAGAACCCAC
CTACAAGACGGACCGGTGGTCTCTT**ATTAACA**ATATCGCTGAAA**ATGTCAACCACAGGAC**GT
ACTGGCAGTTCTGAGTGGCAAACGCCCTTAAACGAAGGAGTTGCGGA**ATGGATGGAGGAGGA**
AGAAGAAGCATATGGATGGATGGACTTCGGCCGCCAGTGCTGAGGAAGGAGACCAGCGTCCC
TAGAACCGAGCTCCAGACCACACCTGAAGACACAGTTTGATCGGCTTAAAGCGGAAGCAGGCC
CCTATAACCTGACC**ATCAGTGAAGTTAGTGTGATTGATG**CACCATTTCCCTCTG**CTCAGC**G
GATCCGTCAACTCAGCCATCAGGTTTCTGCGCAAATATGGCTTTGATGGCTTGAC**CTTG**

Figure S1. Single standard DNA molecule used for qPCR. (a) Schematic representation of the standard DNA molecule. (b) Nucleotide sequence of the single standard DNA. The single standard DNA, 956 bases long, contained cDNA fragments of AMCase, GAPDH, β -actin, pepsinogen A (Pep A), pepsinogen C (Pep C), H⁺/K⁺-ATPase, Gastrin, gastric intrinsic factor (Gif), Mucin and Chit1 in a one-to-one ratio. Primers for qPCR in each target DNA region are shown in underline.

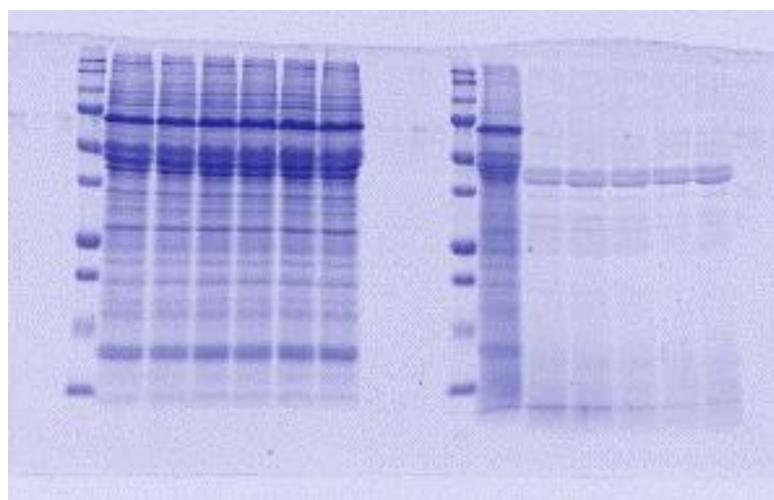
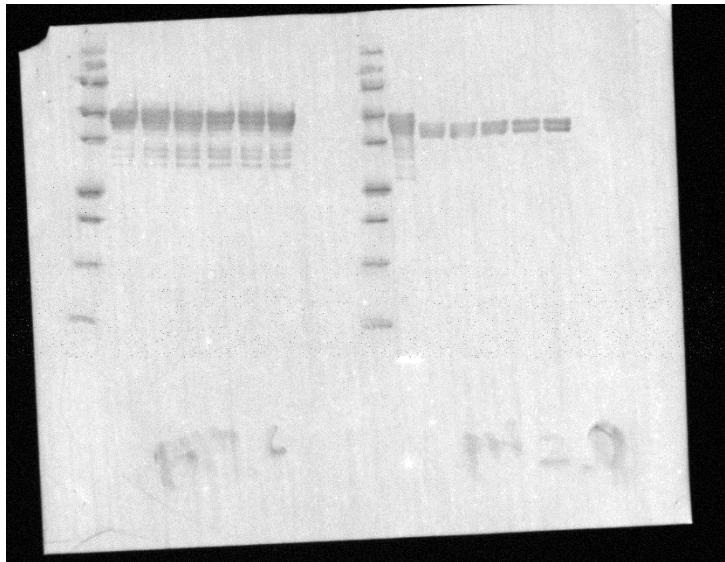


Figure S2. Full-length gel image shown in Fig. 3a. Full-length gel images of SDS-PAGE and CBB staining.

(a)



(b)

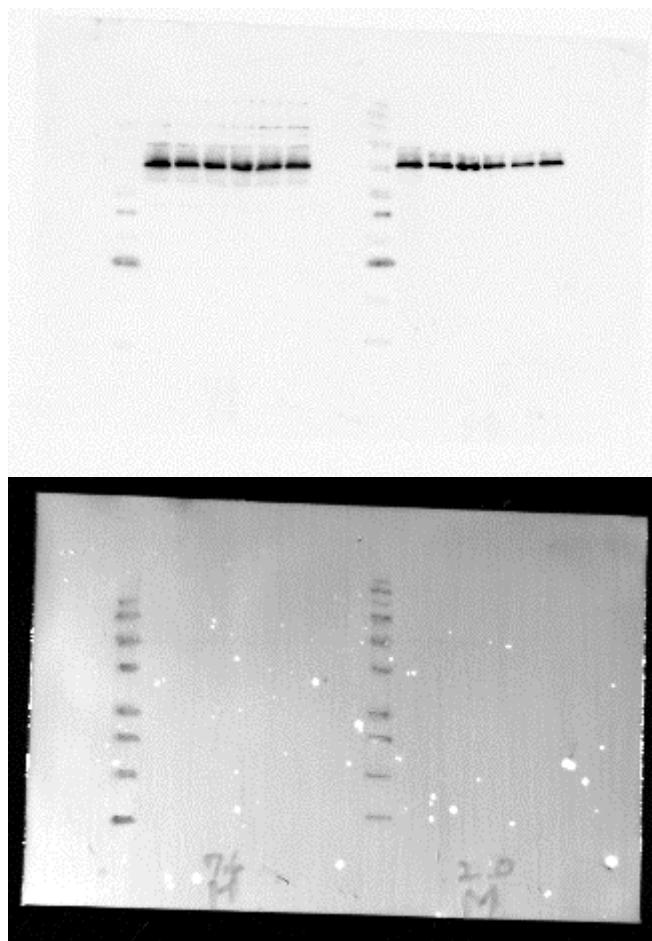


Figure S3. Full-length gel and blots shown in Fig. 3b and c. (a) Western blotting using anti-pepsin and molecular weight markers. (b) Western blotting using anti-AMCase and its molecular weight markers.

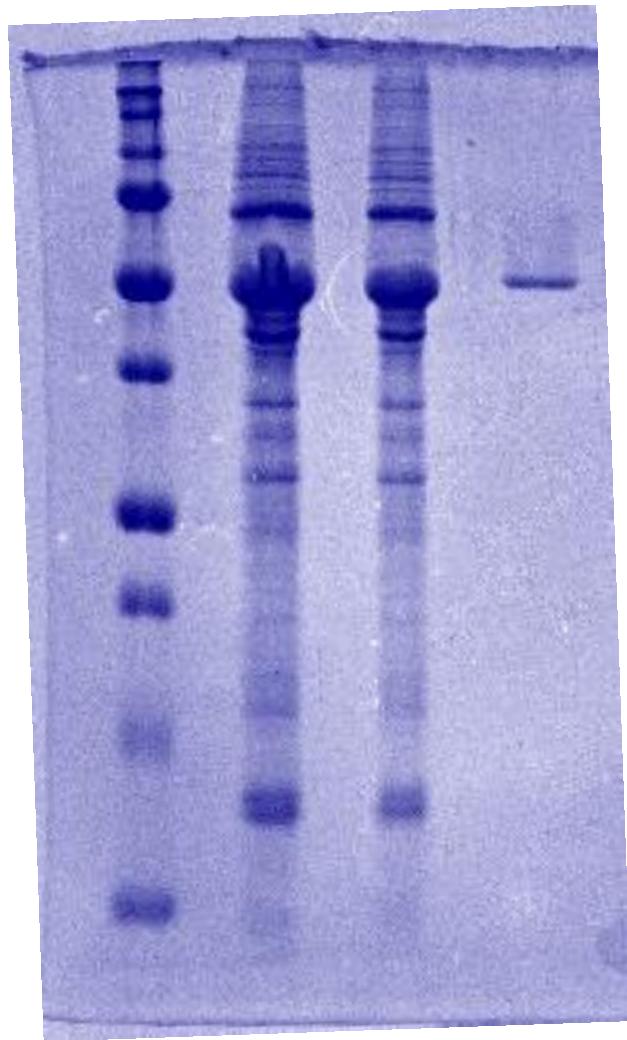
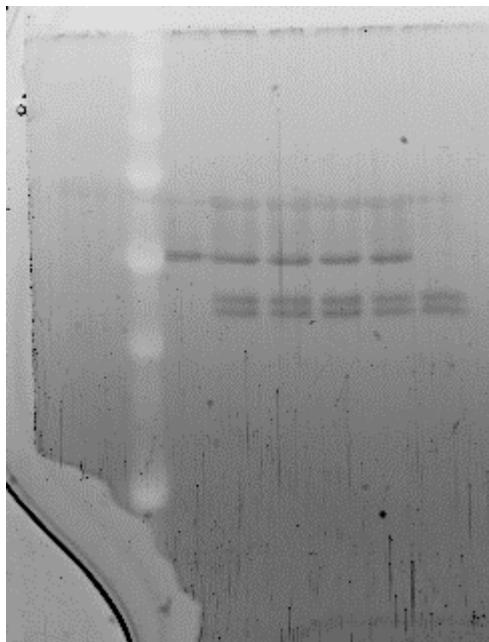
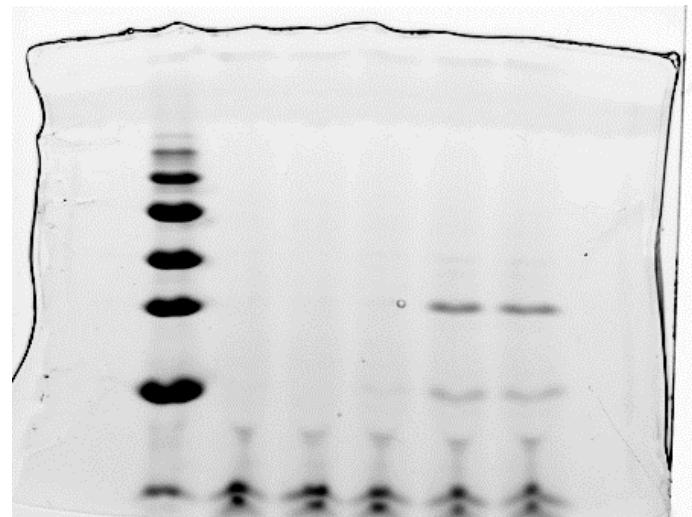


Figure S4. Full-length gel image shown in Fig. 4. Full-length gel image of SDS-PAGE and CBB staining.

(a)



(b)



(c)

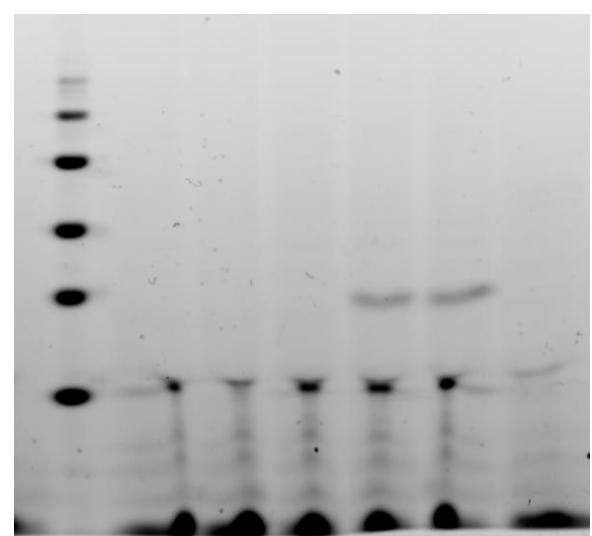
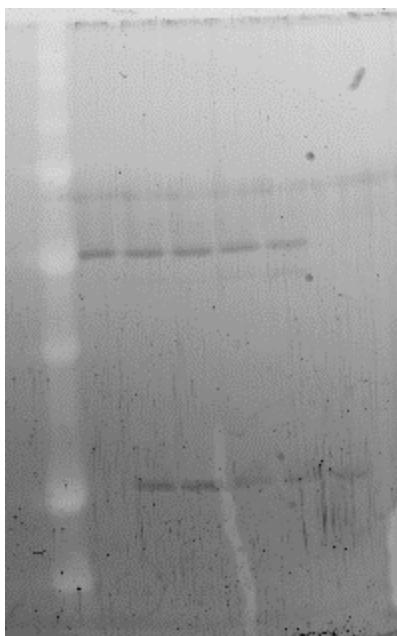
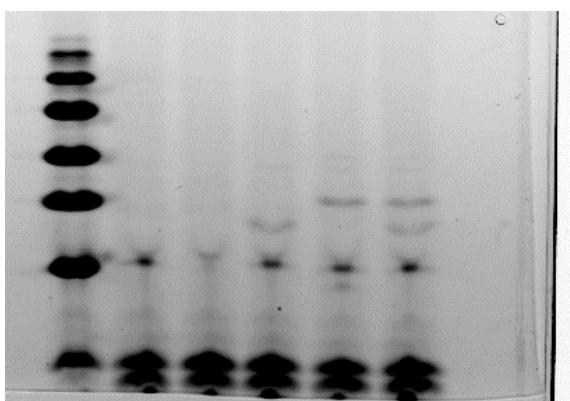


Figure S5. Full-length gel and blots shown in Fig. 5. (a) Full-length gel images of SDS-PAGE and SYPRO Ruby staining in Fig. 5a. (b and c) Full-length gel images of FACE methods in Fig. 5c and d.

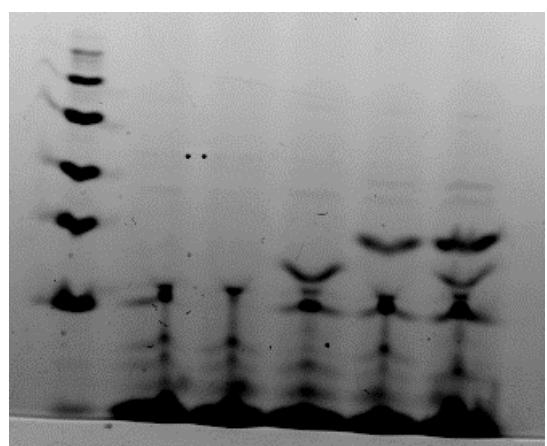
(a)



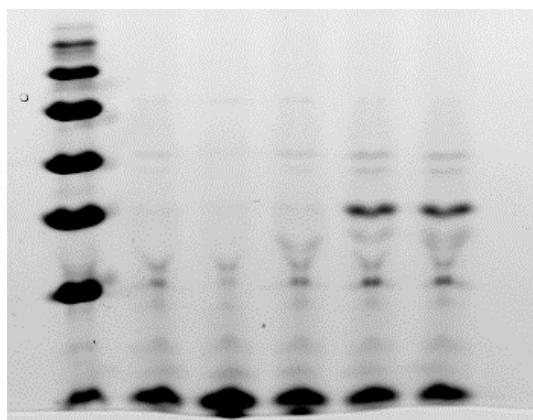
(b)



(d)



(c)



(e)

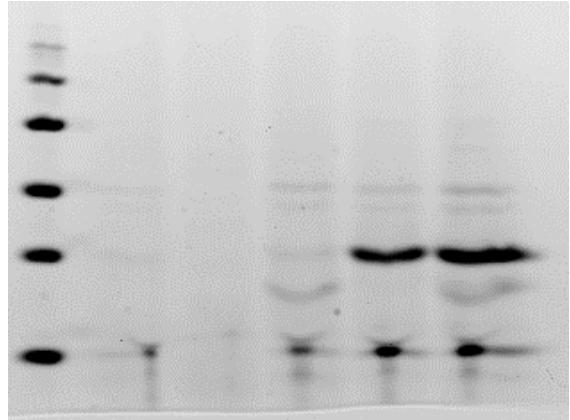


Figure S6. Full-length gel and blots shown in Fig. 6. (a) Full-length gel images of SDS-PAGE and SYPRO Ruby staining in Fig. 6a. (b-e) Full-length gel images of FACE methods in Fig. 6c-f.

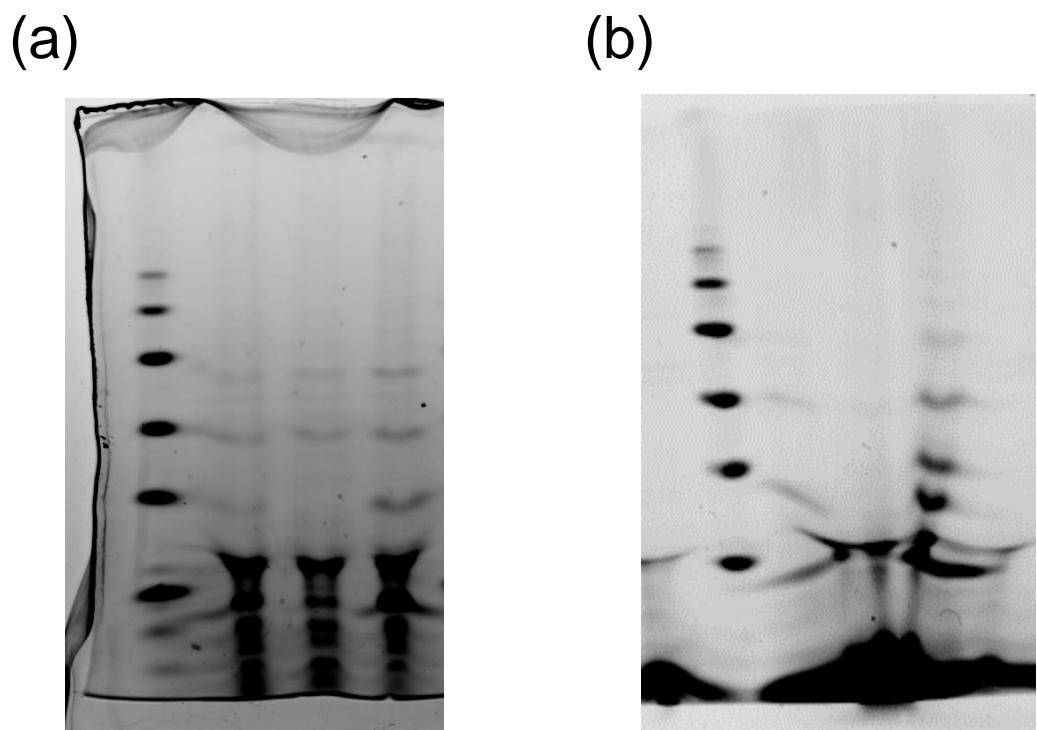


Figure S7. Full-length gel and blots shown in Fig. 7. (a and b) Full-length gel images of FACE methods in Fig. 6a and b.

Table S1. List of qPCR primers.

Gene	Forward primer	Reverse primer
AMCase	TGACTTCACAGGCACTTCT	CGGTGCAACTTGTGCTATT
GAPDH	ACCTCCACTACATGGTCTACA	ATGACAAGCTTCCCCTCTC
β-actin	CTTCTAGGCAGACTGTTAGTG	AGCCATGCCAATCTCATCTC
Pepsinogen A	GGCGGCATCGATTCTTCTTA	ATTACCTGGACAGCATCACC
Pepsinogen C	GAGTTCCCTCAAGACCCACAAG	GATGCTGATCTGCCAAAGTA
H ⁺ /K ⁺ -ATPase	CTGGGTTCACTGACTACTTCAC	GAGAACCAACCACCTACAAGAC
Gastrin	GGACCGGTGGTCTCTTCTATT	TCCGCAACTCCTCGTTAAG
Gif	ATGGATGGAGGAGGAAGAAGA	TCTGGAGCTCGGTTCTAGG
Mucin	CCACAACCTGAAGACACAGT	GCTGAGCAGAGGAAGGAAAT
Chit1	GTCAACTCAGCCATCAGGTT	CAAGGTCAAGGCCATCAA