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Review

Cytoplasmatic Bacterial Membrane Responses to Environmental Perturbations

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Abstract

Bacteria can adapt to various environmental factors such as temperature, pressure, ions, nutrients and toxic substances by modifying their membranes to maintain them in a fluid state. These modifications within the cytoplasmatic membrane particularly result from changes in the fatty acid composition and interaction between proteins and lipids. Fatty acids, mainly phospholipid fatty acids, play a role as a good biomarker of changes of physiological status of microorganisms caused by external factors. A greater understanding of the detailed physiological mechanisms of bacterial membrane lipid adaptation, especially to toxic substances and solvents, are important for researchers who use bacteria in bioremediation and biotransformation processes.

Keywords: environmental factors, bacterial membrane, fatty acids, adaptation

Introduction

Bacteria are unable to insulate themselves from the environment and they react to any fluctuations by changing their own physiological functions. The membrane is the site of the primary contact of the cell with the environment. It reflects both the nature of the intracellular components and the extracellular environmental conditions. The main function of the bacterial membrane is to form permeability barriers regulating the passage of solutes between the cell and the external environment. The membrane keeps essential metabolites and macromolecules inside the cells, it pumps nutrients into the cell and prevents the entry of certain solutes present in the environment [1, 2]. Flexibility and adaptation capability of membrane largely determine the survival ability of the bacteria [3].

Many external factors such as a temperature, pressure, pH, water activity, nutrients, ions, enzyme actions, growth phase of the microbial culture and xenobiotics affect physico-chemical properties of membrane and consequently their functioning. These changes include the balance between bilayer and nonbilayer lipids, stability and fluidity of membrane as well as altering lipid-protein interactions. The understanding of adaptation mechanisms is important in nature as well as in technological applications of microorganisms such as wastewater, waste gas treatment, bioremediation and biocatalysis.

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Chemical Nature of Bacterial Cytoplasmatic Membrane

The cytoplasmatic membrane of a bacterial cell consists of lipids that form a matrix in which enzymes and transport proteins are embedded [4, 5]. Proteins may be located at the periphery (peripheral proteins), span the membrane in part (integral proteins) or completely transverse the membrane (transmembrane proteins). Carbohydrate portions can be attached to proteins or lipids and extend outwards from the membrane [6]. In many membranes 50% of the mass is comprised of lipids [7]. The primary lipid components are the polar glycerophospholipids although other polar lipids such as glyceroglycolipids, sphingophospholipids, sphinglycolypids and neutral lipids are also present in bacterial membrane. Within the membrane, glycerophos-

pholipids are arranged with the head groups oriented externally and the lipid acyl chains directed to the interior of the bilayer. Membrane lipids have saturated and unsaturated fatty acids. The acyl chains have various structures such as branched (*iso*, *anteiso*, hydroxy fatty acids) and cyclopropane rings [8-10].

Fluidity is one of the most important parameters of the cytoplasmatic membrane, which allows keeping the homeostasis of the cell. The fluidity in a membrane is very hard to define because the membrane lipid layer is a special kind of fluid. It is an anisotropic solution and therefore the measurement of membrane fluidity is difficult to perform. The most important fluidity-related parameters are order-disorder transition, or gel to liquid-crystalline phase transition [11, 12]. On the basis of electron paramagnetic resonance (EPR) experiments, Sinensky [13] has suggested that the temperature-induced change in the membrane lipid composition is a mechanism to maintain its certain optimal fluidity (homeoviscous adaptation).

Under normal physiological conditions, the majority of glycerophospholipids in the bacterial membrane are bilayer forming, existing in a liquid-crystalline state [13, 14]. As temperature rises, the lipid molecules are melted and *trans-gauche* rotations (rotation about the carbon-carbon bonds) are able to propagate freely up and down the acyl chain [15, 16]. The acyl chains spread apart and have more conical shape. There is a greater amount of space between the polar head groups and consequently even more space to be allotted to each acyl chain [16, 17]. As temperature drops, the acyl chains adapt all *trans* conformation and are able to pack in a more ordered manner. The lipids become more cylindrical and the membrane takes on a more gel-like structure [18, 19]. Disturbance can also cause shifts to the hexagonal I (H_I) or hexagonal II (H_{II}) phase to occur [1, 20]. Upon transition to the gel state, the lipid chains become stiff and the frequency of *trans-gauche* isomerization is reduced [16]. During transition, the hydrophobic thickness, transmembrane permeability, lateral area compressibility of the membrane, as well as the resistance of the membrane to sheer forces may be affected [21]. The molecular shape and configurations of the membrane phospholipids illustrates Fig. 1.

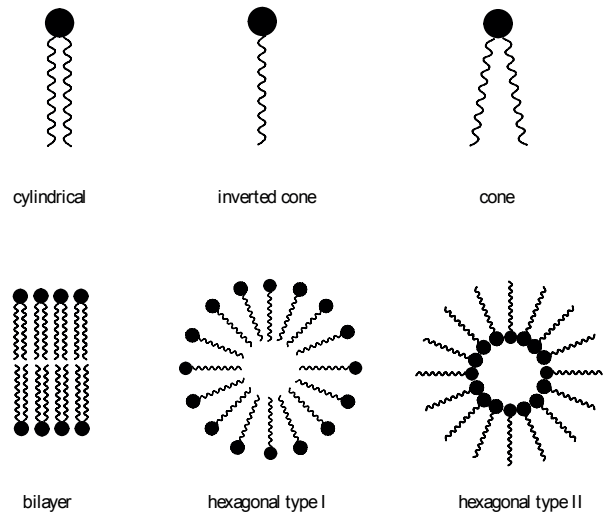

Fig. 1. Molecular shape and configuration of the membrane phospholipids.

Regulation of the membrane fluidity is possible by changing the ratio of saturated to unsaturated fatty acids [19, 22, 23], *cis* to *trans* unsaturated fatty acids [24-26], branched to unbranched structures, type of branching [23, 27, 28] and acyl chain length [23, 29]. In response to perturbations lipids also can modify their polar head groups [30]. These alterations happen less commonly and are less effective in modifying lipid fluidity on the transition temperature [23]. However, changing the head group composition may affect lipid-protein interactions [31]. Lipids are not the only molecules responsive to disturbance. Under stress conditions, the increased amount of specific proteins or *de novo* protein synthesis can result [32-35].

Factors Influencing Membrane Physical Properties

Temperature

Many changes in bacterial fatty acid composition and membrane fluidity occur in response to temperature fluctuations. As growth temperature rises, it is common to observe an increase of the proportion of long-chain and saturated fatty acids within the membrane. Conversely, short-chain, branched and saturated short chain fatty acids are preferred at lower temperature, as cooler temperatures act to solidify the membrane [13, 17, 36]. At low temperature bacteria synthesize longer unsaturated chain fatty acid, for example *cis*-vaccenic acid (18:1*cis*11) is favoured other palmitic acid (16:1) [23]. Changes in branching are more complex and involve an increase of branched fatty acids content as well as an increase in the ratio of the *iso/anteiso* isomers. Freedman [22] reported that the greater proportion of unsaturated or branched fatty acids allows the phase transition to occur at a lower temperature, whereas a greater proportion of saturated fatty acids allows the transition temperature to be elevated.

Henderson et al. [37] observed that temperature had significant effect on a lipid composition of *Vibrio* sp. The proportion of phosphatidyloetanolamine (PE) in total lipid was higher at 5ºC than at 20ºC. In opposite, the proportion of nonesterified fatty acids was lower at 5ºC than at 20ºC, whereas that of phosphatydyloglycerol (PG) was not altered. The levels of saturated fatty acids in total lipid, PE and PG were all decreased by growth at 5ºC. The reduction in growth temperature from 20ºC to 5ºC also caused increased proportions of *trans* 16:1 and 20:5 fatty. No differences were observed with respect to growth temperature in the level of *cis* 16:1, the main monoenoic fatty acid in both PE and PG. Suutari and Laakso [38] studied the changes in branching and unsaturation of fatty acids in *Streptomyces griseus* and *Brevibacterium fermentans* as a response to growth temperature. When temperature was reduced from 35 to 20ºC, changes in branched and

unsaturated fatty acids in *S. griseus* were observed, and below 20ºC only branched fatty acids were changed. In *B. fermentans*, two ranges of growth temperature in which the fatty acid changes were different had been found. Above 30ºC, the chain length of *anteiso*-branched was changed, and below 30ºC fatty acid unsaturation was varied. More complicated biphasic behaviour was observed in *Bacillus megaterium*. The saturated straight-chains and *iso*-branched acids decreased only from 40ºC down to 20 to 26ºC, and *anteiso* acids decreased only from 20 to 26ºC to 10ºC. Unsaturated fatty acids increased over the whole temperature range studied [39].

The temperature-dependent changes in lipid composition have also been detected in bacteria exposed to xenobiotics. It has been reported that pentachlorophenol-degrading strain *Sphingomonas* sp. UG30 after growth at 10, 20 and 30ºC in minimal medium changed percentages of its fatty acids. As the temperature increased, the saturated fatty acids increased from 3 to 12% while the unsaturated fatty acids decreased from 97 to 88% [10].

For some bacteria, changes in temperature did not influence membrane lipid composition. For example, *Staphylococcus aureus* grown at 25 and 37ºC showed no significant changes in fatty acid composition [10].

Pressure

Lipids are particularly sensitive to pressure effects. Generally, pressure causes the membrane lipids to pack more tightly, promoting the transition towards a gel state [19]. Membrane in the fluid phase is more resistant to the effect of pressure while membrane in the gel state characterizes more pressure sensitive [40]. Neutron diffraction experiments indicate that pressure increases bilayer thickness by reducing the "kinking" acyl chains [41].

Many deep-sea organisms modulate their membrane fluidity and composition in response to pressure. Studies with *Photobacteriun profundum* strain SS9 demonstrated that increases in culture pressure changed the proportion of both the monounsaturated fatty acid 18:1 as well as that of the polyunsaturated fatty acids 20:5 and 22:6 [42]. Effect on culture pressure on the proportion of the major fatty acids in *P. profundum* SS9 illustrates Fig. 2. Fang et al. [43] isolated two barophilic DB21MT-2 and DB21MT-5 strains, which characterized wide distribution of 20:5 (in DB21MT-2) and 22:6 (in both) polyunsaturated fatty acids. The presence of polyunsaturated fatty acids is uncommon in most bacteria but present in a higher proportion of isolates from low temperature and deep-sea environments [42]. Polyunsaturated fatty acids are probably produced by deep-sea bacteria for symbiotic interactions with higher deep-sea fauna, where they are needed as essential fatty acids. Alternatively, there may be key differences in the localization of these fatty acids within the membrane lipids. The increasing fatty acid unsaturation with pressure could be to maintain the membrane within a narrow range of viscosity [44].

High-pressure sites are usually coincident with the occurrence of low temperatures. It has been shown that barophilic strains of bacteria are able to change unsaturated fatty acid content and synthesize polyunsaturated fatty acids up to 22 carbons long and with 6 double bonds [19, 45]. The mechanism of producing polyunsaturated fatty acids is not known in detail but it is thought to be similar to existing mechanisms in eucaryotic cells [46]. Such high degrees of unsaturation allows membrane to retain a low gel to liquid-crystalline transition temperature, remaining fluid even under high-pressure and low temperature influence [47, 48].

Ions

Ions such as Ca^{2+}, Mg^{2+} and Fe^{3+} can protect the membrane and prolong the conditions under which bacteria can survive [49]. Martins et al. [50] studied the composition of polar lipid acyl chains of *Bacillus stearothermophilus* as affected by temperature and calcium. The total amount of branched chains decreased with increasing temperature of growth from 48 to 68ºC, whereas the straight chains increased. In the presence of Ca^{2+}, the lipid metabolism favours the biosynthesis of straight acyl chains with depression of branched chains, especially at lower temperatures. Luxo et al. [51] showed that in cells of *Bacillus stearothermophilus* treated with tamoxifen (TAM) and supplemented with calcium the acyl chains and the polar head groups of phospholipids were modified. Calcium ions may compensate for the tamoxifen disorder and transition temperature (T_m) shift or may

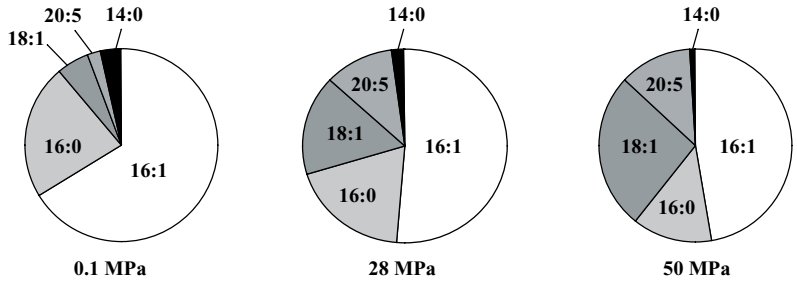

Fig. 2. Effect of culture pressure on the proportion of the major fatty acid species in the deep-sea bacterium *Photobacterium profundum* strain SS9 [44].

Table 1. Percentages of total fatty acids from *Ralstonia eutropha* H850 grown in the presence of fructose or biphenyl [60].

Fatty acid	Growth temperature (°C)			
	Fructose			Biphenyl
	10	30	37	30
	% of total fatty acids			
14:0	0.7	2.7	4.9	5.1
16:0	11.6	29.2	33.6	34.8
17:0 cyclo	ND[a]	1.5	8.0	7.8
14:0 3OH	11.2	11.3	10.1	9.0
16:1	49.7	30.7	27.2	29.5
18:1	26.8	24.6	16.2	13.8
SAT/UNSAT[b]	0.1	0.5	0.9	0.9

[a] Not detected, [b] SAT - saturated fatty acid including straight- and cyclo-chain; UNSAT - unsaturated fatty acid including hydroxylated chain.

decrease the incorporation of TAM into the bilayer. Ca^{2+} induced shift of T_m may also result in a deviation to higher temperatures of the transition from lamellar to a hexagonal phase. Therefore, the addition of TAM to cultures in the Ca^{2+} supplemented medium, may have a less negative impact on bilayer stability. General characteristics of fatty acyl chain distribution of *B. stearothermophilus* lipids as affected by tamoxifen are presented in Table 1. Similar effects promoted by Ca^{2+} and Mg^{2+} have also been reported for a *Pseudomonas putida* strain growing in the presence of repressing solvents [52]. The presence of other bivalent cations (Mn, Co, Cu) also resulted in an increase in membrane stability in the growth rate of *Enterococcus faecalis* cells from 42 to 46ºC [53]. When subjected to high ionic environments, bacteria can increase the negative charge of the membrane as a means of attracting cations Na^+ and H^+ so as to stabilize their membrane structure [54]. High salt concentrations and temperature can also affect fatty acid and phospholipid concentration in membranes of *Listeria monocytogenes*, *Bacillus subtilis* and *Synechocystis* sp. [55, 56].

Nutrients

Some investigations have shown that nutrient status can affect the fatty acid and protein composition of bacterial cells [57, 58]. Nichols et al. [59] showed that growth of *Shevanella gelidimarina* on differing sole carbon sources influenced the percentage and amount of eicosapentaenoic 20:5ω3 (EPA) fatty acid. The highest amounts of 20:5ω3 fatty acid occurred from growth on propionic acid and L-proline. Monounsaturated fatty acid components and EPA were concentrated in phosphatidylglycerol (PG), while the proportion of branched-chain fatty acids was elevated in phosphatidylethanolamine (PE). The association of EPA with 17:1 and 18:0 acyl chains in phospholipids was specific to PG, whereas the association of EPA with 13:0 *iso*; 13:0; 14:0 and 14:0 *iso* was specific to PE. Such acyl chain "tailoring" is indicative of the important role of EPA in bacteria membrane adaptative responses.

The growth medium has also affected the fatty acid composition of Ralstonia eutropha H850 strain grown on fructose and biphenyl. Total saturated straight and cyclo-chain fatty acids represented 33.8% of the total fatty acids in cells grown at 30ºC on fructose, and the ratio of total saturated to unsaturated fatty acids was 0.5. After growth at 30ºC on biphenyl, the saturated fatty acids in *R. eutropha* H850 increased to 47.6%, resulting in a ratio of total saturated to unsaturated fatty acids of 0.9. The increased saturation of membrane fatty acids in biphenyl-grown cells suggests that membranes may be less fluid compared to membrane from fructose-grown cells [60]. Percentages of total fatty acids from *R. eutropha* H850 grown in the presence of fructose or biphenyl demonstrates Table 2. It has been also shown that the Antarctic bacterium, strain JS6 can modify extensively the balance of even-chain, odd-chain, and *iso*-branched odd-chain length fatty acids during growth on different sources in seawater medium. For example, odd-chain fatty acids predominated with <10% branched fatty acids during growth on propionate, whereas growth on leucine gave <50% even-chain and >10% odd-chain fatty acids with most of the remainder being branched fatty acids. As growth temperature is decreased, there was a decrease in odd-chain fatty acids and a corresponding increase in even-chain fatty acids. The changes in branched fatty acids were smaller and complex with first a decrease and then an increase in their proportion as temperature lowered. At low temperature there was also an increase in the polyunsaturated fatty acid 20:5 [61, 62].

In bacteria from the genus *Pseudomonas* and *Vibrio*, exposed to phenolic compounds, the isomerization of *cis* unsaturated fatty acids to *trans* was observed. The *trans*/*cis* ratio of unsaturated fatty acids was defined as the

Table 2. General characteristics of fatty acyl chain distribution of *Bacillus stearothermophilus* lipids as affected by tamoxifen [51].

Additives to the growth medium		Fatty acid composition			
Ca^{2+} (2.5 mM)	Tamoxifen (μM)	Total straight	Total branched	Total *iso*-acids	Total *anteiso*-acids
_	0	33.8±1.35	66.2±1.38	38.2±3.9	27.4±2.4
_	2.5	34.1±1.85	65.8±1.86	40.0±2.0	25.2±0.5
_	5	38.8±1.84	61.1±1.85	40.1±1.1	20.4±1.1
+	0	35.5±0.75	64.5±0.75	35.1±1.4	28.9±0.52
+	7.5	37.0±0.55	62.8±0.54	38.4±1.85	24.0±2.2
+	10	40.4±2.2	59.6±2.2	36.8±1.13	22.2±1.4

ratio between the amounts of two trans unsaturated fatty acids (16:1 and 18:1) and two *cis* unsaturated fatty acids (16:1 and 18:1) of the bacterium [63]. The *trans*/*cis* ratio has been used as an index for the nutrition status of microbial communities [24, 64]. For example, the presence of *trans* fatty acids has been associated with survival at low nutrient levels for cells of *Vibrio cholerae* [65, 66]. Moreover, the *cis*/*trans* isomerization also seems to be a good toxicity test for organic environmental contaminations [63].

Lipophilic Compound Effects on Membrane Composition

Lipophilic compounds accumulate in the lipid bilayer, resulting in alteration of membrane organization and functions. The extent of the toxic effect is related to the actual concentration in the membrane, the location in the membrane and the interaction with the membrane constituents. As a result of accumulation of lipophilic compounds, the membrane loses its integrity, resulting in the dissipation of transmembrane gradients of protons and ions [67]. The hydrocarbons inhibit cellular respiration, growth, and at saturating concentrations may even cause lysis of the cell. Accumulation of lipophilic compounds occurs at varying depths in the bilayer, depending on the hydrophobicity at presence of functional group such as a hydroxyl-, carboxyl-, or phenyl groups. Interaction of these chemicals with the hydrophobic end of the acyl chains has the most pronounced effect on the surface area of the membrane, whereas more hydrophilic compounds like ethanol, phenol effect the hydration of the head groups [68-70]. Ingram [71] observed that the composition of the membranes in the presence of ethanol was altered either by increasing the chain length of the fatty acids or by increasing the proportion of *cis* monounsaturated fatty acids. Relatively small hydrophobic compounds such as tetralin, decalin, biphenyl and anthracene intercalate with the acyl chains disturbing the acyl chain interactions which results in "swelling" of the membrane, and the increase in bilayer fluidity [57]. Large hydrophobic molecules such as longer alkanes do not interact with the head groups and accumulate more deeply in the lipid bilayer. They intercalate with the acyl chains, but probably compensate the disturbing effects by interacting with both the inner and outer leaflet of the lipid bilayer [1, 72].

Bacteria that are able to survive in the presence of lipophilic compounds exhibited adaptation changes in their membrane lipids to compensate for the fluidizing effect of hydrocarbon compounds. In cells of *Rhodococcus opacus* strains GM-14, GM-29 and 1CP, the content of branched (10-methyl) fatty acids was 3- to 10- fold higher of the total fatty acid when the cells grown on benzene, phenol, 4-chlorophenol, 4-chlorobenzene, or toluene as the sole source of carbon and energy, in comparison with cells grown on fructose [73]. The results suggest that methyl-branched fatty acids may participate in the adaptation of bacteria to lipophilic aromatic compounds. Another strain *Rhodococcus* sp. 33 in the presence of benzene increased content of 10Me18:0 fatty acid and ratio of saturated to unsaturated fatty acid from 1.3 to 1.5. Gutierrez et al. [74] claimed that an increase in branched and saturated fatty acids is a possible mechanism to decrease the fluidity of the cell membrane to tolerate benzene. *Pimelobacter* sp. was found to regulate reduced membrane fluidity in a different way. When bacteria are grown on pyridine, the proportion of isopalmitic acid (*iso* 16:0) drastically decreased from 68.4% to 7.7%, while the proportion of anteisoheptadecanoic acid (*anteiso* 17:0) remained almost constant. A decrease of the branched-chain fatty acids was accompanied by the increase of the straight-chain, especially long chain, fatty acids such as 17:0, 18:1, 10Me18:0 and 10Me19:0 [75].

In response to toxic compounds, microorganisms may also increase the abundance of saturated and *trans* unsaturated fatty acids in their cell membranes. In *Escherichia coli* K-12 and *Pseudomonas putida* P8, the degree of saturation can be modified only by growing cells on phenolic compounds and they change the proportions of fatty acids synthesized *de novo* and incorporated into the phospholipid molecules [17, 24]. In some bacteria there apparently exists an alternative way of regulation of membrane fluidity, which is, in contrast to the former, post-synthetic independent of lipid synthesis and thereby of growth of the cells. The conversion of *cis* to *trans* unsaturated fatty acids has the consequence of decreasing the membrane

fluidity. The steric behaviour of *trans* unsaturated fatty acids in membrane is not very distinct from that of saturated acyl chains, which possess a long extended conformation and a small molar volume. For non-growing cells the isomerization of the double bond of unsaturated fatty acids seems to be a suitable and energetically feasible way for membrane fluidity modification compared with a mechanism based on *de novo* synthesis of fatty acids. The increased synthesis in saturated and *trans* unsaturated fatty acids provides cell membranes with a greater rigidity by decreasing the membrane fluidity. This tolerance mechanism effectively reduces the accumulation of toxic compounds in the membrane [57]. Both *de novo* synthesis of various mixtures of saturated fatty acids and the *cis* to *trans* isomerization were observed in *P. putida* P8, *P. putida* S12 and *Vibrio cholerae* strains [24, 63, 65, 76-78].

The formation of cyclopropane fatty acids is also known as a possible post-synthetic modification of cis unsaturated fatty acids. This conversion does not significantly change the physiological properties of the membrane and its physiological function is still not understood [10, 24, 79].

Regarding ecology and biotechnology, the mechanism of isomerization of *cis* to *trans* unsaturated fatty acid is particularly important for microorganisms that are specialized in degradation of toxic and membrane fluidity-influencing compounds, such as phenols. The application of solvent-tolerant microorganisms enables the biodegradation of pollutants with very high toxicity. For example, solvent-tolerant bacteria were used to remove aromatic compounds from wastewater, contaminated soils and sediments. The use of such microorganisms would decrease the costs of biodegradation by reducing the time required for cleaning and in comparison to other traditional physico-chemicals methods seems to be an attractive technology of restoring of polluted sites.

Content of Membrane Protein

Lipids are not the only molecules responsive to environmental disturbances. It has been observed that under stress conditions (i. e. heat or cold shock) bacteria produce the low molecular weight stress proteins [33]. In cold shocked bacteria some of the *de novo* production is attributed to increased desaturase levels, which act to modify membrane lipids [34]. Under heat stress, bacteria modify the fluidity of the membrane through interactions of heat shock proteins with lipids and other membrane proteins [80]. Alteration of protein content has also been observed during starvation. In this state bacteria mediate the production of recovery and degradatory proteins [81]. The formation of individual membrane proteins in the presence of aromatic compounds has also been observed. For example, under phenol-stress conditions, in *Escherichia coli* K-12 only one protein with a molecular weight of 45 kD was expressed in greater amount. Apart from this, several proteins showed reduced expression after phenol addition [82].

Summary

Bacteria in nature are exposed to variations in temperature and are affected by the availability of nutrients, ions and water, and the presence of toxic molecules. Their reactions to these factors require a series of rapid adaptive responses for survival in changing environments. Detailed understanding of these mechanisms helps us to know how environmental disturbances and xenobiotics act to affect the membrane and exert toxic effects on microbial cells. There are still many areas where information is lacking, but in the latest research it would be possible to provide insight into structural changes occurring in the membrane.

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