OptiPrep™ Reference List RS11

Extracellular vesicles from non-mammalian sources

1. Introduction

It is widely recognized that mammalian cells, bacteria, algae and fungi release extracellular vesicles into the surrounding medium; these vesicles are involved in communication between cells and the delivery of biologically and clinically important molecules to other cells. With regard to bacteria the term "extracellular vesicles" (EVs) covers the outer membrane vesicles (OMVs) produced by Gram-negative bacteria and the membrane vesicles (MVs) produced by Gram-positive bacteria and other organisms such as algae and fungi. In all cases; EVs are distinct from the intracellular vesicles present in the cytoplasm. The OMVs from Gram-negative bacteria in particular are widely researched and have been shown to be important in the transfer of virulence factors and the initiation of immune and inflammatory responses in host cells.

- **Section 2** briefly reviews the current OptiPrep™-based methodology; this is described in detail in **Application Sheet S60**, which has its own short reference list.

- The principal aim of this Mini-Review however is to provide a bibliography of all the published papers that have reported the use of an iodixanol gradient. Published papers have been divided into the flowing sections:
- **Section 3a: Gram –ve bacteria**
- **Section 3b: Gram +ve bacteria and mycobacteria**
- **Section 3c: Bacterial exosomes derived from human fluids and waste water**
- **Section 3d: *Haloarchaea* (halobacteria)**
- **Section 3e: Biofilm structures**
- **Section 3f: Mycoplasmas**
- **Section 3g: Protozoa, algae, fungi and archaea**
- **Section 3h: Invertebrates**
- **Section 3i: Trematodes**
- **Section 3j: Nematodes**
- **Section 3k: Arthropoda**
- **Section 3l: Plants**

- **Section 4: References to methodological and functional reviews**

- **All references are listed alphabetically according to first author**

Related research areas that have reported the use of gradients prepared from OptiPrep™ are:

- The analysis of the microvesicles that are expressed from the surface of mammalian cells is covered in **OptiPrep™ Reference List RS10 and OptiPrep™Application Sheet S63**
- The control and organization of membrane trafficking within mammalian cells that permits the movement of vesicles to, and ultimately their fusion with, the plasma membrane or a specific plasma membrane domain is covered in **OptiPrep™ Application Sheet S47**

- These and other **OptiPrep™ Reference Lists** or **OptiPrep™ Application Sheets** can be accessed via the relevant OptiPrep™ Index from the following website: www.Optiprep.com, click on "**Reference Lists**" or "**Methodology**" respectively.

2. Methodological summary

Various forms of pre-gradient processing are employed, during which intact bacteria and aggregated material in the culture medium are mostly removed and the EVs concentrated. This is covered in much greater detail in **Application Sheet S62**

The first step is clarification of the bacterial broth to remove intact cells by centrifugation; the time and *g*-force used varies widely and reflects the size of the organism. Generally rcfs of approx. 10,000 *g* are used for 10-20 min, but there are examples both of lower and higher *g*-forces so that the range spans 4,000-12,000 *g*. Occasionally this first step is carried out in two stages in which the broth is centrifuged for 30 min at 5,000 *g* and then the supernatant at 7,500 *g*. This strategy may minimize the entrapment (and consequent loss) of vesicles into the pellet by the rapidly sedimenting much larger bacteria. Fungal cells appear to sediment satisfactorily at lower speeds such as 2,500 *g* for 10 min.

Commonly the second step is volume reduction since ultimately the vesicles will be sedimented in a fixed-angle ultracentrifuge rotor, prior to gradient purification. Large volumes of clarified medium can pose a problem for this final pre-gradient step. The Beckman 45Ti for example which accommodates 6x94 ml tubes has a maximum *g*-force of 235,000 *g*, but the vesicles at the top of the sample experience initially only 80,000 *g*. Tangential filtration devices (e.g. 70-100 kDa cut-off) are popular or centrifugal filters are popular for this volume reduction step.

Residual bacteria in the broth may then be removed by vacuum filtration through either a 0.45 or 0.22 μm filter or both pore size filters sequentially, before the EVs are sedimented in a fixed-angle rotor; the conditions vary quite widely, from approx. 40,000 *g* for 1 h to 140-150,000 *g* for 2-3 h..

Ammonium sulphate precipitation of OMVs from the clarified broth of Gram-ve bacteria has been used a few cases. The precipitation process may only require about half an hour at 4°C but can be as long as overnight. Moreover the resuspended pellet requires dialysis overnight prior to further processing.

Iodixanol gradient purification of the EVs, regardless of the pre-gradient technology, involves adjustment of the suspension to a density of approx. 1.241 g/ml (sometimes higher 1.267 g/ml or lower 1.215 g/ml).for subsequent flotation through a discontinuous iodixanol gradient; During centrifugation at 100-200,000 *g* for 12-16 h the gradient will become more or less continuous; gradients run for shorter times (e.g. 2 h) will retain some of their discontinuous nature.

The density of MVs in iodixanol gradients is generally >1.11 g/ml, many banding between 1.13 and 1.15 g/ml; although occasionally densities of as a high as 1.20 g/ml have been observed. There is also evidence of heterogeneity amongst EV populations from a single bacterial type.

The first published paper to describe the use of OptiPrep™, of which we have a record, was by Horstman and Kuehn, published in 2000. It documented the purification of OMVs from enterotoxin-containing *Escherichia coli*; the OMVs were shown to contain the pathogenic enterotoxin, lipids and specific proteins characteristic of the outer membrane and proteins from the periplasmic space, but no markers of the cytosol or inner membrane (**see Section 3a - *Escherichia coli***).

3. Bibliography of publications reporting analytical studies on iodixanol-purified OMVs and MVs

References are sorted alphabetically according to first author. Research topics are highlighted in blue in the titles.

3a. Gram-negative bacteria

Acetobacter pasteurianus

Hashimoto, M., Matsumoto, T., Tamura-Nakano, M., Ozono, M., Hashiguchi, S. and Suda, Y. (2018) *Characterization of outer membrane vesicles of Acetobacter pasteurianus NBRC3283* J.Biosci. Bioeng., **125**, 425-431

Acinetobacter baumannii

Chatterjee, S., Mondal, A., Mitra, S. and Basu, S. (2017) *Acinetobacter baumannii transfers the bla_{NDM-1} gene via outer membrane vesicles* J. Antimicrob. Chemother., **72**, 2201–2207

Marion, C.R., Lee, J., Sharma, L., Park, K-S., Lee, C., Liu, W., Liu, P., Feng, J., Gho, Y.S. and Dela Cruz, C.S. (2019) *Toll-like receptors 2 and 4 modulate pulmonary inflammation and host factors mediated by outer membrane vesicles derived from Acinetobacter baumannii* Infect. Immun. **87**: e00243-19

Wachino, J-i., Jin, W., Kimura, K. and Arakawa, Y. (2019) *Intercellular transfer of chromosomal antimicrobial resistance genes between Acinetobacter baumannii strains mediated by prophages* Antimicrob. Agents Chemother., **63**: e00334-19

Aggregatibacter actinomycetemcomitans

Kieselbach, T. and Oscarsson, J. (2017) *Dataset of the proteome of purified outer membrane vesicles from the human pathogen Aggregatibacter actinomycetemcomintans* Data in Brief, **10**, 426-431

Rompikuntal, P.K., Thay, B., Khan, M.K., Alanko, J., Penttinen, A-M., Asikainen, S., Wai, S.N. and Oscarsson, J. (2012) *Perinuclear localization of internalized outer membrane vesicles carrying active cytolethal distending toxin from Aggregatibacter actinomycetemcomitans* Infect. Immun., **80**, 31-42

Alteromonas

Biller, S.J., McDaniel, L.D., Breitbart, M., Rogers, E., Paul, J.H. and Chisholm, S.W. (2017) *Membrane vesicles in sea water: heterogeneous DNA content and implications for viral abundance estimates* ISME J., **11**, 394–404

Bacteroides vulgaris

Maerz, J.K., Steimle, A., Lange, A., Bender, A., Fehrenbacher, B. and Frick, J-S. (2018) *Outer membrane vesicles blebbing contributes to B. vulgatus mpk-mediated immune response silencing* Gut Microbes., **9**(1), 1–12

Borrelia burgdorferi

Coleman, J.L., Crowley, J.T., Toledo, A.M. and Benach, G.L. (2013) *The HtrA protease of Borrelia burgdorferi degrades outer membrane protein BmpD and chemotaxis phosphatase CheX* Mol. Microbiol., **88**, 619–633

Crowley, J.T., Toledo, A.M., LaRocca, T.J., Coleman, J.L., London, E. and Benach, J.L. (2013) *Lipid exchange between Borrelia burgdorferi and host cells* PLoS Pathog., **9**: e1003109

Toledo, A., Coleman, J.L., Kuhlow, C.J., Crowley, J.T. and Benach, J.L. (2012) *The enolase of Borrelia burgdorferi is a plasminogen receptor released in outer membrane vesicles* Infect. Immun., **80**, 359-368

Burkholderia glumae

Kang. Y., Goo, E., Kim, J. and Hwang, I. (2017) *Critical role of quorum sensing-dependent glutamate metabolism in homeostatic osmolality and outer membrane vesiculation in Burkholderia glumae* Sci. Rep., **7**: 44195

Burkholderia pseudomallei

Nieves, W., Heang, J., Asakrah, S., Höner zu Bentrup, K., Roy, C.J. and Morici, L.A. (2010) *Immuno-specific responses to bacterial elongation factor Tu during Burkholderia infection and immunization* PloS One **5**: e14361

Campylobacter jejuni

Jang, K-S., Sweredoski, M.J., Graham, R.L.J., Hess, S. and Clemons Jr., W.M. (2014) *Comprehensive proteomic profiling of outer membrane vesicles from Campylobacter jejuni* J. Proteom., **98**, 90-98

Edwardsiella tarda

Chen, S., Yang, D., Wen, Y., Jiang, Z., Zhang, L., Jiang, J., Chen, Y., Hu, T., Wang, Q., Zhang, Y. and Liu, Q. (2018) *Dysregulated hemolysin liberates bacterial outer membrane vesicles for cytosolic lipopolysaccharide sensing* PLoS Pathog., **14**: e1007240

Edwardsiella piscicida

Wen, Y., Chen, S., Jiang, Z., Wang, Z., Tan, J., Hu, T., Wang, Q., Zhou, X., Zhang, Y., Liu, Q. and Yang, D. (2019) *Dysregulated haemolysin promotes bacterial outer membrane vesicles-induced pyroptotic-like cell death in zebrafish* Cell. Microbiol., **21**: e13010

Escherichia coli

Balsalobre, C., Silvan, J.M., Berglund, S., Mizunoe, Y., Uhlin, B.E. and Wai, S.N. (2006) *Release of the type I secreted α-haemolysin via outer membrane vesicles from Escherichia coli* Mol. Microbiol., **59**, 99-112

Bielaszewska, M., Ruter, C., Kunsmann, L., Greune, L., Bauwens, A., Zhang, W., Kuczius, T., Kim, K.S., Mellmann, A., Schmidt, M.A. and Karch, H. (2013) *Enterohemorrhagic Escherichia coli hemolysin employs outer membrane vesicles to target mitochondria and cause endothelial and epithelial apoptosis* PloS Pathog., **9**: e1003797

Bielaszewska, M., Ruter, C., Kunsmann, L., Greune, L., Bauwens, A., Zhang, W., Kuczius, T., Kim, K.S., Mellmann, A., Schmidt, M.A. and Karch, H. (2013) *Enterohemorrhagic Escherichia coli hemolysin employs outer membrane vesicles to target mitochondria and cause endothelial and epithelial apoptosis* PloS Pathog., **9**: e1003797

Bielaszewska, M., Rüter, C., Bauwens, A., Greune, L., Jarosch, K-A., Steil, D., Zhang, W., He, X. et al (2017) *Host cell interactions of outer membrane vesicle-associated virulence factors of enterohemorrhagic Escherichia coli O157: Intracellular delivery, trafficking and mechanisms of cell injury* PloS Pathog., **13**: e1006159

Bielaszewska, M., Marejková, M., Bauwens, A., Kunsmann-Prokscha, L., Mellmann, A. and Karch, H. (2018) *Enterohemorrhagic Escherichia coli O157 outer membrane vesicles induce interleukin 8 production in human intestinal epithelial cells by signaling via Toll-like receptors TLR4 and TLR5 and activation of the nuclear factor NF-κB* Int. J, Med. Microbiol., **308**, 882–889

Blenkiron, C., Simonov, D., Muthukaruppan, A., Tsai, P., Dauros, P., Green, S., Hong, J., Print, C.G., Swift, S. and Phillips, A.R. (2016) *Uropathogenic Escherichia coli releases extracellular vesicles that are associated with RNA* PLoS One, **11**, e0160440

Chen, L., Valentine, J.L., Huang, C-Jr., Endicott, C.E., Moeller, T.D., Rasmussen, J.A., Fletcher, J.R., Boll, J.M. et al (2016) *Outer membrane vesicles displaying engineered glycotopes elicit protective antibodies* Proc. Natl. Acad. Sci. USA, **113**, E3609–E3618

Daleke-Schermerhorn, M.H., Felix, T., Soprova, Z., ten Hagen-Jongman, C.M., Vikström, D., Majlessi, L., Beskers, J., Follmann, F., de Punder, K., van der Wel, N.N. et al (2014) *Decoration of outer membrane vesicles with multiple antigens by using an autotransporter approach* Appl, Environ. Microbiol., **80**, 5854–5865

Davis, J.M., Carvalho, H.M., Rasmussen, S.B. and O'Brien, A.D. (2006) *Cytotoxic necrotizing factor type 1 delivered by outer membrane vesicles of uropathogenic Escherichia coli attenuates polymorphonuclear leukocyte antimicrobial activity and chemotaxis* Infect. Immun., 74, 4401-4408

Ficurilli, M., Liu, C., Riviello, C., Pozo, M.J. and Meers, P.R. (2015) *Delivery of liposomal contents to outer membrane vesicles from Gram negative bacteria* Biophys. J. **108 (Suppl.1)**, 408a

Ghosal, A., Upadhyaya, B.B., Fritz, J.V., Heintz-Buschart, A., Desai, M.S., Yusuf, D., Huang, D. et al (2015) *The extracellular RNA complement of Escherichia coli* Microbiol.Open, **4,** 252–266

Hong, J., Dauros-Singorenko, P., Whitcombe, A., Payne, L., Blenkiron, C., Phillips, A. and Swift, S. (2019) *Analysis of the Escherichia coli extracellular vesicle proteome identifies markers of purity and culture conditions* J. Extracell. Ves., 8: 1632099

Horstman, A.L. and Kuehn, M.J. (2000) *Enterotoxigenic Escherichia coli secretes active heat-labile enterotoxin via outer membrane vesicles* J. Biol. Chem., 275, 12489-12496

Kesty, N.C. and Kuehn, M.J. (2004) *Incorporation of heterologous outer membrane and periplasmic proteins into Escherichia coli outer membrane vesicles* J. Biol. Chem., **279**, 2069-2076

Kim, J-Y., Doody, A.M., Chen, D.J., Cremona, G.H., Shuler, M.L., Putnam, D. and DeLisa, M.P. (2008) *Engineered bacterial outer membrane vesicles with enhances functionality* J. Mol. Biol., **380**, 51-66

Kim, O.Y., Choi, S.J., Jang, S.C., Park, K-S., Kim, S.R., Choi, J.P., Lim, J.H., Lee, S-W. et al (2015) *Bacterial protoplast-derived nanovesicles as vaccine delivery system against bacterial infection* Nano Lett. **15**, 266−274

Kim, O.Y., Dinh, N.T.H., Park, H.T., Choi, S.J., Hong, K. and Gho, Y.S. (2017) *Bacterial protoplast-derived nanovesicles for tumor targeted delivery of chemotherapeutics* Biomaterials, **113**, 68-79

Kim, O.Y., Park, H.T., Dinh, N.T.H., Choi, S.J., Lee, J., Kim, J.H., Lee, S-W. and Gho, Y.S. (2017) *Bacterial outer membrane vesicles suppress tumor by interferon-γ-mediated antitumor response* Nat. Comm., **8**: 626

Kunsmann, L., Rüter, C., Bauwens, A., Greune, L., Glüder, M., Kemper, B., Fruth, A., Wai, S.N., He, X., Lloubes, R. et al (2015) *Virulence from vesicles: Novel mechanisms of host cell injury by Escherichia coli O104:H4 outbreak strain* Sci. Rep., **5**: 13252

McBroom, A.J., Johnson, A.P., Vemulapalli, S. and Kuehn, M.J. (2006) *Outer membrane vesicle production by Escherichia coli is independent of membrane instability* J. Bacteriol., **188**, 5385-5392

McBroom, A.J. and Kuehn, M.J. (2007) *Release of outer membrane vesicles by Gram-negative bacteria is a novel envelope stress response* Mol. Microbiol., **63**, 545-558

Park, M., Sun, Q., Liu, F., DeLisa, M.P. and Chen, W. (2014) *Positional assembly of enzymes on bacterial outer membrane vesicles for cascade reactions* PloS One, **9**: e97103

Roier, S., Zingl, F.G., Cakar, F., Durakovic, S., Kohl, P., Eichmann, T.O., Kiug, L., Gadermaier, B. et al (2016) *A novel mechanism for the biogenesis of outer membrane vesicles in Gram-negative bacteria* Nat. Comm., **7**: 10515
Roy, K., Hamilton, D.J., Munson, G.P. and Fleckenstein, J.M. (2011) *Outer membrane vesicles induce immune responses to virulence proteins and protect against colonization by enterotoxigenic Escherichia coli* Clin. Vaccine Immunol., **18**, 1803–1808
Tiana, Y., Chen, C., Niu, Q., Zhu, S. and Yan, X. (2019) *Analysis of fluorescent labelling efficiency of extracellular vesicles derived from different kingdoms of life with lipid-binding dyes via nano-flow cytometry* J. Extracell. Ves., **8** (Suppl.1) PF06.06
Valentine, J.L., Chen, L., Perregaux, E.C., Weyant, K.B., Rosenthal, J.A., Heiss, C., Azadi, P., Fisher, A.C., Putnam, D. et al (2016) *Immunization with outer membrane vesicles displaying designer glycotopes yields class-switched, glycan-specific antibodies* Cell Chem. Biol., **23**, 655–665
Zhao, H. and Martinis, S.A. (2017) *Isolation of bacterial compartments to track movement of protein synthesis factors* Methods, **113**, 120–126

Flavobacterium columnare
Laanto, E., Penttinen, R.K., Bamford, J.K.H. and Sundberg, L-R. (2014) *Comparing the different morphotypes of a fish pathogen - implications for key virulence factors in Flavobacterium columnare* BMC Microbiol., **14**:170

Francisella novicida
McCaig, W.D., Koller, A. and Thanassi, D.G. (2013) *Production of outer membrane vesicles and outer membrane tubes by Francisella novicida* J. Bacteriol., **195**, 1120-1132

Francisella tularensis
Chen, L., Valentine, J.L., Huang, C-Jr., Endicott, C.E., Moeller, T.D., Rasmussen, J.A., Fletcher, J.R., Boll, J.M. et al (2016) *Outer membrane vesicles displaying engineered glycotopes elicit protective antibodies* Proc. Natl. Acad. Sci. USA, **113**, E3609–E3618
Chen, F., Cui, G., Wang, S., Nair, M.K.M., He, L., Qi, X., Han, X., Zhang, H., Zhang, J-R. and Su, J (2017) *Outer membrane vesicle-associated lipase FtlA enhances cellular invasion and virulence in Francisella tularensis LVS* Emerg. Microbes Infect., **6**: e66
Sampath, V., McCaig, W.D. and Thanassi, D.G. (2018) *Amino acid deprivation and central carbon metabolism regulate the production of outer membrane vesicles and tubes by Francisella* Mol.Microbiol., **107**, 523–541

Fusobacterium nucleatum
Liu, J., Hsieh, C-L., Gelincik, O., Devolder, B., Sei, S., Zhang, S., Lipkin, S.M. and Chang, Y.F. (2019) *Proteomic characterization of outer membrane vesicles from gut mucosa derived Fusobacterium nucleatum* J. Proteom., **195**, 125–137

Haemophilus influenzi
Roier, S., Blume, T., Klug, L., Wagner, G.E., Elhenawy, W., Zangger, K., Prassl, R., Reidl, J., Daum, G., Feldman, M.F. and Schild, S. (2015) *A basis for vaccine development: comparative characterization of Haemophilus influenzae outer membrane vesicles* Int. J. Med. Microbiol., **305**, 298–309
Roier, S., Zingl, F.G., Cakar, F., Durakovic, S., Kohl, P., Eichmann, T.O., Kiug, L., Gadermaier, B. et al (2016) *A novel mechanism for the biogenesis of outer membrane vesicles in Gram-negative bacteria* Nat. Comm., **7**: 10515
Sharpe, S.W., Kuehn, M.J. and Mason, K.M. (2011) *Elicitation of epithelial cell-derived immune effectors by outer membrane vesicles of non-typeable haemophilus influenzae* Infect. Immun., **79**, 4361-4369

Haemophilus parasuis
McCaig, W.D., Loving, C.L., Hughes, H.R. and Brockmeier, S.L. (2016) *Characterization and vaccine potential of outer membrane vesicles produced by Haemophilus parasuis* PLoS One **11**: e0149132

Helicobacter pylori
Choi, H-I., Choi, J-P., Seo, J., Kim, B.J., Rho, M., Han, J.K. and Kim, J.G. (2017) *Helicobacter pylori-derived extracellular vesicles increased in the gastric juices of gastric adenocarcinoma patients and induced inflammation mainly via specific targeting of gastric epithelial cells* Exp. Mol. Med., **49**, e330
Liu, Q., Li, X., Zhang, Y., Song, Z., Li, R., Ruan, H. and Huang, X. (2019) *Orally-administered outer-membrane vesicles from Helicobacter pylori reduce H. pylori infection via Th2-biased immune responses in mice* Pathog. Dis., **77**: ftz050

Zavan, L., Bitto, N.J., Johnston, E.L., Greening, D.W. and Kaparakis-Liaskos, M. (2019) *Helicobacter pylori growth stage determines the size, protein composition, and preferential cargo packaging of outer membrane vesicles* Proteomics, **19**: 1800209

Klebsiella pneumoniae
Cahill, B.K., Seeley, K.W., Gutel, D. and Ellis, T.N. (2015) *Klebsiella pneumoniae O antigen loss alters the outer membraneprotein composition and the selective packaging of proteins into secreted outer membrane vesicles* Microbiol. Res., **180**, 1–10

Legionella pneumophila
Fernandez-Moreira, E., Helbig, J.H. and Swanson, M.S. (2006) *Membrane vesicles shed by Legionella pneumophila inhibit fusion of phagosomes with lysosomes* Infect. Immun., **74**, 3285-3295

Lysobacter enzymogenes
Meers, P.R., Liu, C., Chen, R., Bartos, W. Davis, J. Dziedzic, N. Orciuolo, J., Kutyla, S., Jose, M. et al (2018) *Vesicular delivery of the antifungal antibiotics of Lysobacter enzymogenes* C3 Appl. Environ. Microbiol., 84, e01353-18

Marinobacter guineae
See "*Shewanella livingstonensis*"

Mycobacteria
See Section 3b

Neisseria gonorrhoeae
Pérez-Cruz, C., Delgado, L., López-Iglesias, C. and Mercade, E. (2015) *Outer-inner membrane vesicles naturally secreted by Gram-negative pathogenic bacteria* PLoS One, **10**: e0116896
Deo, P., Chow, S.H., Hay, I.D., Kleifeld, O., Costin, A., Elgass, K.D., Jiang, J-H., Ramm, G. et al (2018) *Outer membrane vesicles from Neisseria gonorrhoeae target PorB to mitochondria and induce apoptosis* PLoS Pathog., 14: e1006945

Neisseria meningitides
Matthias, K.A., Strader, M.B., Nawar, H.F., Gao, Y.S., Lee, J., Patel, D.S., Im, W. and Bash, M.C. (2017) *Heterogeneity in non-epitope loop sequence and outer membrane protein complexes alters antibody binding to the major porin protein PorB in serogroup B Neisseria meningitides* Mol. Microbiol., **105**, 934–953

Porphyromonas gingivalis
Cecil, J.D., O'Brien-Simpson, N.M., Lenzo, J.C., Holden, J.A., Chen, Y-Y., Singleton, W., Gause. K.T., Yan, Y., Caruso, F. and Reynolds, E.C. (2016) *Differential responses of pattern recognition receptors to outer membrane vesicles of three periodontal pathogens* PLoS One **11**: e0151967

Prochlorococcus
Biller, S.J., McDaniel, L.D., Breitbart, M., Rogers, E., Paul, J.H. and Chisholm, S.W. (2017) *Membrane vesicles in sea water: heterogeneous DNA content and implications for viral abundance estimates* ISME J., **11**, 394–404

Pseudoalteromona
See "*Shewanella livingstonensis*"

Pseudomonas aeruginosa
Ballok, A.E., Filkins, L.M., Bomberger, J.M., Stanton, B.A. and O'Toole, G.A. (2014) *Epoxide-mediated differential packaging of Cif and other virulence factors into outer membrane vesicles* J. Bacteriol., **196**, 3633–3642
Barnaby, R., Koeppen, K. and Stanton B.A. (2018) *Cyclodextrins reduce the ability of Pseudomonas aeruginosa outer-membrane vesicles to reduce CFTR Cl^- secretion* Am. J. Physiol. Lung Cell Mol. Physiol., **316**, L206–L215
Bauman, S.J. and Kuehn, M.J. (2006) *Purification of outer membrane vesicles from Pseudomonas aeruginosa and their activation of an IL-8 response* Microbes Infect., **8**, 2400-2408
Bauman, S.J. and Kuehn, M.J. (2009) *Pseudomonas aeruginosa vesicles associate with and are internalized by human lung epithelial cells* BMC Microbiol., **9**:26

Bomberger, J.M., MacEachran, D.P., Coutermarsh, B.A., Ye, S., O'Toole, G.A. and Stanton, B.A. (2009) *Long-distance delivery of bacterial virulence factors by Pseudomonas aeruginosa outer membrane vesicles* PLoS Pathog., **5**:e1000382
Couto, N., Schooling, S.R., Dutcher, J.R. and Barber, J. (2015) *Proteome profiles of outer membrane vesicles and extracellular matrix of Pseudomonas aeruginosa biofilms* J. Proteome Res., **14**, 4207−4222
Ellis, T.N., Leiman, S.A. and Kuehn, M.J. (2010) *Naturally produced outer membrane vesicles from Pseudomonas aeruginosa elicit a potent innate immune response via combined sensing of both lipopolysaccharide and protein components* Infect. Immun., **78**, 3822-3831
Esoda, C.N., Kuehn, M.J. (2019) *Pseudomonas aeruginosa leucine aminopeptidase influences early biofilm composition and structure via vesicle-associated antibiofilm activity* Host-Microbe Biol., **10**: e02548-19
Koeppen, K., Hampton, T.H., Jarek, M., Scharfe, M., Gerber, S.A., Mielcarz, D.W., Demers, E.G., Dolben, E.L. et al (2016) *A novel mechanism of host-pathogen interaction through sRNA in bacterial outer membrane vesicles* PloS Pathog., **12**: e1005672
Koeppen, K., Barnaby, R., Jackson, A.A., Gerber, S.A., Hogan, D.A. and Stanton, B.A. (2019) *Tobramycin reduces key virulence determinants in the proteome of Pseudomonas aeruginosa outer membrane vesicles* PLoS One, **14**: e0211290
MacDonald, I.A. and Kuehn, M.J. (2013) *Stress-induced outer membrane vesicle production by Pseudomonas aeruginosa* J. Bacteriol., **195**, 2971–2981
MacEachran, D.P., Ye, S., Bomberger, J.M., Hogan, D.A., Swiatecka-Urban, A., Stanton, B.A. and O'Toole, G.A. (2007) *The Pseudomonas aeruginosa secreted protein PA2934 decreases apical membrane expression of the cystic fibrosis transmembrane conductance regulator* Infect. Immun., **75**, 3902-3912
Schooling, S.R., Hubley, A. and Beveridge, T.J. (2009) *Interactions of DNA with biofilm-derived membrane vesicles* J. Bacteriol., **191**, 4097-4102
Tashiro, Y., Sakai, R., Toyofuku, M., Sawada, I., Nakajima-Kambe, T., Uchiyama, H. and Nomura, N. (2009) *Outer membrane machinery and alginate synthesis regulators control membrane vesicle production in Pseudomonas aeruginosa* J. Bacteriol., **191**, 7509-7519
Tashiro, Y., Ichikawa, S., Shimizu, M., Toyofuku, M., Takaya, N., Nakajima-Kambe, T., Uchiyama, H. and Nomura, N. (2010) *Variation of physiochemical properties and cell association activity of membrane vesicles with growth phase in Pseudomonas aeruginosa* Appl. Environ. Microbiol., **76**, 3732-3239
Toyofuku, M., Roschitzki, B., Riedel, K. and Eberl, L. (2012) *Identification of proteins associated with the Pseudomonas aeruginosa biofilm extracellular matrix* J. Proteome Res., **11**, 4906−4915
Toyofuku, M., Zhou, S., Sawada, I., Takaya, N., Uchiyama, H. and Nomura, N. (2014) *Membrane vesicle formation is associated with pyocin production under denitrifying conditions in Pseudomonas aeruginosa* PAO1 Environ. Microbiol., **16**, 2927–2938
Turnbull, L., Toyofuku, M., Hynen, A.L., Kurosawa, M., Pessi, G., Petty, N.K., Osvath, S.R., Cárcamo-Oyarce, G. et al (2016) *Explosive cell lysis as a mechanism for the biogenesis of* bacterial membrane *vesicles and biofilms* Nat. Comm., **7**: 11220
Zhao, K., Deng, X., He, C., Yue, B. and Wu, M. (2013) *Pseudomonas aeruginosa outer membrane vesicles modulate host immune responses by targeting the Toll-like receptor 4 signaling pathway* Infect. Immun., **81**, 4509-4518

***Pseudomonas panacis* (from faeces)**

Choi, Y., Kwon, Y., Kim, D-K., Jeon, J., Jang, S.C., Wang, T., Ban, M., Kim, M-H., Jeon, S.G. et al (2015) *Gut microbe-derived extracellular vesicles induce insulin resistance, thereby impairing glucose metabolism in skeletal muscle* Sci. Rep., **5**: 15878

Psychrobacter fozii
See "*Shewanella livingstonensis*"

Salinicola

Biller, S.J., McDaniel, L.D., Breitbart, M., Rogers, E., Paul, J.H. and Chisholm, S.W. (2017) *Membrane vesicles in sea water: heterogeneous DNA content and implications for viral abundance estimates* ISME J., **11**, 394–404

Salmonella enterica

Bai, J., Kim, S.I., Ryu, S. and Yoon, H. (2014) *Identification and characterization of outer membrane vesicle-associated proteins in Salmonella enterica Serovar Typhimurium* Infect. Immun., **82**, 4001–4010
Bonnington, K.E. and Kuehn, M.J. (2016) *Outer membrane vesicle production facilitates LPS remodeling and outer membrane maintenance in Salmonella during environmental transitions* mBio, **7**: e01532-16

Daleke-Schermerhorn, M.H., Felix, T., Soprova, Z., ten Hagen-Jongman, C.M., Vikström, D., Majlessi, L., Beskers, J., Follmann, F., de Punder, K., van der Wel, N.N. et al (2014) *Decoration of outer membrane vesicles with multiple antigens by using an autotransporter approach* Appl, Environ. Microbiol., **80**, 5854–5865
Habier, J., May, P., Heintz-Buschart, A., Ghosal, A., Wienecke-Baldacchino, A.K., Nolte-'t Hoen, E.N.M., Wilmes, P. and Fritz, J.V. (2018) *Extraction and analysis of RNA isolated from pure bacteria-derived outer membrane vesicles* In Bacterial Regulatory RNA: Methods and Protocols, Methods in Mol. Biol., **1737** (eds. Arluison, V. and Valverde, C.) Springer Science+Business Media, LLC, pp 213-230
Kim, O.Y., Park, H.T., Dinh, N.T.H., Choi, S.J., Lee, J., Kim, J.H., Lee, S-W. and Gho, Y.S. (2017) *Bacterial outer membrane vesicles suppress tumor by interferon-γ-mediated antitumor response* Nat. Comm., **8**: 626
Kitagawa, R., Takaya, A., Ohya, M., Mizunoe, Y., Takade, A., Yoshida, S-i., Isogai, E. and Yamamoto, T. (2010) *Biogenesis of Salmonella enterica serovar Typhimurium membrane vesicles provoked by induction of PagC* J. Bacteriol., **192**, 5645–5656
Liu, Q., Liu, Q., Yi, J., Liang, K., Liu, T., Roland, K.L., Jiang, Y. and Kong, Q. (2016) *Outer membrane vesicles derived from Salmonella Typhimurium mutants with truncated LPS induce cross-protective immune responses against infection of Salmonella enterica serovars in the mouse model* Int. J. Med. Microbiol., **306**, 697–706
Muralinath, M., Kuehn, M.J., Roland, K.L. and Curtiss III, R. (2011) *Immunization with Salmonella enterica serovar Typhimurium-derived outer membrane vesicles delivering the pneumococcal protein PspA confers protection against challenge with Streptococcus pneumoniae* Infect. Immun., **79**, 887–894

Shewanella livingstonensis

Frias, A., Manresa, A., de Oliveira, E., López-Iglesias, C. and Mercade, E. (2010) *Membrane vesicles: a common feature in the extracellular matter of cold-adapted Antarctic bacteria* Microb. Ecol., **59**, 476–486
Yokoyama, F., Kawamoto, J., Imai, T. and Kurihara, T. (2017) *Characterization of extracellular membrane vesicles of an Antarctic bacterium, Shewanella livingstonensis Ac10, and their enhanced production by alteration of phospholipid composition* Extremophiles **21**: 723–731

Shewanella veisiculosa

Frias, A., Manresa, A., de Oliveira, E., López-Iglesias, C. and Mercade, E. (2010) *Membrane vesicles: a common feature in the extracellular matter of cold-adapted Antarctic bacteria* Microb. Ecol., **59**, 476–486
Pérez-Cruz, C., Carrión, O., Delgado, L., Martinez, G., López-Iglesias, C. and Mercade, E. (2013) *New type of outer membrane vesicle produced by the Gram-negative bacterium Shewanella vesiculosa M7T: implications for DNA content* Appl. Environ. Microbiol., **79**, 1874-1881

Tannerella forsythia

Cecil, J.D., O'Brien-Simpson, N.M., Lenzo, J.C., Holden, J.A., Chen, Y-Y., Singleton, W., Gause. K.T., Yan, Y., Caruso, F. and Reynolds, E.C. (2016) *Differential responses of pattern recognition receptors to outer membrane vesicles of three periodontal pathogens* PLoS One **11**: e0151967
Veith, P.D., Chen, Y-Y., Chen, D., O'Brien-Simpson, N.M., Cecil, J.D., Holden, J.A., Lenzo, J.C. and Reynolds, E.C. (2015) *Tannerella forsythia outer membrane vesicles are enriched with substrates of the type IX secretion system and TonB-dependent receptors* J. Proteome Res., **14**, 5355−5366

Thalassospria

Biller, S.J., McDaniel, L.D., Breitbart, M., Rogers, E., Paul, J.H. and Chisholm, S.W. (2017) *Membrane vesicles in sea water: heterogeneous DNA content and implications for viral abundance estimates* ISME J., **11**, 394–404

Treponema denticola

Cecil, J.D., O'Brien-Simpson, N.M., Lenzo, J.C., Holden, J.A., Chen, Y-Y., Singleton, W., Gause. K.T., Yan, Y., Caruso, F. and Reynolds, E.C. (2016) *Differential responses of pattern recognition receptors to outer membrane vesicles of three periodontal pathogens* PLoS One **11**: e0151967
Veith, P.D., Glew, M.D., Gorasia, D.G., Chen, D., O'Brien-Simpson, N.M. and Reynolds, E.C. (2019) *Localization of outer membrane proteins in Treponema denticola by quantitative proteome analyses of outer membrane vesicles and cellular fractions* J. Proteome Res., **18**, 1567−1581

Vibrio cholerae

Bitar, A., Aung, K.M., Wai, S.N., and Hammarström, M-L. (2019) *Vibrio cholerae derived outer membrane vesicles modulate the inflammatory response of human intestinal epithelial cells by inducing microRNA-146a* Sci. Rep., **9**: 7212

Elluri, S., Enow, C., Vdovikova, S., Rompikuntal, P.K., Dongre, M., Carlsson, S., Pal, A., Uhlin, B.E., Wai, S.N. (2014) *Outer membrane vesicles mediate transport of biologically active Vibrio cholerae cytolysin (VCC) from V. cholerae strains* PLoS One, **9**: e106731
Kohl, P., Zingl, F.G., Eichmann, T.O. and Schild, S. (2018) *Isolation of outer membrane vesicles including their quantitative and qualitative analyses* In *Vibrio Cholerae*: Methods and Protocols, Methods in Mol. Biol., **1839**, (ed. Sikora, A.E.), Springer Science+Business Media, LLC, pp 117-134
Mondal, A., Tapader, R., Chatterjee, N.S., Ghosh, A., Sinha, R., Koley, H., Saha, D.R., Chakrabarti, M.K., Wai, S.N. and Pala, A. (2016) *Cytotoxic and inflammatory responses induced by outer membrane vesicle-associated biologically active proteases from Vibrio cholerae* Infect. Immun., **84**, 1478-1490
Roier, S., Zingl, F.G., Cakar, F., Durakovic, S., Kohl, P., Eichmann, T.O., Kiug, L., Gadermaier, B. et al (2016) *A novel mechanism for the biogenesis of outer membrane vesicles in Gram-negative bacteria* Nat. Comm., **7**: 10515
Sjöström, A.E., Sandblad, L., Uhlin, B.E. and Wai, S.N. (2015) *Membrane vesicle-mediated release of bacterial RNA* Sci. Rep., **5**: 15329

Vibrio shilonii
Li, J., Azam, F. and Zhang, S. (2016) *Outer membrane vesicles containing signalling molecules and active hydrolytic enzymes released by a coral pathogen Vibrio shilonii AK1* Environ. Microbiol., **18**, 3850–3866

Vibrio tasmaniensis
Vanhove, A.S., Duperthuy, M., Charrière, G.M., Le Roux, F., Goudenège, D., Gourbal, B., Kieffer-Jaquinod, S., Couté, Y., Wai, S.N. and Destoumieux-Garzón, D. (2015) *Outer membrane vesicles are vehicles for the delivery of Vibrio tasmaniensis virulence factors to oyster immune cells* Environ. Microbiol., **17**, 1152–1165

Yersinia pestis
Eddy, J.L., Gielda, L.M., Caulfield, A.J., Rangel, S.M. and Lathem, W.W. (2014) *Production of outer membrane vesicles by the plague pathogen Yersinia pestis* PloS One, **9**: e107002

Yersinia pseudotuberculosis
Monnappa, A.K., Bari, W., Seo, J.K. and Mitchell, R.J. (2018) *The cytotoxic necrotizing factor of Yersinia pseudotuberculosis (CNFy) is carried on extracellular membrane vesicles to host cells* Sci. Rep., 8:14186

3b. Gram-positive bacteria and mycobacteria
Acholeplasma
Medvedeva, E.S., Mouzykantov, A.A.,Baranova, N.B., Dramchini, M.A., Chernova, O.A. and Chernov, V.M. (2019) *Data on proteomic profiling of cells and extracellular vesicles of the melittin-resistant Acholeplasma laidlawii strain* Data in brief, **25**, 104169

Bacillus anthracis
Wolf, J.M., Rivera, J. and Casadevall, A. (2012) *Serum albumin disrupts Cryptococcus neoformans and Bacillus anthracis extracellular vesicles* Cellular Microbiology (2012) 14(5), 762–773

Bacillus subtilis
Brown, L., Kessler, A., Cabezas-Sanchez, P., Luque-Garcia, J.L. and Casadevall, A. (2014) *Extracellular vesicles produced by the Gram-positive bacterium Bacillus subtilis are disrupted by the lipopeptide surfactin* Mol. Microbiol., **93**, 183–198
Prados-Rosales, R., Brown, L., Casadevall, A., Montalvo-Quiros, S. and Luque-Garcia, J.L. (2014) *Isolation and identification of membrane vesicle-associated proteins in Gram-positive bacteria and mycobacteria* MethodsX, **1**, 124–129

Clostridium perfringens
Jiang. Y., Kong, Q., Roland, K.L. and Curtiss III, R. (2014) *Membrane vesicles of Clostridium perfringens type A strains induce innate and adaptive immunity* Int. J, Med. Microbiol., **304**, 431–443
Obana, N., Nakao, R., Nagayama, K., Nakamura, K., Senpuku, H. and Nomuraa, N. (2017) *Immunoactive clostridial membrane vesicle production is regulated by a sporulation factor* Infect. Immun., 85: e00096-17

Enterococcus faecium
Wagner, T., Joshi, B., Janice, J., Askarian, F., Škalko-Basnet, N., Hagestad, O.C., Mekhlif, A., Wai, S.N., Hegstad, K. and Johannessen, M. (2018) *Enterococcus faecium produces membrane vesicles containing virulence factors and antimicrobial resistance related proteins* J.Proteom., **187**, 28–38

Lactobacillus acidophilus

Kim, O.Y., Park, H.T., Dinh, N.T.H., Choi, S.J., Lee, J., Kim, J.H., Lee, S-W. and Gho, Y.S. (2017) *Bacterial outer membrane vesicles suppress tumor by interferon-γ-mediated antitumor response* Nat. Comm., **8**: 626

Lactobacillus sakei

Yamasaki-Yashiki, S., Yuki Miyoshi, Y., Nakayama, T., Kunisawa, J. and Katakura, Y. (2019) *IgA-enhancing effects of membrane vesicles derived from Lactobacillus sakei subsp. sakei NBRC15893* Biosci. Microbiota Food Health, **38**, 23–29

Listeria monocytogenes

Coelho, C., Brown, L., Maryam, M., Vij, R., Smith, D.F.Q., Burnet, M.C., Kyle, J.E., Heyman, H.M., Ramirez, J. et al (2019) *Listeria monocytogenes virulence factors, including listeriolysin O, are secreted in biologically active extracellular vesicles* J. Biol. Chem., **294**, 1202–1217

Mycobacterium smegmatis

Dauros Singorenko, P., Chang, V., Whitcombe, A., Simonov, D., Hong, J., Phillips, A., Swift, S. and Blenkiron, C. (2017) *Isolation of membrane vesicles from prokaryotes: a technical and biological comparison reveals heterogeneity* J. Extracell. Ves., **6**: 1324731

Mycobacterium tuberculosis

D'Lima, N.G. and Teschke, C.M. (2015) *A method to investigate protein association with intact sealed mycobacterial membrane vesicles* Anal. Biochem. **485**, 109–111

Lee, J., Kim, S-H., Choi, D-S., Lee, J.S., Kim, D-K., Go, G., Park, S-M., Kim, S.H., Shin, J.H., Chang, C.L. and Gho, Y.S. (2015) *Proteomic analysis of extracellular vesicles derived from Mycobacterium tuberculosis* Proteomics, **15**, 3331–3337

Palacios, A., Sampedro, L., Sevilla, I.A., Molina, E., Gil, D., Azkargorta, M., Elortza, F., Garrido, J.M., Anguita, J. and Prados-Rosales, R. (2019) *Mycobacterium tuberculosis extracellular vesicle-associated lipoprotein LpqH as a potential biomarker to distinguish paratuberculosis infection or vaccination from tuberculosis infection* BMC Vet. Res., **15**: 188

Prados-Rosales, R., Weinrick, B.C., Piqué, D.G., Jacobs, Jr., W.R., Casadevall,A. and Rodriguez, G.M. (2014) *Role for Mycobacterium tuberculosis membrane vesicles in iron acquisition* J. Bacteriol., **196**, 1250–1256

Prados-Rosales, R., Brown, L., Casadevall, A., Montalvo-Quiros, S. and Luque-Garcia, J.L. (2014) *Isolation and identification of membrane vesicle-associated proteins in Gram-positive bacteria and mycobacteria* MethodsX, **1**, 124–129

Ratha, P., Huang, C., Wang, T., Wang, T., Li, H., Prados-Rosales, R., Elemento, O., Casadevall, A. and Nathan, C.F. (2013) *Genetic regulation of vesiculogenesis and immunomodulation in Mycobacterium tuberculosis* Proc. Natl. Acad. Sci. USA, **110**, E4790–E4797

Proprionibacterium acnes

Jeon, J., Park, S.C., Her, J., Lee, J.W., Han, J-K., Kim, Y-K., Kim, K.P. and Ban, C, (2018) *Comparative lipidomic profiling of the human commensal bacterium Propionibacterium acnes and its extracellular vesicles* RSC Adv., **8**, 15241–15247

Staphylococcus aureus

Askarian, F., Lapek Jr., J.D., Dongre, M., Tsai, C-M., Kumaraswamy, M., Kousha, A., Valderrama, J.A., Ludviksen, J.A. et al (2018) *Staphylococcus aureus membrane-derived vesicles promote bacterial virulence and confer protective immunity in murine infection models* Front. Physiol., **9**: 262

Askarian, F., Lapek Jr., J.D., Dongre, M., Tsai, C-M., Kumaraswamy, M., Kousha, A., Valderrama, J.A., Ludviksen, J.A. et al (2018) *Staphylococcus aureus membrane-derived vesicles promote bacterial virulence and confer protective immunity in murine infection models* Front. Physiol., **9**: 262

Streptococcus haemolyticus

Cavanagh, J.P., Askarian, F., Pain, M., Bruun, J-A., Urbarova, I., Wai, S.N., Schmidt, F. and Johannessen, M. (2019) *Proteome profiling of secreted and membrane vesicle associated proteins of an invasive and a commensal Staphylococcus haemolyticus isolate* Data in Brief, **22**, 914–919

Cavanagh, J.P., Pain, M., Askarian, F., Bruune, J-A., Urbarova, I., Waif, S.N., Schmidt, F. and Johannessen, M. (2019) *Comparative exoproteome profiling of an invasive and a commensal Staphylococcus haemolyticus isolate* J. Proteom., **197**, 106–114

Cavanagh, J.P., Askarian, F., Pain, M., Bruun, J-A., Urbarova, I., Wai, S.N., Schmidt, F. and Johannessen, M. (2019) *Proteome profiling of secreted and membrane vesicle associated proteins of an invasive and a commensal Staphylococcus haemolyticus isolate* Data in Brief, **22**, 914–919
Cavanagh, J.P., Pain, M., Askarian, F., Bruune, J-A., Urbarova, I., Waif, S.N., Schmidt, F. and Johannessen, M. (2019) *Comparative exoproteome profiling of an invasive and a commensal Staphylococcus haemolyticus isolate* J. Proteom., **197**, 106–114
Askarian, F., Lapek Jr., J.D., Dongre, M., Tsai, C-M., Kumaraswamy, M., Kousha, A., Valderrama, J.A., Ludviksen, J.A. et al (2018) *Staphylococcus aureus membrane-derived vesicles promote bacterial virulence and confer protective immunity in murine infection models* Front. Physiol., **9**: 262

Streptococcus haemolyticus

Cavanagh, J.P., Askarian, F., Pain, M., Bruun, J-A., Urbarova, I., Wai, S.N., Schmidt, F. and Johannessen, M. (2019) *Proteome profiling of secreted and membrane vesicle associated proteins of an invasive and a commensal Staphylococcus haemolyticus isolate* Data in Brief, **22**, 914–919
Cavanagh, J.P., Pain, M., Askarian, F., Bruune, J-A., Urbarova, I., Waif, S.N., Schmidt, F. and Johannessen, M. (2019) *Comparative exoproteome profiling of an invasive and a commensal Staphylococcus haemolyticus isolate* J. Proteom., **197**, 106–114
Lee, E-Y., Choi, D-Y., Kim, D-K., Kim, J-W., Park, J O., Kim, S., Kim, S-H., Desiderio, D.M., Kim, Y-K., Kim, K-P- and Gho, Y.S. (2009) *Gram-positive bacteria produce membrane vesicles: Proteomics-based characterization of Staphylococcus aureus-derived membrane vesicles* Proteomics, **9**, 5425-5436
Kim, O.Y., Park, H.T., Dinh, N.T.H., Choi, S.J., Lee, J., Kim, J.H., Lee, S-W. and Gho, Y.S. (2017) *Bacterial outer membrane vesicles suppress tumor by interferon-γ-mediated antitumor response* Nat. Comm., **8**: 626
Schlatterer, K., Beck, C., Hanzelmann, D., Lebtig, M., Fehrenbacher, B., Schaller, M., Ebner, P., Nega, M. et al (2018) *The mechanism behind bacterial lipoprotein release: phenol-soluble modulins mediate toll-like receptor 2 activation via extracellular vesicle release from Staphylococcus aureus* mBio, **9**, e01851-18
Thay, B., Wai, S.N. and Oscarsson, J. (2013) *Staphylococcus aureus α-toxin-dependent induction of host cell death by membrane-derived vesicles* PloS One, **8**: e54661
Tiana, Y., Chen, C., Niu, Q., Zhu, S. and Yan, X. (2019) *Analysis of fluorescent labelling efficiency of extracellular vesicles derived from different kingdoms of life with lipid-binding dyes via nano-flow cytometry* J. Extracell. Ves., **8** (Suppl.1) PF06.06
Wang, X., Thompson, C.D., Weidenmaier, C. and Lee, J.C. (2018) *Release of Staphylococcus aureus extracellular vesicles and their application as a vaccine platform* Nat. Comm., **9**: 1379

Streptococcus pneumoniae

Codemo, M., Muschiol, S., Iovino, F., Nannapaneni, P., Plant, L., Wai, S.N. and Henriques-Normark, B. (2018) *Immunomodulatory effects of pneumococcal extracellular vesicles on cellular and humoral host defenses* mBio **9**: e00559-18
Jhelum, H., Sori, H. and Sehgal, D. (2018) *A novel extracellular vesicle associated endodeoxyribonuclease helps Streptococcus pneumoniae evade neutrophil extracellular traps and is required for full virulence* Sci. Rep., **8**: 7985
Olaya-Abrila, A., Prados-Rosales, R., McConnell, M.J., Martín-Peña, R., González-Reyes, J.A., Jiménez-Munguía, I., Gómez-Gascón, L.., Fernández, J., et al (2014) *Characterization of protective extracellular membrane-derived vesicles produced by Streptococcus pneumoniae* J. Proteomics, **106**, 46-60

Streptomyces

Hoefler, B.C., Stubbendieck, R.M., Josyula, N.K., Moisan, S.M., Schulze, E.M. and Straight, P.D. (2017) *A link between linearmycin biosynthesis and extracellular vesicle genesis connects specialized metabolism and bacterial membrane physiology* Cell Chem. Biol., **24**, 1238–1249

3c Bacterial exosomes derived from human fluids and waste water

Choi, Y., Kwon, Y., Kim, D-K., Jeon, J., Jang, S.C., Wang, T., Ban, M., Kim, M-H., Jeon, S.G. et al (2015) *Gut microbe-derived extracellular vesicles induce insulin resistance, thereby impairing glucose metabolism in skeletal muscle* Sci. Rep., **5**: 15878
Choi, Y.J., Lee, D.H., Kim, H.S. and Kim, Y-K. (2018) *An exploratory study on the effect of daily fruits and vegetable juice on human gut microbiota* Food Sci. Biotechnol., **27**, 1377–1386
Maestre-Carballa, L., Gomez, M.L., Navarro, A.A., Garcia-Heredia, I., Martinez-Hernandez, F. and Martinez-Garcia, M. (2019) *Insights into the antibiotic resistance dissemination in a wastewater effluent microbiome: bacteria, viruses and vesicles matter* Environ. Microbiol., **21**, 4582–4596
Tulkens, J., De Wever, O. and Hendrix, A. (2020) *Analyzing bacterial extracellular vesicles in human body fluids by orthogonal biophysical separation and biochemical characterization* Nat. Protoc., **15**, 40–67

3d. Halobacteria

Erdmann, S., Tschitschko, B., Zhong, L., Raftery, M.J. and Cavicchioli, R. (2017) *A plasmid from an Antarctic haloarchaeon uses specialized membrane vesicles to disseminate and infect plasmid-free cells* Nat. Microbiol., **1446**, 1446–1455

3e. Biofilms

Ficurilli, M., Liu, C., Riviello, C., Pozo, M.J. and Meers, P.R. (2015) *Delivery of liposomal contents to outer membrane vesicles from Gram negative bacteria* Biophys. J. **108 (Suppl.1)**, 408a

Laanto, E., Penttinen, R.K., Bamford, J.K.H. and Sundberg, L-R. (2014) *Comparing the different morphotypes of a fish pathogen - implications for key virulence factors in Flavobacterium columnare* BMC Microbiol., **14**:170

Turnbull, L., Toyofuku, M., Hynen, A.L., Kurosawa, M., Pessi, G., Petty, N.K., Osvath, S.R., Cárcamo-Oyarce, G. et al (2016) *Explosive cell lysis as a mechanism for the biogenesis of bacterial membrane vesicles and biofilms* Nat. Comm., **7**: 11220

3f. Mycoplasmas

Acholeplasma laidlawii

Chernov,, V.M., Mouzykantov, A.A., Baranova, N.B., Medvedeva, E.S., Yu Grygorieva, T., Trushina, M.V., Vishnyakov, I.E., Sabantsev, A.V., Borchsenius, S.N. and Chernova, O.A. (2014) *Extracellular membrane vesicles secreted by mycoplasma Acholeplasma laidlawii PG8 are enriched in virulence proteins* J. Proteom., **110**, 117–128

3g. Protozoa, algae, fungi and archaea

Caeiro, L.D., Alba-Soto, C.D., Rizzi, M., Solana, M.E., Rodriguez, G., Chidichimo, A.M., Rodriguez, M.E. et al (2018) *The protein family TcTASV-C is a novel Trypanosoma cruzi virulence factor secreted in extracellular vesicles by trypomastigotes and highly expressed in bloodstream forms* PLoS Negl. Trop. Dis., **12**: e0006475

Camacho, E., Vij, R., Chrissian, C., Prados-Rosales, R., Gil, D., O'Meally, R.N., Cordero, R.J.B., Cole, R.N., McCaffery, J.M., Stark, R.E. and Casadevall, A. (2019) *The structural unit of melanin in the cell wall of the fungal pathogen Cryptococcus neoformans* J. Biol. Chem., **294**, 10471–10489

Johnson, T.B., Mach, C., Grove, R., Kelly, R., Van Cott, K. and Blum, P. (2018) *Secretion and fusion of biogeochemically active archaeal membrane vesicles* Geobiology, 16, 659–673

Sampaio, N.G., Cheng, L. and Eriksson, E.M. (2017) *The role of extracellular vesicles in malaria biology and pathogenesis* Malar. J., **16** 245

Long, H., Zhang, F., Xu, N., Liu, G., Diener, D.R., Rosenbaum, J.L. and Huang, K. (2017) *Comparative analysis of ciliary membranes and ectosomes* Curr. Biol., **26**, 3327–3335

Oliveira, D.L., Nimrichter, L., Miranda, K., Frases, S., Faull, K.F., Casadevall, A. and Rodrigues, M.L. (2009) *Cryptococcus neoformans cryoultramicrotomy and vesicle fractionation reveals an intimate association between membrane lipids and glucuronoxylomannan* Fungal Genet. Biol., **46**, 956–963

Regev-Rudzki, N., Wilson, D.W., Carvalho, T.G., Sisquella, X., Coleman, B.M., Rug, M., et al (2013) *Cell–cell communication between malaria-infected red blood cells via exosome-like vesicles* Cell, **153**, 1120–1133

Rodrigues, M.L., Oliveira, D.L., Vargas, G., Girard-Dias, W., Franzen, A.J., Frasés, S., Miranda, K. and Nimrichter, L. (2016) *Analysis of yeast extracellular vesicles* In Unconventional Protein Secretion: Methods and Protocols, Methods Mol. Biol., **1459** (ed. Pompa, A. and De Marchis, F.), Springer Science+Business Media New York, pp 175-190

Schatz, D., Rosenwasser, S., Malitsky, S., Wolf, S.G., Feldmesser, E. and Vardi, A. (2017) *Communication via extracellular vesicles enhances viral infection of a cosmopolitan alga* Nat. Microbiol., **2**, 1485–1492

Wolf, J.M., Rivera, J. and Casadevall, A. (2012) *Serum albumin disrupts Cryptococcus neoformans and Bacillus anthracis extracellular vesicles* Cellular Microbiology (2012) 14(5), 762–773

3h. Invertebrates

Matusek, T., Wendler, F., Polès, S., Pizette, S., D'Angelo, G., Fürthauer, M. and Thérond, P.P. (2014) *The ESCRT machinery regulates the secretion and long-range activity of Hedgehog* Nature, **516**, 99-103

Shibata, T., Hadano, J., Kawasaki, D., Dong, X. and Kawabata, S-i. (2017) *Drosophila TG-A trans-glutaminase is secreted via an unconventional Golgi-independent mechanism involving exosomes and two types of fatty acylations* J. Biol. Chem., **292**, 10723–10734

Thoene, J., Goss, T., Witcher, M., Mullet, J., N'Kuli, F., Van Der Smissen, P., Courtoy, P. and Hahn, S.H. (2013) *In vitro correction of disorders of lysosomal transport by microvesicles derived from baculovirus-infected Spodoptera cells* Mol. Genet. Metab., **109**, 77–85

Vora, A., Zhou, W., Londono-Renteria, B., Woodson, M., Sherman, M.B., Colpitts, T.M., Neelakanta, G. and Sultana, H. (2018) *Arthropod EVs mediate dengue virus transmission through interaction with a tetraspanin domain containing glycoprotein Tsp29Fb* Proc. Natl. Acad. Sci. USA, **115**, E6604–E6613
Zhou, W., Woodson, M., Neupane, B., Bai, F., Sherman, M.B., Choi, K.H., Neelakanta, G., and Sultana, H. (2018) *Exosomes serve as novel modes of tick-borne flavivirus transmission from arthropod to human cells and facilitates dissemination of viral RNA and proteins to the vertebrate neuronal cells* PLoS Pathog., **14**: e1006764

3i. Trematodes

Schistosoma mansonii

Meningher, T., Lerman, G., Regev-Rudzki, N., Gold, D., Ben-Dov, I.Z., Sidi, Y., Avni, D. and Schwartz, E. (2017) *Schistosomal microRNAs isolated from extracellular vesicles in sera of infected patients: a new tool for diagnosis and follow-up of human schistosomiasis* J. Infect. Dis., **215**, 378–86
Sotillo, J., Pearson, M., Potriquet, J., Becker, L., Pickering, D., Mulvenna, J. and Loukas, A. (2016) *Extra-cellular vesicles secreted by Schistosoma mansoni contain proteinvaccine candidates* Int. J. Parasitol., **46**, 1–5

3j Nematodes

Eichenberger, R.M., Talukder, H., Field, M.A., Wangchuk, P., Giacomina, P., Loukas, A. and Sotillo, J. (2018) *Characterization of Trichuris muris secreted proteins and extracellular vesicles provides new insights into host–parasite communication* J. Extracell. Ves., **7**: 1428004
Eichenberger, R.M., Talukder, H. Field, M.A., Wangchuk, P., Giacomin, P.R., Loukas, A. and Sotillo, J. (2018) *Extracellular vesicles from the parasitic nematode Trichuris muris: new insights into host–parasite communications* J. Extracell. Ves., **7**, Suppl. 1, Abstr. # PT01.09

3k. Arthropoda

Zaborowski, M.P., Cheah, P.S., Zhang, X., Bushko, I., Lee, K., Sammarco, A., Zappulli, V., Maas, S.L.N., Allen, R.M. et al (2019) *Membrane-bound Gaussia luciferase as a tool to track shedding of membrane proteins from the surface of extracellular vesicles* Sci. Rep., **9**: 17387

3l. Arabidopsis

Rutter, B.D. and Innes, R.W. (2017) *Extracellular vesicles isolated from the leaf apoplast carry stress-response proteins* Plant Physiol., **173**, 728–741

4. Methodology and functional reviews

Chutkan, H., MacDonald, I., Manning, A. and Kuehn, M.J. (2013) *Quantitative and qualitative preparations of bacterial outer membrane vesicles* In, Bacterial cell surfaces: Methods and Protocols, Methods Mol, Biol., **966** (ed. Delcour, A.H.) Springer Science+Business Media, pp 259-272
Coelho, C. and Casadevall, A. (2019) *Answers to naysayers regarding microbial extracellular vesicles* Biochem. Soc. Trans., **47**, 1005–1012
Dauros Singorenko, P., Chang, V., Whitcombe, A., Simonov, D., Hong, J., Phillips, A., Swift, S. and Blenkiron, C. (2017) *Isolation of membrane vesicles from prokaryotes: a technical and biological comparison reveals heterogeneity* J. Extracell. Ves., **6**: 1324731
Gnopo, Y.M.D., Watkins, H.C., Stevenson, T.C., DeLisa, M.P. and Putnama, D. (2017) *Designer outer membrane vesicles as immunomodulatory systems - Reprogramming bacteria for vaccine delivery* Advanced Drug Delivery Rev., **114**, 132–142
Kim, O.Y., Lee, J. and Gho, Y.S. (2017) *Extracellular vesicle mimetics: Novel alternatives to extracellular vesicle-based theranostics, drug delivery, and vaccines* Semin.Cell Dev. Biol., **67**, 74–82
Klimentová, J. and Stulík, J. (2015) *Methods of isolation and purification of outer membrane vesicles from gram-negative bacteria* Microbiol., Res.,**170**, 1–9
D'Lima, N.G. and Teschke, C.M. (2015) *A method to investigate protein association with intact sealed mycobacterial membrane vesicles* Anal. Biochem. **485**, 109–111
Qing, G., Gong, N., Chen, X., Chen, J., Zhang, H., Wang, Y., Wang, R., Zhang, S., Zhang, Z. (2019) *Natural and engineered bacterial outer membrane vesicles* Biophys. Rep., **5**, 184–198
Toyofuku, M., Tashiro, Y., Hasegawa, Y., Kurosawa, M. and Nomura, N. (2015) *Bacterial membrane vesicles, an overlooked environmental colloid: Biology, environmental perspectives and applications* Adv. Colloid Interface Sci., **226**, 65–77
Tulkens, J., De Wever, O. and Hendrix, A. (2020) *Analyzing bacterial extracellular vesicles in human body fluids by orthogonal biophysical separation and biochemical characterization* Nat. Protoc., **15**, 40–67