Chapter 9
The Nature and Relevance of Solvent Stress in Microbes and Mechanisms of Tolerance

Mike Manefield, Matthew Lee and Joanna Koenig

Abstract Solvent stress in microbiology refers to exposure of microorganisms to chemical compounds with relatively low polarity. Environments in which solvent stress is intense are traditionally grouped with other extreme environments with hazardous temperatures, pressures, salinity, acidity and radiation. Extreme Environments with respect to solvents include natural oil or organohalide contaminated environments and industrial settings in which microbes are used to produce solvents or other compounds in dual phase reactor systems. Stress is typically thought to be exerted by interference with membrane function but the ability of solvents to interfere with protein structure is perhaps an underestimated target for solvent stress. It is a significant concern that selection for efflux pumps through exposure to solvents is likely to select for resistance to antimicrobials. Other solvent tolerance mechanisms include membrane adaptation and solvent biodegradation along with more generic strategies such as biofilm formation, motility and endospore formation. Whilst mechanisms of tolerance in aerobic bacteria have been extensively studied, less work has been done on anaerobic bacteria and archaea. An understanding of the nature of solvent stress and microbial strategies to adapt has relevance in natural and biotechnology settings.

9.1 Introduction

Habitats with pH, temperature, pressure or salinity outside the norm are the usual suspects when considering extreme environments, however microbial communities living in the presence of high dissolved or free phase organic solvents also fit the remit. Such environments can be naturally occurring or contrived by humankind either by accident or in biotechnological settings. Much is now known about

M. Manefield (✉) · M. Lee · J. Koenig
School of Biotechnology and Biomolecular Sciences, University of NSW, Sydney, Australia
e-mail: manefield@unsw.edu.au

M. Manefield
Urban Water Systems Engineering, Technical University Munich, Garching, Germany

C. Chénard and F.M. Lauro (eds.), *Microbial Ecology of Extreme Environments*,
DOI 10.1007/978-3-319-51686-8_9

microbial responses to solvent stress, with a variety of model systems having generated a wealth of information. In this book chapter the nature of solvent stress is described along with the relevance and molecular mechanisms of tolerance. This knowledge is routinely exploited in the bioproduction of organic solvents and the bioremediation of solvent polluted environments.

9.2 The Nature of Solvent Stress

Organic solvents encompass natural and anthropogenic hydrocarbons with varying degrees of non-polar character. Examples include oil or petroleum hydrocarbons, which are mostly of natural origin though processed and redistributed on mass by humankind, and halogenated hydrocarbons, which are widespread in nature but mass produced and distributed anthropogenically. Chemical classes include alcoholic, aromatic or aliphatic hydrocarbons.

Organic solvents impose stress on biological systems principally as a consequence of the polarity of the offending molecule and interactions with cell membranes and proteins (Isken and de Bont 1998). There are other more specific mechanisms by which organic solvents impact on biological systems, for example through competitive inhibition of protein-ligand binding sites affecting respiration, interference with gene expression or through transformative activation in mammalian systems to toxic or mutagenic compounds. Competitive inhibition has been reported where VC competes with cis-DCE for reducing equivalents (Kitanidis and McCarty 2012). Chloroform is known to affect gene transcription in *Desulfitobacterium* species (Futagami et al. 2013). In pure cultures of *Desulfitobacterium dehalogenans* and *Desulfitobacterium hafniense* strains grown on 3-chloro-4hydroxyphenylacetate as electron acceptor, chloroform inhibited transcription of the reductive dehalogenase *cprA*. Other specific inhibitory mechanisms were proposed with chlorophenol and chloroethene respiring *Desulfitobacterium* lineages displaying different sensitivities (Futagami et al. 2013). It is also well known that solvents can be highly mutagenic, but generally only after some form of metabolic activation. For example, perchloroethene is not mutagenic in the standard Ames test, however preincubation with glutathione-*S*-transferases results in formation of TCVG and a striking mutagenic response in the Ames test (Vamvakas et al. 1989).

Whilst vinyl chloride, chloroform and perchloroethene are all solvents and in these examples cause growth inhibition or mutation in specific bacterial strains, the described phenomena are not a unique consequence of their polarity. These phenomena are often strain specific and not related to solvent toxicity where polarity is the central determinant.

The impacts of organic solvents increase with concentration. Many organic solvents have a limit to their aqueous solubility and can reach concentrations where a separate phase develops, analogous to a salt falling out of solution. In dual phase systems, solvent stress is related to concentration by virtue of the surface area of the phase divide, rather than the aqueous concentration, which remains constant as

more solvent is applied. Organic solvents in aqueous solutions can also form isolated droplets and emulsions on agitation and interact readily with hydrophobic surfaces. Organic solvents are typically separated from hydrophilic surfaces by a layer of water.

Polarity dictates biological availability via aqueous solubility as quantified with the octanol: water partition coefficient (log P_{OW}). Solvents with a log P_{OW} between 1 and 5 inhibit the growth and metabolic activity of microorganisms in a concentration dependent manner because they are soluble enough in water to interact with microbes but have low enough polarity to partition into cellular membranes. Polarity of the solvent dictates the location in lipid membranes and ultimately how membrane structure is deformed. Note that the relationship between log P_{ow} and membrane solubility is heavily impacted by the specific lipid composition of a lipid bilayer (Sikkema et al. 1995). Partitioning into triacylglyceride lipid bilayers results in disruption of membrane structure and ultimately function (Sikkema et al. 1995). Organic solvent accumulation in the area of a lipid bilayer containing acyl chains or in the area between opposing monolayers of lipid bilayers results in increases in the area occupied by each phospholipid molecule and membrane thickness (Sikkema et al. 1995) (Fig. 9.1). Solvent impacted membranes become permeable to ions (e.g. protons) and larger biomolecules (e.g. ATP) thereby antagonising generation of proton motive force and storage of energy in metabolic nucleotides (Heipieper et al. 1991). Typically, bacterial cells with dual membrane structure (Gram negative) can tolerate higher solvent concentrations compared to those with a cell structure involving a single lipid bilayer membrane (Gram positive) because outer membranes and the intervening periplasm offer some degree of protection to the inner cytoplasmic membrane (Torres et al. 2011; Segura et al. 2012).

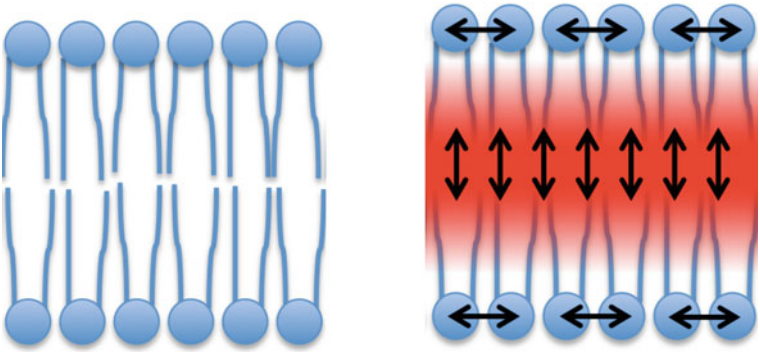

Fig. 9.1 Non-polar solvent impacts on lipid bilayers. Lipid head groups and acyl chains presented in *blue*. *Red* represents accumulated non-polar solvent. *Arrows* indicate forces acting on the bilayer in the presence of solvent. Depending on polarity solvents will penetrate membranes and acculumulate amongst the acyl chains creating forces that separate the phospholipid heads (e.g. hexane) and between the opposing monolayers of acyl chains forcing the membrane to increase in width (e.g. dodecane to hexadecane). Ultimately the natural order is disrupted leading to greater permeability and loss of function

Additionally, organic solvents disrupt protein structure and function. This can occur indirectly through interference with membrane lipids, which constitute the environment in which many important membrane associated proteins fold and function, including the respiratory machinery of microbes (White and Wimley 1999). Solvents additionally disrupt protein function directly owing to displacement of water molecules implicated in protein architecture and catalysis and in the thermodynamics, cooperativity and specificity of ligand binding (Mattos and Ringe 2001). Whilst the impact of aqueous organic mixtures are known to disrupt protein structure and function, it is important to note that many proteins can maintain structure and function in pure organic solvents (Griebenow and Klibanov 1996). Examples of intensively studied proteins in biocatalysis research showing activity in the presence of organic solvents or in neat organic solvents include elastase, thermolysin and lysozyme (Griebenow and Klibanov 1996; Mattos and Ringe 2001). The polarity of the organic solvent as well as the ability to compete for hydrogen bonding dictate the impact of a solvent on protein structure.

Aside from cell integrity maintained by single or double lipid bilayer membranes and anabolic and catabolic catalysis carried out by proteins there is little evidence that solvents impact negatively on other cellular structures or functions. Whilst several organic solvents are known to cause damage in DNA in higher organisms it is understood that this damage occurs as a consequence of processing by mammalian enzymes not present in microorganisms (Irving and Elfarra 2013). To date there is no evidence that organic solvents interfere with the integrity or function of DNA or RNA directly.

9.3 The Relevance of Solvent Stress

Solvent stress and tolerance in microorganisms are relevant in natural and human derived settings. It is not clear when organic solvents were first present on Earth in an abundance that could impart stress and have selected for solvent tolerance in microbes. Organic molecules such as light alkanes (e.g. propane, butane) can be formed abiotically under heat and pressure and are liquid or dissolved under pressure. Such chemistry has been stored 2–3 km into the subsurface in billion year old groundwater environments inhabited by microbes, predominantly sulfate reducing bacteria (Lollar and Ballentine 2009). These may be the earliest environments in which microbes were exposed to solvent stress.

The oil deposits of the Earth, which contain organic solvents, were mostly laid down during the Mesozoic era and are in the order of 100 million years old (Moldowan and Dahl 1994). This is likely the first time microorganisms were widely exposed to solvent stress, with mechanisms of tolerance co-adapted from other forms of stress. Meckenstock and coworkers recently described microbial communities living in small water droplets of deep subsurface origin embedded in a natural asphalt lake (Meckenstock et al. 2014). The communities were dominated by members of the orders Burkholderiales and Enterobacteriales, which contain

many of the extensively studied solvent tolerant bacterial lineages. In environments such as this and others including traditional oil deposits, oil sands, oil shale and marine and terrestrial oil seeps solvent tolerance is a requisite for microbial activity and replication. The characteristics of these hydrocarbon deposits have been influenced by microbes over geological time (Head et al. 2003), which could not have occurred without the ability to tolerate organic solvent stress. Indeed, if microorganisms were unable to tolerate and degrade the 1.3 million tons of oil that enters the environment each year mostly through natural seeps our coastlines would be awash with oil (Head et al. 2006).

Microbes in the environment are also exposed to solvent stress in marine and subsurface environments as a consequence of anthropogenic pollution. The microbiology of infamous marine oil spills such as in the Prince William Sound and the Gulf of Mexico have been studied extensively (Atlas and Hazen 2011), with the ability of microbial communities and the higher trophic levels dependent on microorganisms to rebound in part owing to solvent tolerance. Petroleum leakage from underground storage tanks (BTEX) from omnipresent domestic fuel distribution infrastructure is another anthropogenic phenomenon requiring solvent tolerance in groundwater microorganisms for environmental restoration.

In addition to oil derived solvents, chlorinated aliphatic hydrocarbons are widely distributed in the environment as a consequence of human industry. Organochlorine solvents used extensively as industrial solvents for dry cleaning of fabrics, degreasing engines and in the production of plastics and other chemicals have entered subsurface soil and groundwater environments. Bioremediation of organochlorine contaminated sites has emerged as the most cost effective means of ameliorating the human and environmental health risks of such contamination. This requires aerobic and anaerobic subsurface microorganisms to tolerate high dissolved concentrations of organic solvents and non-aqueous phase pollutant mass.

Solvent tolerance in microorganisms also has relevance in industrial and biomedical chemical production including biofuel production. There are several advantages to performing biocatalytical reactions in biphasic or nonaqueous systems relating to the solubility of reactants and products, removal of toxic products and prevention of contamination (Sardessai and Bhosle 2004). The commercial significance of such bioproduction systems have been a major driver in the isolation and genetic manipulation of solvent tolerant bacteria and research into the mechanisms of solvent tolerance.

Solvent tolerance also has relevance to other tolerances displayed by microorganisms. It has been shown that there is a correlation between solvent and temperature tolerance (Ramos et al. 2002). This suggests that adaptation to high temperature also results in an increase in tolerance to organic solvent stress and is likely related to membrane composition adaptation. A correlation between organic solvent tolerance and resistance to antibiotics has also been observed (Asako et al. 1997). This is important because exposure of microorganisms to organic solvents may increase antibiotic resistance, with implications to infectious disease control and human health. This correlation is related not to membrane structure but to the efficacy and activity of efflux pumps (Fernandes et al. 2003).

9.4 Organic Solvent Tolerant Microorganisms and Mechanisms of Tolerance

There are broad differences in the ability of different microorganisms to tolerate solvent stress that are not in any way related to microbial phylogeny. Indeed solvent tolerant microbes along with solvent sensitivity are well distributed throughout the phylogenetic tree, although detailed information on the mechanisms of solvent tolerance tend to focus on easy to handle laboratory stalwarts such as *Escherichia* and *Pseudomonas* species. Tolerance mechanisms can be broadly divided into two categories. The first encompasses generic mechanisms microbes use to defend themselves against environmental insults generally. The second encompasses mechanisms that are specific to solvent stress Fig. 9.2 summarises generic and specific defence mechanisms against solvent stress.

In environmental settings, microbes protect themselves from environmental stress through aggregation and biofilm formation. Aggregation is mediated by cell surface properties (e.g. hydrophobicity), electrostatic interactions with cations, extracellular DNA (Das et al. 2014) and specific ligand-receptor interactions (sugar-lectin binding) (Rickard et al. 2002). Aggregated cells represent a diffusion barrier to most chemicals, so cells central to the aggregate will be protected from

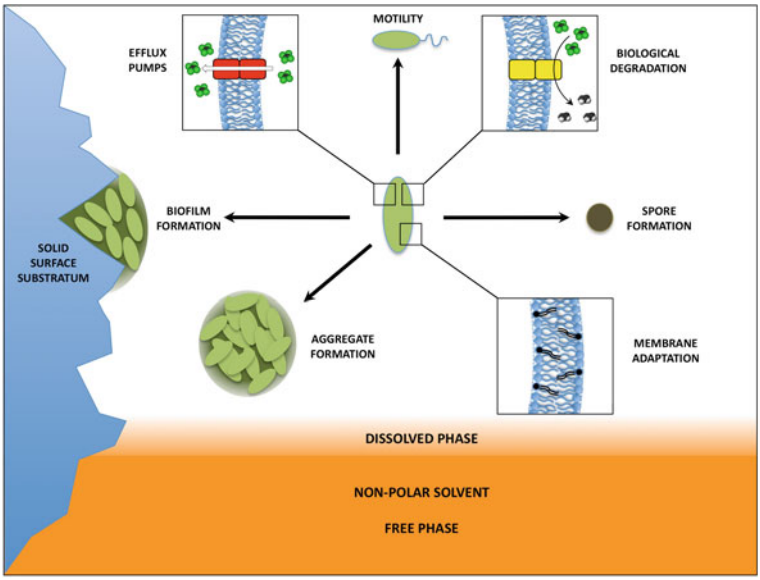

Fig. 9.2 Mechanisms of solvent tolerance dictating the effect of solvent stress on the behaviour and composition of microbial communities in a natural setting (sediment or subsurface). Mechanisms fall into two broad categories. Generic mechanisms include biofilm and aggregate formation, spore formation and motility. Specific mechanisms include increased membrane rigidity, efflux pumps and biological degradation

increases in solvent concentration in the surrounding bulk aqueous phase for a period of time. Surface associated biofilms also consist of aggregated cells with the additional protection of a solid surface, representing a spatial dimension from which solvents cannot access cells, like protecting your back by standing against a wall (Hall-Stoodley et al. 2004). Aggregated cells in flocculates and in biofilms attached to surfaces also generate an extracellular matrix that limits diffusion. These matrices, composed principally of polysaccharides, nucleic acids and peptides protect cells from negative impacts of the surrounding environment (Das et al. 2013). If cells in aggregates or biofilms can degrade the solvent, protection may last indefinitely. Whilst challenging to exhaustively demonstrate, there are no bacteria specifically known for the inability to produce biofilms or aggregates. Cells in biofilms can display low metabolic activity as a consequence of limited access to nutrients and energy but are generally considered to be metabolically active (Hall-Stoodley et al. 2004).

Endospore formation represents another general mechanism by which microbes protect themselves from stress including solvent stress, however this is a dormant, metabolically inactive state (Nicholson et al. 2000). Endospore formation is only recognized within the Firmicutes phylum, though members of this phylum are diverse and widely distributed in the environment, including the *Bacillus* and *Clostridium* genera (Maczulak 2011; Martin and Travers 1989). Endospore formation likely evolved before solvent stress was a major selective advantage, but certainly spore formation will be selected for in complex environmental communities exposed to natural or anthropogenic solvent stress. Simplistically spores consist of an outer coat, a cortex and a core, all of which presumably serve to limit access of solvents to the inner membrane within the cortex and proteins stored in the core, though exactly what imparts solvent tolerance to spores is not known. Bacteria outside the Firmicutes phylum display stress responses analogous to spore formation, whereby cell size reduces, metabolic activity is reduced to a minimum and cell structure becomes more tolerant to insults. This genetic program and resting state mediated principally through ppGpp accumulation and sigma factors (RpoS) has been most intensively studied as a response to starvation, but offers cross protection against solvent exposure (Shimizu 2015).

If one approach to surviving solvent stress is to batten down the hatches (aggregate, biofilm, endospore formation) another is to flee. Many microbes possess sophisticated sensory systems controlling motility (Bren and Esienbach 2000). Motility is most commonly associated with flagella production and action and examples exist whereby solvent challenges to cultures upregulate genes encoding motility (Tomas et al. 2003). Motility is a general response to stress where unicellular organisms attempt to move away from the stressful condition. This is not much help in homogenous culture media bioreactor systems, but potentially useful in escaping down solvent concentration gradients in contaminated saturated subsurface environments (Fig. 9.3).

One of the most extensively studied areas of solvent tolerance is the adaptation of cell membrane composition (Liu 2011). The bulk of this work has concentrated on *Pseudomonas* species, relevant to solvent bioproduction and petroleum product

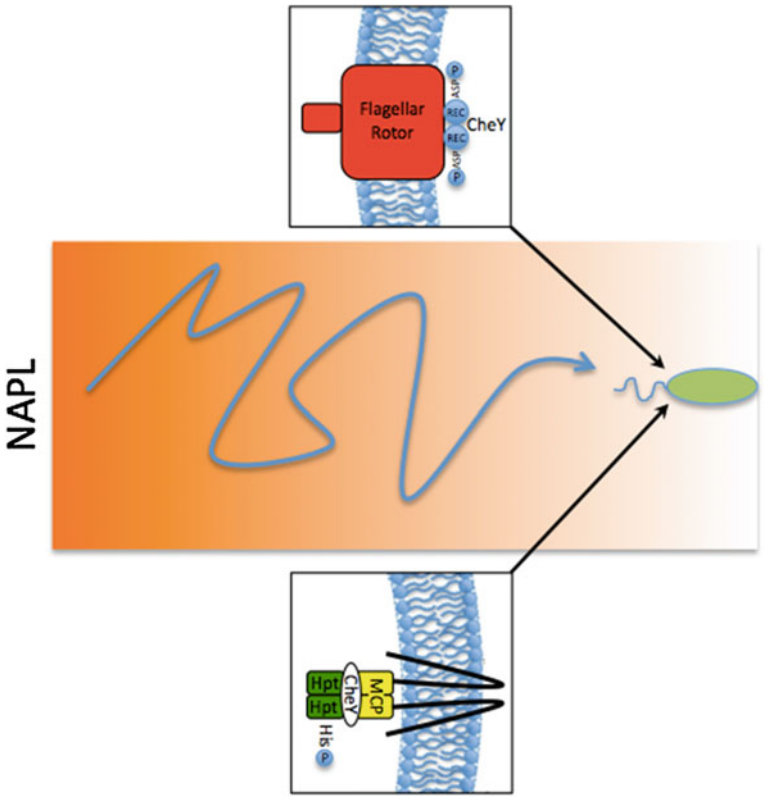

Fig. 9.3 Illustration of a motile bacterial cell with a polar flagellum controlling movement down an organic solvent gradient away from non-aqueous phase liquid (DNAPL or LNAPL). The lower inset shows membrane associated sensory proteins based on the well studied Che system in *Escherichia coli*. When activated by the presence of solvent the sensory system phosphorylates enzymes controlling flagella rotor rotation (upper inset). The bacterial cell tumbles to change direction

bioremediation alike. The toluene tolerant model organism *Pseudomonas putida* and others have been shown to alter outer membrane lipid content to reduce permeability and increase rigidity and hydrophobicity (Ramos et al. 2002; Segura et al. 2004). These changes to membrane properties are achieved through changing the proportions of proteins versus lipids in the membrane and converting lipids with cis (kinked) conformation to the trans (linear) conformation (Pinkart and White 1997). Such adaptations enable survival of short-term exposure to solvents or mild solvent concentrations and require active replication to manifest. The genetic response for membrane and protein adaptation is related to the heat shock response which also requires a decrease in membrane rigidity. The response is orchestrated by the sigma factor RpoH.

The other intensively studied mechanism of solvent tolerance is the use of efflux pumps (Torres et al. 2011). Efflux pumps are enzyme transporter complexes embedded in the cytoplasmic membrane that use energy to transfer out of the cytoplasm molecules that interfere with cellular function. Consequently, solvent stress can confer cross protection and resistance to antimicrobials, including disinfectants and antibiotics. Solvent efflux pumps have been extensively studied in *Pseudomonas putida* (Kieboom et al. 1998) *Pseudomonas aeruginosa* (Li et al. 1998), *Escherichia coli* (Asako et al. 1997) and *Bacillus cereus* (Matsumoto et al. 2002). As long as cells remain metabolically active, efflux pumps offer tolerance to solvents indefinitely and this is one of the reasons why efflux pumps represent possibly the most important mechanism of solvent tolerance, especially in the field of bioproduction. The importance of efflux pumps to solvent tolerance raises an interesting question about the mechanism of solvent toxicity. It is widely believed that solvents principally exert their effects through interactions with membranes (Isken and de Bont 1998), but if this were the case then removal of solvents from the cytoplasm would not be a particularly effective defense strategy. This remains one of the last general paradoxes remaining in the research area of solvent tolerance.

Another widely recognized mechanism of solvent tolerance relates to the ability of microbes to alter or degrade the offending solvent. In the context of bioproduction this is generally undesirable but in the context of bioremediation it is very much the end game. Selection for solvent tolerance through biodegradation to harmless end products is a mean of developing bacterial strains for bioaugmentation of contaminated sites. Again, *Pseudomonas* species have featured heavily in this research area in the context of oxidative oil degradation but perhaps the most interesting examples lie in low redox conditions where members of the Firmicutes and Chloroflexi exploit chlorinated organic solvents as electron acceptors in a process known as reductive dechlorination (Koenig et al. 2014). Because chlorinated solvents have a higher density than water, they are predominantly found deep in groundwater setting where oxygen does not permeate.

So called organochlorine respiring bacteria (ORB) have been shown to tolerate, and be metabolically active in, the presence of some of the highest priority pollutants known (Lee et al. 2012). For example, chloroform is a widespread groundwater contaminant globally and is recognized for its ability to inhibit microbial activity at 10 mg/L through interference with membrane function (Chidthaisong and Conrad 2000). Recently, members of the *Dehalobacter* genus have been isolated that can reduce chloroform to dichloromethane thereby reducing the solvent stress imposed (Grostern et al. 2010; Lee et al. 2012). These *Dehalobacter* strains show tolerance to chloroform above 100 mg/L. Whilst degradation is considered an important defense mechanism, the role of efflux pumps and membrane adaptation are yet to be determined for strictly anaerobic bacteria.

Indeed, the solvent tolerance of strictly anaerobic bacteria or bacteria grown under anaerobic conditions generally is an area of neglect in this research field. In a recent study, the effects of perchloroethene, carbon tetrachloride, chloroform and 1,2-dichloroethane on the growth of four fermentative, one nitrate-reducing, one

iron-reducing and one sulphate-reducing species was examined (Koenig and Groissmeier et al. 2014). The octanol-water partition coefficient or log $P_{o/w}$ of the solvents proved to be a generally satisfactory measure of their toxicity. Interestingly, toxicity also correlated well with growth rates observed in solvent-free cultures, with fast-growing organisms displaying higher tolerance. This suggests the ability to harvest energy from the environment and short doubling times underlies the ability to tolerate solvent stress in anaerobic bacteria. It stands to reason that the more energy and resources an organism has at its disposal the greater response it can mount via all known tolerance mechanisms.

Yung et al. (2016) recently conducted an interesting study on the transcriptional response (RNA sequencing) of *E. coli* to sub-growth-inhibitory concentrations of eight volatile organic compounds (n-butanol, N-cyclohexyl-pyrrolidone, cyclopentanone, N,N-dimethylacetamide, dimethyl sulphide, 1-methyl-2-pyrrolidone, N-methyl succinimide and toluene). Whilst each compound generated unique transcriptional responses in *E. coli* several general responses emerged.

Firstly, transcription of genes involved in Fe/S cluster biogenesis were upregulated in response to many of the solvents. The iron-sulfur cluster (ISC) and sulfur mobilization (SUF) systems which carry out biogenesis and maturation of all Fe/S clusters in prokaryotes, were variously upregulated. IscR, responsible for regulating Fe/S homeostasis and expression of a number of Fe/S proteins was upregulated in response to all compounds tested. Transcription of iscR itself is upregulated by diminishing Fe/S cluster availability but also by oxidative stress.

Secondly, transcription of genes involved in oxidative stress responses were upregulated suggesting the solvents tested at sub-growth-inhibitory concentrations impose stress on *E. coli* equivalent to oxidizing agents such as paraquat and other superoxide generators. Transcription of the SoxRS controlled genes encoding the PqiAB enzyme and genes encoding the YhcN and MntS enzymes associated with the presence of or resistance to hydrogen peroxide were upregulated.

Thirdly, transcription of genes encoding transport proteins were significantly upregulated. Whilst predictably this included multidrug efflux pumps encoded by *mdtI*, *mdtJ* and *emrB,* a swathe of transporters for importing amino acids (*oppABCDF*), inorganic ions (*feoA*, *feoB* and *efeO*) and siderophores (*exbBD*, *yncD* and *fhuF*) were upregulated at the transcriptional level. This interesting response appears to suggest that E. coli not only responds to solvents by exporting from the cytoplasm but by importing elements and compounds apparently essential to counter the solvent stress.

9.5 Conclusions

Solvent exposed environments are generally considered to be extreme. Solvents encompass a broad range of chemical compounds with relatively low polarity. These environments include natural oil or organohalide contaminated environments and industrial settings in which microbes are used to produce solvents or other

compounds in dual phase reactor systems where organic solvents are used to partition products out of the aqueous phase. Stress is exerted by interference with membrane function, the integrity of which is crucial for cell integrity, reproduction and activity. The ability of solvents to interfere with protein structure is perhaps an underestimated target for solvent stress, as implied by the central role of efflux pumps in solvent tolerance. It is a significant concern that selection for efflux pumps through exposure to solvents is likely to select for resistance to antimicrobials. Other solvent tolerance mechanisms include membrane adaptation and solvent biodegradation along with more generic strategies such as biofilm formation, motility and endospore formation. Whilst mechanisms of tolerance in aerobic bacteria have been extensively studied, less work has been done on anaerobic bacteria and archaea.

Acknowledgements JK and ML were supported by Australian Research Council Linkage Project LP110200610. MM was supported by an August Wilhelm Scheer Visiting Professorship.

References

Asako H, Nakajima H et al (1997) Organic solvent tolerance and antibiotic resistance increased by overexpression of *marA* in *Escherichia coli*. Appl Environ Microbiol 63(4):1428–1433

Atlas RM, Hazen TC (2011) Oil biodegradation and bioremediation: a tale of the two worst spills in U.S. history. Environ Sci Technol 45(16):6709–6715

Bren A, Eisenbach M (2000) How signals are heard during bacterial chemotaxis: protein-protein interactions in sensory signal propagation. J Bacteriol 182(24):6865–6873

Chidthaisong A, Conrad R (2000) Specificity of chloroform, 2-bromoethanesulfonate and fluoroacetate to inhibit methanogenesis and other anaerobic processes in anoxic rice field soil. Soil Biol Biochem 32(7):977–988

Futagami T, Fukaki Y et al (2013) Evaluation of the inhibitory effects of chloroform on ortho-chlorophenol- and chloroethene-dechlorinating *Desulfitobacterium* strains. AMB Express 3(1):30

Das T, Sehar S, et al. (2014). Influence of calcium in extracellular DNA mediated bacterial aggregation and biofilm formation. *Plos One* 9(3)

Das T, Sehar S et al (2013) The roles of extracellular DNA in the structural integrity of extracellular polymeric substance and bacterial biofilm development. Environ Microbiol Rep 5 (6):778–786

Fernandes P, Ferreira BS et al (2003) Solvent tolerance in bacteria: role of efflux pumps and cross-resistance with antibiotics. Int J Antimicrob Agents 22:211–216

Griebenow K, Klibanov AM (1996) On protein denaturation in aqueous-organic mixtures but not in pure organic solvents. J Am Chem Soc 118(47):11695–11700

Grostern A, Duhamel M et al (2010) Chloroform respiration to dichloromethane by a *Dehalobacter* population. Environ Microbiol 12(4):1053–1060

Hall-Stoodley L, Costerton JW et al (2004) Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2(2):95–108

Head IM, Jones DM et al (2003) Biological activity in the deep subsurface and the origin of heavy oil. Nat 426(6964):344–352

Head IM, Jones DM et al (2006) Marine microorganisms make a meal of oil. Nat Rev Microbiol 4 (3):173–182

Heipieper HJ, Keweloh H et al (1991) Influence of phenols on growth and membrane permeability of free and immobilized *Escherichia coli*. Appl Environ Microbiol 57(4):1213–1217
Irving RM, Elfarra AA (2013) Mutagenicity of the cysteine S-conjugate sulfoxides of trichloroethylene and tetrachloroethylene in the Ames test. Toxicol 306:157–161
Isken S, de Bont JAM (1998) Bacteria tolerant to organic solvents. Extremophiles 2:229–238
Kieboom J, Dennis JJ et al (1998) Identification and molecular characterization of an efflux pump involved in *Pseudomonas putida* S12 solvent tolerance. J Biol Chem 273:85–91
Kitanidis PK, McCarthy PL (2012) Delivery and mixing in the subsurface: processes and design principles for in situ remediation. Springer, New York
Koenig JC, Groissmeier KD et al (2014a) Tolerance of anaerobic bacteria to chlorinated solvents. Microbes Environ 29(1):23–30
Koenig J, Lee M, Manefield M (2014b) Aliphatic organochlorine degradation in sub-surface environments. Rev Environ Sci Bio/Technology 14(1):49–71
Lee M, Low A et al (2012) Complete chloroform dechlorination by organochlorine respiration and fermentation. Environ Microbiol 14(4):883–894
Li XZ, Zhang L et al (1998) Role of the multidrug efflux systems of *Pseudomonas aeruginosa* in organic solvent tolerance. J Bacteriol 180(11):2987–2991
Liu ZL (ed) (2011) Microbiology monographs: microbial stress tolerance for biofuels—Systems biology. Springer
Lollar BS, Ballentine CJ (2009) Insights into deep carbon derived from noble gases. Nat Geosci 2 (8):543–547
Maczulak A (2011) Clostridium. *Encycl Microbiol* 168–173
Martin PAW, Travers RS (1989) Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. Appl Environ Microbiol 55(10):2437–2442
Matsumoto M, de Bont JAM et al (2002) Isolation and characterization of the solvent-tolerant *Bacillus cereus* strain R1. J Biosci Bioeng 94:45–51
Mattos C, Ringe D (2001) Proteins in organic solvents. Curr Opin Struct Biol 11(6):761–764
Meckenstock RU, Von Netzer F et al (2014) Water droplets in oil are microhabitats for microbial life. Sci 345(6197):673–676
Moldowan JM, and Dahl J (1994) The molecular fossil record of oleanane and its relation to angiosperms. Sci 265(5173):768–71
Nicholson WL, Munakata N et al (2000) Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microbiol Mol Biol Rev 64(3):548–572
Pinkart HC, White DC (1997) Phospholipid biosynthesis and solvent tolerance in *Pseudomonas putida* strains. J Bacteriol 179(13):4219–4226
Ramos JL, Duque E et al (2002) Mechanisms of solvent tolerance in gram-negative bacteria. Ann Rev Microbiol 56:743–768
Rickard AH, Leach SA et al (2002) Phylogenetic relationships and coaggregation ability of freshwater biofilm bacteria. Appl Environ Microbiol 68(7):3644–3650
Sardessai YN, Bhosle S (2004) Industrial potential of organic solvent tolerant bacteria. Biotechnol Prog 20(3):655–660
Segura A, Duque E et al (2004) Fatty acid biosynthesis is involved in solvent tolerance in *Pseudomonas putida* DOT-T1E. Environ Microbiol 6(4):416–423
Segura A, Molina L et al (2012) Solvent tolerance in Gram-negative bacteria. Curr Opin Biotechnol 23(3):415–421
Shimizu K (2015) Metabolic regulation and coordination of the metabolism in bacteria in response to a variety of growth conditions. Adv Biochem Eng Biotechnol 1–51. doi: 10.1007/10_2015_320
Sikkema J, de Bont JAM et al (1995) Mechanisms of membrane toxicity of hydrocarbons. Microbiol Rev 59(2):201–222
Tomas CA, Welker NE et al (2003) Overexpression of groESL in *Clostridium acetobutylicum* results in increased solvent production and tolerance, prolonged metabolism, and changes in the cell's transcriptional program. Appl Environ Microbiol 69(8):4951–4965

Torres S, Pandey A et al (2011) Organic solvent adaptation of Gram positive bacteria: applications and biotechnological potentials. Biotechnol Adv 29(4):442–452

Vamvakas S, Herkenhoff M et al (1989) Mutagenicity of tetrachloroethene in the ames test—metabolic activation by conjugation with glutathione. J Biochem Toxicol 4:21–27

White SH, Wimley WC (1999) Membrane protein folding and stability: physical principles. Annu Rev Biophys Biomol Struct 28:319–365

Yung PY, Grasso LL et al (2016) Global transcriptomic responses of Escherichia coli K-12 to volatile organic compounds. Sci Rep 6:19899