Published in final edited form as:
Proteomics. 2011 October ; 11(19): 3935–3941. doi:10.1002/pmic.201100103.

A reference map of the membrane proteome of *Enterococcus faecalis*

Gianluca Maddalo[1], **Peter Chovanec**[2], **Filippa Stenberg-Bruzell**[3], **Hailyn V. Nielsen**[4], **Michael I. Jensen-Seaman**[2], **Leopold L. Ilag**[1], **Kimberly A. Kline**[4], and **Daniel O. Daley**[3]

[1]Department of Analytical Chemistry, Stockholm University, SE-106 91 Stockholm, Sweden

[2]Department of Biological Sciences, Duquesne University, Pittsburgh, PA 15282, USA

[3]Center for Biomembrane Research, Department of Biochemistry and Biophysics, Stockholm University, SE-106 91 Stockholm, Sweden

[4]Center for Women's Infectious Disease Research, Department of Molecular Microbiology, Washington University School of Medicine, 660 S. Euclid Ave., Campus Box 8230, Saint Louis, MO 63110-1093, USA

Abstract

Enterococcus faecalis is a gram-positive bacterium that is part of the indigenous microbiotica of humans and animals as well as an opportunistic pathogen. In this study we have fractionated the membrane proteome of *E. faecalis* and identified many of its constituents by mass spectrometry. We present BN-/SDS-PAGE reference maps that contain 102 proteins. These proteins are important for cellular homeostasis, virulence, and antibiotic intervention. Intriguingly, many proteins with no known function were also identified, indicating that there are substantial gaps in knowledge of this organism's biology. On a more limited scale we also provide insight into the composition of membrane protein complexes. This study is a first step toward elucidating the membrane proteome of *E. faecalis*which is critical for a better understanding of how this bacterium interacts with a host and with the extracellular *milieu*.

Keywords

Enterococcus faecalis; membrane proteome; protein complex; BN-PAGE; nano LC-ESI-MS/MS

Enterococci are gram-positive, facultatively anaerobic bacteria that inhabit the gastrointestinal tract of humans and animals [1, 2]. Although they are part of the indigenous microbiotica, enterococci are also opportunistic pathogens that can cause a variety of diseases (*e.g.* endocarditis, bacteremia, meningitis, wound infections, and urinary tract infections) and are one of the leading causes of nosocomial infections [1–3]. In the United States alone, an estimated 800,000 enterococcal infections occur each year [2]. These infections are predominantly caused by *Enterococcus faecalis* and *Enterococcus faecium* [1]. While relatively little is known about the pathogenic mechanisms of these organisms, it is known that many virulence proteins are localized in the cell envelope [4]. Some of these proteins can facilitate adherence to the host tissue, colonization, biofilm formation, evasion

Corresponding author: Daniel O. Daley (Associate Professor), Center for Biomembrane Research, Department of Biochemistry and Biophysics, Stockholm University, SE-106 91 Stockholm, Sweden. ddaley@dbb.su.se. Phone: +46-8-16 29 10 Fax: +46-8-15 36 79.

The mass spectrometry data set has been deposited into the PRIDE database (accession no. 17167, username: review18709, password: Kn2smN#B).

The authors declare no conflict of interests.

of the immune system, and antibiotic resistance [1, 2]. Some proteins embedded in the cell envelope are also essential for importing nutrients and maintaining cellular homeostasis [4–6] and, therefore, enable enterococci to colonize a wide range of niches. The importance of many cell envelope proteins for cellular homeostasis, pathogenesis and drug resistance makes them an obvious target for the development of novel therapeutics [7]. For these reasons, proteomics characterization of the *E. faecalis* cell envelope is of great interest. To this end, recent studies on secreted and surface proteins of *E. faecalis* have only identified 9 membrane embedded proteins (~1.5% of the predicted membrane embedded proteome) [8, 9].

The aim of this study was to gain insight into the composition of the membrane proteome of *E. faecalis*. We used *E. faecalis* OG1X, a laboratory strain commonly used as a model system [10]. The study was carried out using a proteomics platform that separates membrane proteins by three orthogonal principles, enabling identification by mass spectrometry [11] (Figure 1A). From three biological replicate experiments we detected 294 protein spots (Supplementary Figure 1). Analysis of these spots by nano LC-ESI-MS/MS (Supplementary Table 1 and 2) indicated that they corresponded to 102 unique proteins (Table 1). Bioinformatics analysis of the amino acid sequences for these proteins was carried out with SignalP3.0 [12] (to identify cleavable signal peptides), SCAMPI [13] (to identify transmembrane proteins), and PRED-LIPO [14] (to identify lipoproteins). This analysis predicted that 64 membrane embedded proteins, 9 lipoproteins and 29 soluble proteins (Figure 1B). A large fraction of the soluble proteins (16/29) were known from the literature as components of membrane protein complexes. Indeed, 11 of these aligned in a vertical channel with a membrane embedded protein in our study, thus justifying their localization at the membrane. To our knowledge, all other proteomics studies on *E. faecalis* have focused on the soluble sub-proteomes (*i.e.* cytoplasmic and surface proteins) [8, 9, 15–18]. This study therefore represents a first-step towards a reference map of the membrane embedded proteome of *E. faecalis* and complements current knowledge on the cytoplasmic and surface proteomes of this important nosocomial bacterium.

To approximate the depth-of-coverage we achieved, we downloaded the proteome of OG1RF [5] from the Pathosystems Resource Integration Centre (http://patricbrc.vbi.vt.edu/portal) and then generated a predicted membrane proteome using SCAMPI [13] (note that OG1RF contains essentially the same gene content as OG1X [10]). We then compared our list of experimentally identified membrane embedded proteins to the predicted membrane proteome. Using this measure, we estimate that we have experimentally identified ~10% of the membrane embedded proteome (~620 proteins). Retrospective analysis of replicate experiments (D, E and F in Figure 1A) indicated that the depth-of-coverage could be improved if narrower fractions from the anion exchange step were analyzed by BN-PAGE. This conclusion was based on the fact that most proteins were identified in the narrowest fraction and the fewest proteins from the largest fraction (61, 43, and 36 proteins in fractions F, D and E, respectively). The analysis also indicated that the experiment was reasonably reproducible, as fractions D and E gave 46% overlap (i.e. 25/54 proteins), even though they were not equal in size. Complete depth-of-coverage for bacterial membrane embedded proteomes is notoriously difficult to achieve [19–25]. Employing a single experimental approach usually identifies 10 – 20% of the predicted membrane embedded proteome (Supplementary Table 3). This can be improved using combinations of different approaches, however, no single study that we are aware of has exceeded 50% coverage of a membrane proteome.

To investigate protein functions in the membrane proteome, we examined the functional annotations of the identified proteins (taken from the homologous protein in the V583 strain [4] or the OG1RF strain [5]) (Supplementary Table 1). The majority of the identified

proteins are predicted to be involved in transport processes (Figure 1C, Table 1) and include 13 ABC-type transporters, 6 PTS-type transporters, 2 IT-type transporters, and 2 MFS-type transporters (data not shown). The genome sequence of *E. faecalis* V583 contains a large number of transport systems compared to other bacteria [4], and it was therefore not surprising that we detected so many. Intriguingly, a large fraction of the proteins that we identified had no known function (Figure 1C, Table 1). The presence of these proteins indicates that there is biology in the membrane of *E. faecalis* that we do not yet understand. We also identified 17 proteins that are potentially of biomedical interest, as they have been implicated by other studies to play roles in biofilm formation (Supplementary Table 4) and virulence (Supplementary Table 5). Together with approaches that address soluble and surface proteomes, our platform will be useful for understanding proteomes in pathogenic and antibiotic resistant strains of *E. faecalis*.

In addition to identification of single membrane constituents we were able to resolve membrane protein complexes. This was possible because we had utilized a mild detergent to extract the proteins from the membrane and native conditions to separate them during anion exchange chromatography and BN-PAGE. Membrane complexes resolved as a band in the non-denaturing BN-PAGE, and constituent proteins resolved as spots in a vertical channel in the denaturing BN-/SDS-PAGE (Figure 1A). For example, we could clearly recognize three ABC transporter complexes (Figure 2A – C), a mannose PTS transporter (Figure 2D), and the DivIVA-EzrA cell divison complex (Figure 2E). Unfortunately the composition of many complexes was difficult to assign from the gels without prior biological knowledge, as some vertical channels were clearly over-lapping (Figure 2F). The molecular mass of these complexes in the BN-PAGE indicated that the two complexes had the same electrophoretic mobility rather than being super-complexes. However, the data show that most proteins resolved at a higher molecular mass in the BN-PAGE than in the denaturing SDS-PAGE, indicating that they exist in a high molecular mass complex (Supplementary Figure 1). If no prior biological knowledge is available, then the vertical channels can simply be used to suggest a list of ‘putative’ interacting partners. As these data were generated from natively expressed proteins that are not tagged or over-expressed, it is complementary to other protein-protein interaction methodologies. We therefore believe that it will be useful to the scientific community for the prediction and/or confirmation of novel protein interactions at the membrane.

In conclusion, we have presented a first reference map of the membrane proteome of *E. faecalis* OG1X. This map contains proteins important for cell homeostasis, virulence, and antibiotic intervention. It also contains a large number of proteins with no known function, indicating that much is left to learn about the biology of this important sub-proteome. On a more limited scale, we were also able to provide insight into membrane protein complexes. Taken together, the information we have presented is a first step towards understanding how *E. faecalis* uses its membrane proteome to interact with the host and with the extracellular *milieu*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Scott Hultgren (Washington University in St. Louis) and Birgitta Henriques-Normark (Karolinska Institute) for technical assistance and support. This work was supported by grants from the Swedish Research Council and the NIH (R01 GM081827-01) to DOD.

Non-standard Abbreviations

ABC	ATP binding cassette
AEX	anion exchange
BN-PAGE	blue native-polyacrylamide gel electrophoresis
DDM	*n*-dodecyl-β-D-maltoside
IT	ion transport
MFS	major facilitator superfamily
PTS	phosphoenolpyruvate-dependent transferase system

References

1. Fisher K, Phillips C. The ecology, epidemiology and virulence of *Enterococcus*. Microbiology. 2009; 155:1749–1757. [PubMed: 19383684]
2. Tendolkar PM, Baghdayan AS, Shankar N. Pathogenic enterococci: new developments in the 21st century. Cell Mol Life Sci. 2003; 60:2622–2636. [PubMed: 14685687]
3. Hidron AI, Edwards JR, Patel J, Horan TC, et al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention. 2006–2007. Infect Control Hosp Epidemiol. 2008; 29:996–1011. [PubMed: 18947320]
4. Paulsen IT, Banerjei L, Myers GS, Nelson KE, et al. Role of mobile DNA in the evolution of vancomycin-resistant *Enterococcus faecalis*. Science. 2003; 299:2071–2074. [PubMed: 12663927]
5. Bourgogne A, Garsin DA, Qin X, Singh KV, et al. Large scale variation in *Enterococcus faecalis* illustrated by the genome analysis of strain OG1RF. Genome Biol. 2008; 9:R110. [PubMed: 18611278]
6. Scott JR, Barnett TC. Surface proteins of gram-positive bacteria and how they get there. Annu Rev Microbiol. 2006; 60:397–423. [PubMed: 16753030]
7. Hurdle JG, O'Neill AJ, Chopra I, Lee RE. Targeting bacterial membrane function: an underexploited mechanism for treating persistent infections. Nat Rev Microbiol. 2011; 9:62–75. [PubMed: 21164535]
8. Benachour A, Morin T, Hebert L, Budin-Verneuil A, et al. Identification of secreted and surface proteins from *Enterococcus faecalis*. Can J Microbiol. 2009; 55:967–974. [PubMed: 19898536]
9. Bohle LA, Riaz T, Egge-Jacobsen W, Skaugen M, et al. Identification of surface proteins in *Enterococcus faecalis* V583. BMC Genomics. 2011; 12:135. [PubMed: 21362161]
10. Ike Y, Craig RA, White BA, Yagi Y, Clewell DB. Modification of *Streptococcus faecalis* sex pheromones after acquisition of plasmid DNA. Proc Natl Acad Sci U S A. 1983; 80:5369–5373. [PubMed: 6412228]
11. Maddalo G, Stenberg Bruzell F, Gotzke H, Toddo S, et al. Systematic analysis of native membrane protein complexes in *Escherichia coli*. J Proteome Res. 2011; 10(4):1848–1859. [PubMed: 21210718]
12. Bendtsen JD, Nielsen H, von Heijne G, Brunak S. Improved prediction of signal peptides: SignalP 3.0. J Mol Biol. 2004; 340:783–795. [PubMed: 15223320]
13. Bernsel A, Viklund H, Falk J, Lindahl E, et al. Prediction of membrane-protein topology from first principles. Proc Natl Acad Sci U S A. 2008; 105:7177–7181. [PubMed: 18477697]
14. Bagos PG, Tsirigos KD, Liakopoulos TD, Hamodrakas SJ. Prediction of lipoprotein signal peptides in gram-positive bacteria with a Hidden Markov Model. J Proteome Res. 2008; 7:5082–5093. [PubMed: 19367716]
15. Bohle LA, Faergestad EM, Veiseth-Kent E, Steinmoen H, et al. Identification of proteins related to the stress response in *Enterococcus faecalis* V583 caused by bovine bile. Proteome Sci. 2010; 8:37. [PubMed: 20579342]

16. Giard JC, Laplace JM, Rince A, Pichereau V, et al. The stress proteome of *Enterococcus faecalis*. Electrophoresis. 2001; 22:2947–2954. [PubMed: 11565789]
17. Heim S, Lleo MM, Bonato B, Guzman CA, Canepari P. The viable but nonculturable state and starvation are different stress responses of *Enterococcus faecalis* as determined by proteome analysis. J Bacteriol. 2002; 184:6739–6745. [PubMed: 12426365]
18. Wang X, He X, Jiang Z, Wang J, et al. Proteomic analysis of the *Enterococcus faecalis* V583 strain and clinical isolate V309 under vancomycin treatment. J Proteome Res. 2010; 9:1772–1785. [PubMed: 20128627]
19. Bernsel A, Daley DO. Exploring the inner membrane proteome of *Escherichia coli*: which proteins are eluding detection and why? Trends Microbiol. 2009; 17:444–449. [PubMed: 19766000]
20. Eichacker LA, Granvogl B, Mirus O, Muller BC, et al. Hiding behind hydrophobicity. Transmembrane segments in mass spectrometry. J Biol Chem. 2004; 279:50915–50922. [PubMed: 15452135]
21. Fischer F, Poetsch A. Protein cleavage strategies for an improved analysis of the membrane proteome. Proteome Sci. 2006; 4:2. [PubMed: 16512920]
22. Santoni V, Molloy M, Rabilloud T. Membrane proteins and proteomics: un amour impossible? Electrophoresis. 2000; 21:1054–1070. [PubMed: 10786880]
23. Stenberg, F.; Daley, DO. Exploring membrane proteomes. In: Hagen, v, editor. Proteomics Sample Preparation. Wiley-VCH; 2008. p. 303-312.
24. Weiner JH, Li L. Proteome of the *Escherichia coli* envelope and technological challenges in membrane proteome analysis. Biochim Biophys Acta. 2008; 1778:1698–1713. [PubMed: 17904518]
25. Griffin NM, Schnitzer JE. Overcoming key technological challenges in using mass spectrometry for mapping cell surfaces in tissues. Mol Cell Proteomics. 2011; 10:R110 000935. [PubMed: 20548103]

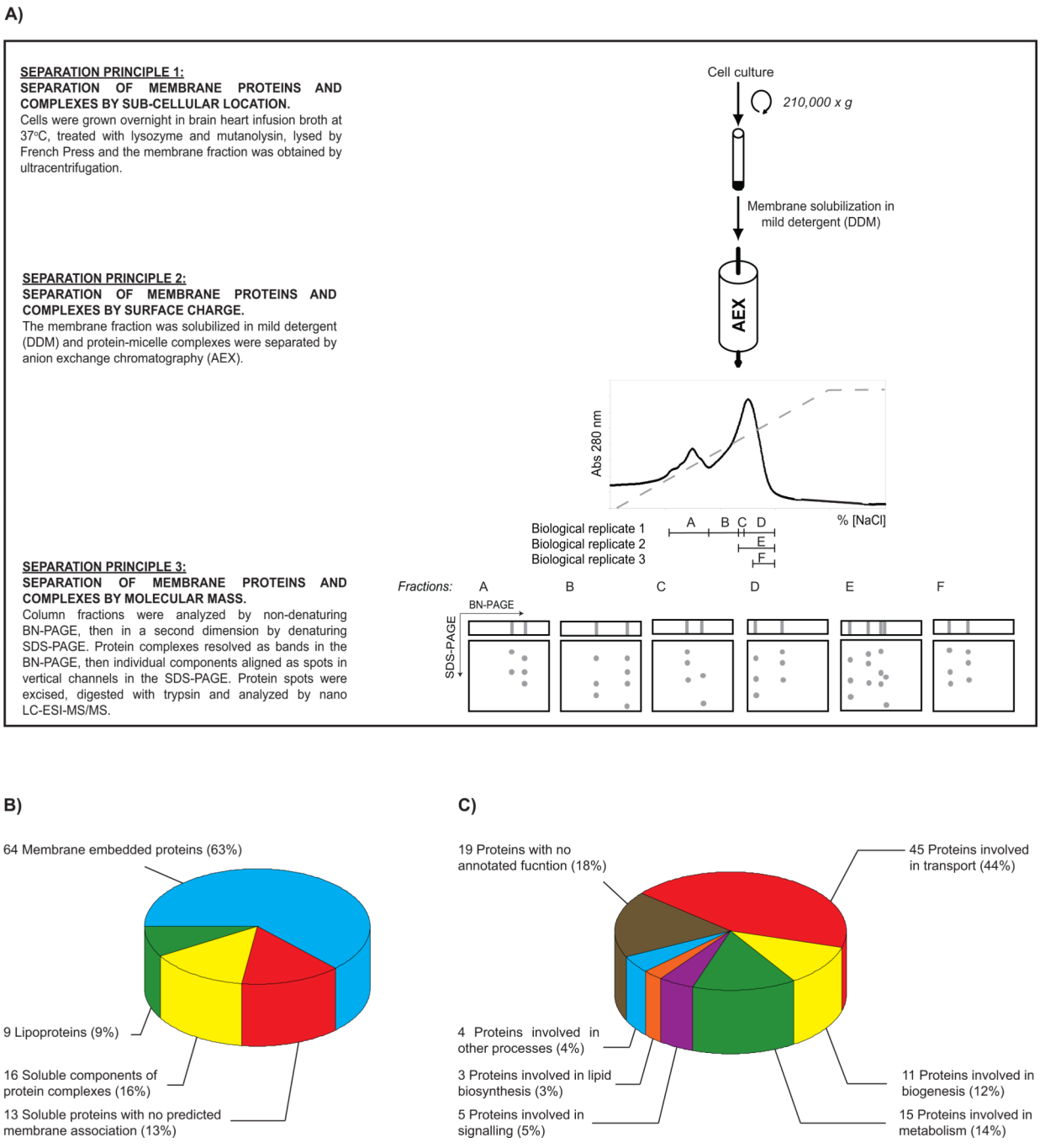

Figure 1. A birds-eye view of the membrane proteome of *E. faecalis* OG1X

(A) An overview of the methodology used in this study. A detailed description of all methodology is available in Supplementary Materials and Methods. Identified proteins were classified by (B) cellular location and (C) function.

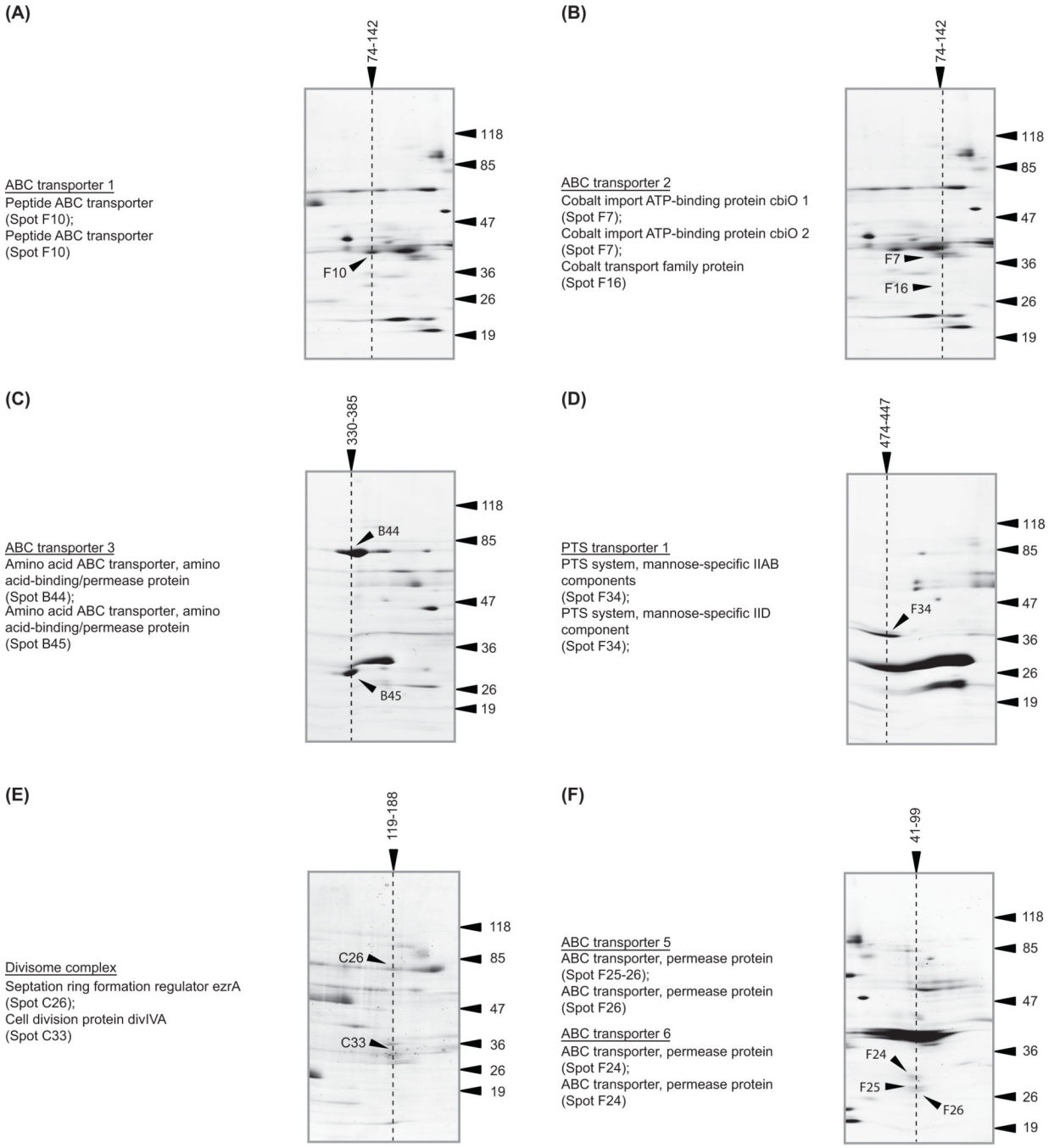

Figure 2. Examples of membrane protein complexes that were identified

Cropped sections from the BN-/SDS-PAGE showing ‘vertical channels’ that contain proteins in known complexes. Only spots in a precise vertical channel can be part of the same complex. For the sake of clarity we have omitted annotations for proteins that are in close (but unrelated) channels. These spots were identified and are shown in Supplementary Figure 1. Soluble and membrane proteins were used to calibrate the protein complexes in the BN-PAGE (Supplementary Materials and Methods). As these two independent sets of proteins give significantly different calibration curves, we have calculated the molecular mass for each protein complex using both curves and reported a molecular mass range. All molecular mass markers are in kDa.

Table 1

Unique proteins found in this study. Salient features of mass spectrometry experiments are contained in Supplementary Table 1. Peptides matched in Mascot searches are listed in Supplementary Table 2. Fragmentation spectra for single-peptide protein indentifications are shown in Supplementary Figure 2.

Protein name	Locus in *Enterococcus faecalis* OG1RF *(a)*	Cellular location *(b)*	TMs *(c)*	Type *(d)*
Proteins involved in transport				
Peptide ABC transporter	Contig 35 (24586–25509)	M	6	ABC1
Peptide ABC transporter	Contig 35 (26499–27443)	S	0	ABC1
Peptide ABC transporter	Contig 35 (27451–28450)	S	0	ABC1
Cobalt import ATP-binding protein CbiO 1	Contig 10 (31446–32285)	S	0	ABC2
Cobalt import ATP-binding protein CbiO 2	Contig 10 (30601–31476)	S	0	ABC2
Cobalt transport family protein	Contig 10 (29807–30604)	M	5	ABC2
Amino acid ABC transporter	Contig 5 (209776–210513)	S	0	ABC3
Amino acid ABC transporter	Contig 5 (211684–212683)	M	6	ABC3
ABC transporter, ATP-binding protein	Contig 10 (72643–73276)	S	0	ABC4
ABC transporter, permease protein	Contig 10 (71091–72090)	M	7	ABC4
ABC transporter	Contig 27 (10556–10969)	M	5	ABC5
ABC transporter	Contig 27 (10974–11849)	M	6	ABC5
ABC transporter	Contig 36 (11529–12271)	L	0	ABC6
ABC transporter	Contig 36 (12552–13460)	M	5	ABC6
ABC transporter	Contig 36 (13487–14425)	M	4	ABC6
Amino acid ABC transporter	Contig 10 (23469–24468)	M	3	ABC7
ABC transporter	Contig 5 (242535–243096)	M	5	ABC8
ABC transporter	Contig 5 (243812–244811)	M	5	ABC8
Peptide ABC transporter	Contig 4 (9909–10908)	L	0	ABC9
Phosphate import ATP-binding protein pstB 1	Contig 8 (185123–185881)	S	0	ABC10
Phosphate import ATP-binding protein pstB 2	Contig 8 (184303–185112)	S	0	ABC10
Cell division ABC transporter	Contig 8 (180445–181329)	M	4	ABC11
ABC transporter	Contig 27 (23767–24766)	M	5	ABC12
ABC transporter	Contig 27 (25491–26490)	M	6	ABC12
Glycine betaine/L-proline ABC transporter	Contig 32 (97401–99107)	M	7	ABC13
PTS system, mannose-specific IIAB components	Contig 11 (5988–6947)	S	0	PTS1
PTS system, mannose-specific IIC component	Contig 11 (7015–7818)	M	7	PTS1
PTS system, mannose-specific IID component	Contig 11 (7841–8752)	M	5	PTS1
PTS system, fructose-specific family, IIABC components	Contig 5 (165638–166637)	M	8	PTS2
PTS family glucose porter, IICBA component	Contig 3 (52672–53671)	M	9	PTS3
PTS system, IIC component	Contig 7 (40824–41823)	M	8	PTS4
PTS system, IIC component	Contig 3 (73328–74327)	M	10	PTS5
PTS system, IID component	Contig 8 (141368–142177)	M	5	PTS6
C4-dicarboxylate transporter, putative	Contig 11 (97235–98159)	M	8	IT1
Citrate transporter	Contig 23 (2321–3320)	M	10	IT2

Protein name	Locus in *Enterococcus faecalis* OG1RF [(a)]	Cellular location [(b)]	TMs [(c)]	Type [(d)]
Major facilitator family transporter	Contig 11 (65570–66569)	M	11	MFS1
Amino acid permease family protein	Contig 37 (11438–12437)	M	12	MFS2
Amino acid permease family protein	Contig 5 (76782–77781)	M	11	
Formate/nitrite transporter family protein	Contig 41 (2254–3057)	M	5	
Mn2+/Fe2+ transporter, NRAMP family	Contig 13 (12302–13301)	M	12	
Na+/H+ antiporter	Contig 26 (63390–64389)	M	11	
Cation-transporting ATPase, E1–E2 family	Contig 8 (39552–40551)	M	8	
Large-conductance mechanosensitive channel	Contig 35 (74751–75200)	M	2	
V-type ATPase, subunit K	Contig 3 (30122–30595)	M	3	
V-type ATPase, subunit I	Contig 3 (28115–29114)	M	8	
Proteins involved in biogenesis				
Preprotein translocase, SecY subunit	Contig 10 (39044–40043)	M	9	
OG1RF_Possible O-antigen polymerase	Contig 30 (31004–32003)	M	12	
Septation ring formation regulator ezrA	Contig 26 (26980–27979)	M	1	
Cell division protein DivIVA	Contig 7 (57857–58558)	S	0	
Penicillin-binding protein 4	Contig 32 (211716–212715)	S	0	
Penicillin-binding protein 2A	Contig 5 (126101–127100)	M	1	
Penicillin-binding protein 2B	Contig 18 (13934–14933)	M	1	
Preprotein translocase, YajC subunit	Contig 4 (20567–20944)	M	1	
Amidase, putative	Contig 5 (187646–188645)	S	0	
DltD protein	Contig 17 (5604–6603)	S	0	
Foldase protein prsA	Contig 5 (134096–134800)	L	0	
Proteins involved in metabolism				
ATP synthase subunit alpha	Contig 32 (122707–123706)	S	0	
ATP synthase subunit beta	Contig 32 (125269–126268)	S	0	
ATP synthase gamma chain	Contig 32 (124279–125187)	S	0	
ATP synthase epsilon chain	Contig 32 (126692–127111)	S	0	
ATP synthase subunit b	Contig 32 (121619–122149)	M	1	
Oxidoreductase, pyridine nucleotide-disulfide family	Contig 34 (7771–8770)	M	5	
Formate acetyltransferase	Contig 39 (81299–82298)	S	0	
Enolase	Contig 8 (18945–19944)	S	0	
Glucose-6-phosphate isomerase	Contig 3 (25333–26332)	S	0	
Ornithine carbamoyltransferase	Contig 11 (94338–95337)	S	0	
Decarboxylase	Ccontig 5 (74669–75668)	S	0	
2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	Contig 10 (58445–59131)	S	0	
Glyceraldehyde 3-phosphate dehydrogenases	Contig 8 (15688–16687)	S	0	
Thiamin biosynthesis lipoprotein ApbE	Contig 34 (5310–6309)	L	0	
Fumarate reductase flavoprotein subunit	Contig 32 (179927–180597)	L	0	
Proteins involved in signalling				
Probable protease eep	Contig 38 (3317–4016)	M	4	
Serine/threonine-protein kinase PrkC	Contig 35 (43308–44307)	M	1	

Protein name	Locus in *Enterococcus faecalis* OG1RF [(a)]	Cellular location [(b)]	TMs [(c)]	Type [(d)]
Sensor histidine kinase	Contig 7 (797–1796)	M	2	
Pheromone cAD1 lipoprotein	Contig 34 (6564–7272)	L	0	
Pheromone binding protein	Contig 28 (61901–62900)	L	0	
Proteins involved in lipid biosynthesis				
Glycerophosphoryl diester phosphodiesterase family protein	Contig 8 (75630–76629)	M	9	
(3R)-hydroxymyristoyl-[acyl-carrier-protein] dehydratase 1	Contig 43 (1042–1476)	S	0	
(3R)-hydroxymyristoyl-[acyl-carrier-protein] dehydratase 2	Contig 18 (35776–36201)	S	0	
Proteins involved in other processes				
Regulatory protein pfoR, putative	Contig 11 (86464–86967)	M	8	
Transcriptional regulator, PSR protein	Contig 39 (33224–33935)	M	1	
Transcriptional regulator lytR	Contig 1 (102661–103572)	S	0	
Bacterial sugar transferase	Contig 30 (42555–43341)	M	5	
Proteins with no annotated function				
hypothetical protein EF2929	Contig 27 (32738–33728)	M	11	
Putative uncharacterized protein	Contig 39 (151365–151832)	M	1	
Membrane protein	Contig 11 (20435–21434)	M	12	
hypothetical protein OG1RF_0150 [Enterococcus faecalis OG1RF]	Contig 30 (18903–19902)	M	10	
Membrane protein	Contig 30 (1279–1750)	M	10	
Putative uncharacterized protein	Contig 15 (106536–106931)	M	3	
YitT family protein	Contig 39 (20040–20923)	M	6	
Putative uncharacterized protein	Contig 27 (19139–19838)	M	1	
Putative uncharacterized protein	Contig 15 (54450–55181)	M	3	
Putative uncharacterized protein	Contig 18 (7102–7677)	M	4	
Putative uncharacterized protein	Contig 8 (194308–194714)	M	1	
conserved hypothetical protein [Enterococcus faecalis ATCC 29200]	Contig 32 (88786–89658)	M	12	
Phage infection protein	Contig 4 (65550–68213)	M	6	
PIN domain protein	Contig 11 (28478–29477)	M	4	
Cell wall surface anchor family protein	Contig 35 (105859–106858)	M	1	
Putative uncharacterized protein	Contig 30 (12932–13931)	S	0	
Putative uncharacterized protein	Contig 1 (61273–61944)	S	0	
Basic membrane protein family	Contig 10 (73934–74933)	L	0	
Basic membrane protein family	Contig 10 (75061–76060)	L	0	

*(a)*As the OG1X sequence is not publically available we verified the location of each protein in the closely related OG1RF strain (http://blast.hgsc.bcm.tmc.edu/blast.hgsc?organism=EfaecalisOG1).

*(b)*Membrane (M), soluble (S), lipoprotein (L).

*(c)*The number of predicted transmembrane helices for each protein was obtained by analyzing the amino acid sequence with SCAMPI (http://scampi.cbr.su.se/) and SignalP 3.0 (http://www.cbs.dtu.dk/services/SignalP/).

*(d)*Type of transporter: ABC, amino acid binding cassette; PTS, phosphoenolpyruvate:sugar phosphotransferase system; IT, ion transporter; MFS, major facilitator superfamily. Transporters were grouped according to the Transporter Classification Database (http://www.tcdb.org/superfamily.php) and numbered arbitrarily.