



Supplemental Figure 1 Enzymatic degradation of gliadins in mouse chow *in vitro* detected by immunoblotting. Mouse chow was supplemented with *R. aeria* (Ra+) and without (Ra-) and incubated at 37°C for 0, 2 and 4 hr. Aliquots (10 µl) of 60% ethanol-extracted sample supernatants were loaded. Lane1: Gliadin control (Gli, 15 µg); Lane 2: Ra (-) at 0 hour; Lane 3: Ra (-) at 2 hr; Lane 4: Ra (-) at 4 hr; Lane 5: Ra (+) at 0 hr; Lane 6: Ra (+) at 2 hr; Lane 7: Ra (+) at 4 hr, blue (arrow) points to the gliadin bands at 37 and 50 kDa.

MIC Susceptibility of Two *Rothia* Species to Antimicrobics

Category	Antimicrobics	MIC ($\mu\text{g/ml}$)		
		Rm	Ra	
Aminoglycosides	Amikacin	<4	<4	
	Gentamicin	<1	<1	
	Streptomycin	<1000	<1000	
	Tobramycin	4	8	
Beta-lactams	Cephalosporins	Cefepime	<2	<2
		Cefotaxime	<1	<1
		Ceftazidime	4	8
		Ceftaroline	<0.12	<0.12
	Carbapenems	Ceftriaxone	<8	<8
		Doripenem	<0.12	0.25
		Ertapenem	<0.25	0.5
		Imipenem	<1	<1
	Monobactams	Meropenem	<1	<1
	Penicillins	Aztreonam	>16	>16
		Ampicillin	<0.12	<0.12
		Oxacillin+2%NaCl	<0.25	<0.25
	Glycopeptides	Penicillin	<0.06	<0.06
		Vancomycin	<1~1	2
Telavancin		<0.06	0.12	
Glycylcyclines	Tigecycline	<0.06	0.12	
Lincosamides	Clindamycin	<0.5~0.5	>2	
Lipopeptides	Daptomycin	0.5~1	4	
Macrolides	Erythromycin	<0.25	4	
Nitrofurans	Nitrofurantoin	<32	<32	
Oxazolidinones	Linezolid	<1~1	1	
Polymyxins	Colistin	>4	>4	
	Polymixin B	>4	>4	
Quinolones	Ciprofloxacin	2~4	2~4	
	Gatifloxacin	<1	<1	
	Levofloxacin	<1~2	1	
	Moxifloxacin	<0.25	0.5	
Rifamycins	Rifampin	<0.5	<0.5	
Tetracyclins	Doxycycline	<2	<2	
	Minocycline	<2	<2	
	Tetracycline	<2	<2	
Others	Chloramphenicol	<2	<2	
Combinations	Piperacillin / tazobactam constant 4	<8/4	<8/4	
	Quinupristin / dalfopristin	0.25	0.12	
	Ticarcillin / clavulanic acid constant 2	<16/2	<16/2	
	Trimethoprim / sulfamethoxazole	<0.5/9.5	<0.5/9.5	

Supplemental Figure 2, MIC (minimal inhibitory concentration) susceptibility of two *Rothia* species to antibiotics. The experiment was performed by using MIC susceptibility plates (Sensititre, Thermofisher Scientific/ Trek diagnostic systems). MIC was recorded as the lowest concentration of antibiotic that inhibits visible growth. Rm: *Rothia musinoginosa*; Ra: *Rothia aeria*.

There are some concerns that *Rothia* bacteria may cause side effects in patients if they colonize non-naturally areas of the human body. Here data shows that Rm and Ra are susceptible to antibiotics and to 70% ethanol (Figure 2 A). Notably, the dead Rm/Ra still exhibit gluten degrading activity (Figure 2 B-D), indicating that these inactivated Rm and Ra maintain gluten proteolytic activity to benefit CD patients.

>BAV86562.1 glycerol-3-phosphate ABC transporter [*Rothia aeria*]

MAITAGLPATAAPAGDPDTPVAQDIARNSREHAVLSDSMKKAEGNIPVFFVQFKGKGAYEQTQSPAVLANKQAPTNNKQ
AEVQAIKTQVQSQAQAAAQSTGAKTLYTTHNIMRGVALQGDAAQIRALANNPEVERITPIVPKKKQNAGSVVDTGAA
ENWARENSGYT**GKDV**KIA**VVD****S**GIDYTHADFGGPGTVEAFNKATKL**TEMPA**AD**S**GLY**DAK**KY**I**GGYDLV**GDS**YDGTN
QTAPDN**NPIDCS**AGGHGTHVAGTAAGYGV**NQD**GTTFRGDY**SKL**TAEQL**NQMKIG**PGAA**PEA**QLY**SFRV**FGCTGTTGV
VVQALDR**TLD**PNGDGDFSDRANIV**NLS**IGGEF**SPPDD**ADAYAVESLNRQ**GV**LAVVSAGNATDYYGRGDTYSDSG**QPA**
NAVSALTVANSIGSSYAVDSMEIQAPANVAGKVPGDYTVSYTYTGAKPEAL**TGT**VVTP**SE**SNK**FGCE**AFSAEDA**AKI**
KDKWV**FLE**WANADGSLPCGSKVRFDNVEKAGGKGVVLSSEEEKPALPIGGNESIPGFRVAKSASAKVREAAANGELK
VRLG**TDL**KE**SLR**PSNKKD**QLT**ASSARGY**H**GTYGYTKPDVAAPGNNISSARVGTGTDG**ISY**TGT**S**MSAPFAAGVAAQ
VLQANQSYGPTQLKAAIMNSANHDV**RTAD**GNVYAVDRVGS**GRIDAKAA**AETKVLLYNADRPAQVSQTFGVLE**YAV**NE
GKQTLTREM**TVEN**FD**SHT**HTY**NI**SYAGSTDMPGVEFSLPSNITVNPGEKKNFTVTITIDPAAMEKTMDPAMEK**THNS**
VDPYGDGTEL**VPEQ**YRQFIASESGRILLTEGAATLRAPIHAAPK**PASAM**KVEGSSVEIPAGEHQANLKL**TG**TELNQR
GYK**LLGAF**EHGAS**IERT**SPVKLDVSSNAKANMQHVGAASTAPALKASGGNPNDG**LLAF**GISTWANWDVVSTENTFT
VNIDTDGNNRADYMLVTDRAK**GID**FP**IV**RLYGYKNGNLEQ**IA**YYPLNNAWGDTDTNMMDSNALVMAVPLKDLGLSAE
KTKDIKYSVSATTQYAWTNVSETGW**IN**YRPFDPK**LW**FSGTAATVPGFFADAP**SSEL**VAHRAEGATDV**KAL**FLHM**HNT**
TGDLSGLNGAAGNRAQVLEVTEQQQLDPAPSRFTDVPAENQFYAEINWLAQRRIT**TG**YPDG**TFR**PGENVERGAMAAY
FYRLAGTPQFTAPDNPTFS**DVP**KSHPFYKEIEWMAARGIT**TG**YGDG**TFR**PSASVNRDAMAAFFYRYAN**SPQFA**APAA
SPFKDVPANSQFYKEIAWLAEQGITKGWDDGTYR**PGE**PIHRDAMAAFLYRYSDKVLK

Supplemental Figure 3. Amino acid sequence of *R. aeria* BAV86562.1. In bold: domain cd07474 representing the peptidase S8 family domain in Vpr-like proteins. BAV86562.1 is not an ABC transporter protein as its name suggests, since it lacks the LSGGQ domains characteristic of the ABC transporter family. The highlighted D, S and H residues comprise the catalytic triad of the subtilisin enzyme.