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minireview

Microbial Ecophysiology of *Vibrio ruber*

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Summary

Bacteria use different adaptation strategies to survive environmental perturbations. In this minireview, adaptation strategies of new red-pigmented *Vibrio ruber* isolated from coastal environments to different environmental stresses (*i.e.* salinity, viscosity, UV light, mitomycin C, nutrient availability and temperature) are reviewed. To cope with environmental stresses *Vibrio ruber* uses several different adaptive strategies. For example, lipid composition as well as phase behaviour are strongly dependent on salt concentration. *Vibrio ruber* membrane has no hydroxy fatty acids, but exceptionally high lysolipid content compared to other related *Vibrio* species. Inorganic nutrient uptake by bacteria is selective, depends on environmental conditions and varies several fold with environmental perturbations. Protein composition, carbon flow through the central metabolic pathways, energy generation as well as secondary metabolite production adapt readily to stress conditions. The activity of glucose-6-phosphate dehydrogenase proved to be a good indicator of *Vibrio ruber* stress. Cells are able to modulate their local viscosity in response to variations of environmental viscosity. The bacterium harbours several viral genetic elements in its genome, which could be induced by mitomycin C. Environmental conditions during growth of bacteria have a significant effect on lysate carbon turnover. Secondary metabolite prodigiosin confers protection against UV in the environment, which adds to the known repertoire of prodigiosin ecophysiological functions. In conclusion, *Vibrio ruber* in its short acquaintance with the scientific community (less than ten years) has proven to be an immensely valuable model system for ecophysiological studies of bacteria.

Key words: ecophysiology, secondary metabolites, prodigiosin, *Vibrio ruber*, marine bacteria

Introduction

Bacteria usually live in an environment that is constantly perturbed and have to coordinate their metabolic processes in order to maximize their efficiency. Marine and coastal environments are attractive and highly diverse, which enables a rich microbial community to thrive. A common strategy in this environment is synthesis of metabolites that do not affect producer's growth and development (*1*,*2*), but have diverse survival functions (*3*). The adaptive mechanisms include production of exopolymers, biofilm formation, quorum sensing, antimicrobial agents, maintenance of cellular metabolism and membrane integrity (*2*).

Among bacteria, genus *Vibrio* is probably the most widely distributed group of microorganisms in marine, coastal and estuarine environments. It is a group of highly diverse bacteria, which contains both free-living bacteria and bacteria associated with eukaryotes (*4*). Some species infect marine animals, others cause intestinal or extraintestinal infections in humans (*5*). In natural environment vibrios often face unfavourable conditions due to fluctuations of salinity, temperature and radiation. One of the adaptation strategies is to enter into a viable but non-culturable (VBNC) state, when cells decrease metabolic activity and alter their cell shape (*6*,*7*). Vibrios also produce a diverse array of secondary metabolites, which help them to adapt to changing environment and

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to outcompete other bacteria. For a recent review about active secondary metabolites produced by vibrios, a reader is advised to read Mansson *et al*. (*8*). In this minireview, we will focus on *Vibrio ruber*, a new producer of a red-pigmented secondary metabolite prodigiosin, which confers versatile ecological advantages to the producer. It also has excellent potential for biotechnological and medical applications (*9*). Many vibrios like *Vibrio gazogenes (10*), *Vibrio psychroerythrus* (*11*), *Vibrio ruber* (*12*) and *Vibrio rhizosphaerae* (*13*) produce prodigiosin. A decade ago, a new red-pigmented *Vibrio ruber* was isolated from shallow marine coastal regions (*12*,*14*). This organism proved to be a very good model system to study bacterial ecophysiology. In this paper, recent developments in the study of *Vibrio ruber* and several of its adaptation strategies to changing environmental conditions will be reviewed.

Vibrio ruber

Red-pigmented bacterium *Vibrio ruber* DSM 14379 was isolated from estuarine waters of the Northern Adriatic Sea (*14*) approximately at the same time as *Vibrio ruber* JCM 11486 from a shallow coastal region in Taiwan (*12*). *Vibrio ruber* DSM 14379 is able to grow in a wide salinity range from 0.05 to 17 % NaCl, with the optimal growth rate at around 2.5 % NaCl. There is no growth in the absence of NaCl (*15*,*16*). When growing in a minimal growth medium, it can utilise glucose, glutamate, acetate, trehalose, galactose, mannose, sucrose, mannitol, citrate, L-aspartate, L-glutamate, L-alanine, L-arginine or L-ornithine as a sole carbon source (*17*). In the minimal growth medium with glucose, the highest growth rate was obtained at low glucose concentration of 0.5 g/L (*17*). It grows at temperatures between 10 and 44 °C with an optimum at around 40 °C. It is not able to grow at 4 and 45 °C (*17*,*18*). The strain grows at pH=5.0–8.0 with the optimum at 7.0 (*17*,*18*). Since *Vibrio ruber* DSM 14379 is a facultative anaerobe, it is well adapted to changing oxygen levels in its natural environment. Under anaerobic conditions, this bacterium ferments glucose, fructose, mannose, maltose, sucrose, galactose, trehalose and sorbitol, but it is not able to ferment rhamnose, lactose, dulcitol and inositol (*17*). *Vibrio ruber* DSM 14379 can grow in a viscous environment ranging from 0.8 to 29.4 mPa·s without a significant change in the growth rate (*19*). There are some characteristics that differentiate *Vibrio ruber* DSM 14379 from *Vibrio ruber* JCM 11486. *Vibrio ruber* JCM 11486 is not able to grow at 0.1 % NaCl, it is not able to utilize trehalose, sorbitol, L-alanine, L-arginine or L-ornithine, and it is not susceptible to vibriostatic agent O129. On the other hand, it is able to utilize lactose, whereas *Vibrio ruber* DSM 14379 is not. Their genomes show (82.6±0.5) % DNA similarity (*20*). Both strains are also highly similar according to multilocus sequence typing (MLST) analysis on 16S rRNA and 6 housekeeping genes (*i.e. rpoA*, *recA*, *ftsZ*, *gapA*, *gyrB* and *mreB*) (*19*). Although *Vibrio ruber* JCM 11486 was first described by Shieh *et al*. (*12*), not much is known about its physiological response to environmental parameters. On the other hand, ecophysiological response of *Vibrio ruber* DSM 14379 is better described and will be further reviewed.

Ecophysiological Response of *Vibrio ruber* DSM 14379 to Different Environmental Factors

Salinity

Changes of salinity are frequent in *Vibrio ruber* natural environment and they affect lipid and protein composition significantly. Analysis of lipid extracts showed that *Vibrio ruber* has four different polar lipid headgroups in the membrane: phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE) and lysophosphatidylethanolamine (lyso-PE). The amount fraction of a given polar lipid headgroup depends on the salt concentration in the medium (*15*). Membrane is composed of (in mole percentage): PE 45.4, PG 27.5, lyso-PE 19.1 and DPG 8.0 at optimal salt level (3 % NaCl). At 3 % NaCl, the amount fractions of anionic lipids (PG and DPG) and lyso-PE were the highest, while the amount fraction of PE was the lowest. Surprisingly, the amount fraction of lysolipids was exceptionally high compared to other related *Vibrio* species (*21*). These lipids, together with PE, have a tendency to form non-bilayer lipid structures and increase membrane permeability (*22*,*23*). Lipid extracts from *Vibrio ruber* formed mixtures of lamellar and normal cubic-like phases as a result of high amount fraction of lyso-PE and PE at all salt concentrations. Similarly, the acyl chain composition was also affected by salt concentration in the medium (*15*). The major fatty acid in *Vibrio ruber* lipid extract is oleic acid (18:1), the mole fraction of which decreased from 71 to 53 with the increase of salinity. The total amount of unsaturated fatty acids (18:1 and 16:1) decreased, while the total amount fraction of saturated fatty acids (18:0 and 16:0) increased with the increase of salinity. Unexpectedly, there were no hydroxy fatty acids present in lipid extracts, which are typical for *Vibrio* species (*24*). It has been shown that salinity affects membrane reduction potential (*15*). The maximal rate of TEMPONE reduction was observed under optimal salt conditions and decreased asymmetrically at low and high salt concentrations. A more pronounced decrease of TEMPONE reduction was observed towards low salt concentrations. Apart from changes in lipid composition and membrane activity, *Vibrio ruber* also modifies protein composition in response to salinity changes. The major cell element (C, N, P and S) content decreased with increased salinity (*16*). There was no change in the C/N/P ratio, while C/S ratio increased with increased salinity. Bacterial cells accumulated different amounts of minor and trace elements and showed different 2D protein profiles in response to salinity (*16*,*18*). Central metabolic pathways (glycolysis, tricarboxylic acid cycle, and respiratory chain) of *Vibrio ruber* were modulated by salinity (*25*). At low salt concentrations, the total endogenous respiration, dehydrogenase activity and net intracellular adenosine triphosphate (ATP) concentration increased, while the phosphofructokinase (PFK) and pyruvate kinase (PK) activity decreased. At high salt concentrations, G6PD activity significantly increased and correlated with a 10-fold increase in the concentration of osmoprotectant L-proline. The L-proline biosynthesis is a reduction pathway that proceeds in five metabolic steps from the α-ketoglutarate precursor (*26*), three of which require NADPH as a redox equivalent. The production of L-proline increased the ratio of intracellular NADP/NADPH, which

regulates carbon flux through the oxidative pentose phosphate pathway. In addition, the total endogenous respiration and dehydrogenase activity increased, while the net intracellular ATP concentration, PFK or PK activity remained unchanged at high salt concentrations. The total dehydrogenase activity positively correlated with total endogenous respiration. This is consistent with the notion that the more capable the cells are to reduce tetrazolium salts (substrate for dehydrogenases), the more CO_2 they release per cell (*27*). *Vibrio ruber* metabolic activity responded to low and high salt concentrations asymmetrically. At low salinities, there was a severe metabolic imbalance resulting in decreased glycolytic activity and higher electron transport activity. At high salinities, there was an increase in glycolytic activity and production of reducing equivalents, which were partly used for biosynthesis and growth and partly for maintenance. In general, cells spent more energy on maintenance than on growth. Salinity also had an effect on the secondary metabolite production (*28*). The highest amount of prodigiosin occurred at optimal salt concentration. There was an inhibition of prodigiosin production at low and high salt concentrations. All these results show the extent, richness, and magnitude of adaptation mechanisms in *Vibrio ruber* in response to changing salt concentration in the environment.

Viscosity

Vibrio ruber can live in planktonic and biofilm forms. Due to different viscosities in these two environments, cells experience different molecular diffusion and transport phenomena. Environmental viscosity influences the production of primary and secondary metabolites in *Vibrio ruber* (*19*). At the level of primary metabolism, cells increased their metabolic activity (*i.e.* endogenous respiration and total dehydrogenase activity) to maintain the same growth rate at viscosities higher than 2.4 mPa·s. At still further increased viscosities in the environment, the pentose phosphate pathway activity (*i.e.* G6PD) was notably elevated, indicating an increasing demand for biosynthetic intermediates or reducing equivalents. At viscosities higher than 8 mPa·s, cells decreased the prodigiosin synthesis up to 5-fold and pigmentation dynamics changed significantly. Prodigiosin synthesis started later and lasted for a shorter time. This led to smaller amounts of prodigiosin produced at high viscosities. Another unexpected strategy that *Vibrio ruber* employs is the ability to change the viscosity of its local environment (*19*). At low environmental viscosities up to 2.4 mPa·s, *Vibrio ruber* slightly increases the viscosity of the medium, producing extracellular polymeric substances. On the other hand, *Vibrio ruber* actively decreases local viscosity at environmental viscosities above 8.1 mPa·s. How bacteria maintain viscous homeostasis in the environment is not well understood yet.

UV light

UV stress has a huge influence on bacterial survival. During evolution, bacterial cells have developed many different mechanisms which help them survive UV stress. Prodigiosin extracted from *Vibrio ruber* cells has a broadband absorption range in the visible spectrum between 400 and 600 nm with a maximal absorbance at a wavelength of 530 nm, and a smaller absorption band in the UV range from 240 to 400 nm (*20*,*29*–*31*). Due to absorption in the UV range, prodigiosin could provide a protection of *Vibrio ruber* cells from UV irradiation. It has been shown that non-pigmented mutant strain is dramatically more susceptible to UV stress than the wild-type (*20*). Pigmented cells survived the highest UV dose applied (324 J/m^2), approx. 1000-fold better than the non-pigmented cells. The protective role of prodigiosin was more evident at higher UV doses and positively correlated with prodigiosin concentration in the cell (*20*,*28*). Survival of *Vibrio ruber* cells under UV stress was dependent on *Vibrio ruber* physiological state and UV doses applied (*20*). Cell resistance to UV increased from lag to stationary growth phase. When non-pigmented cells were grown in co-cultures with the wild-type cells, they overgrew the wild-type strain under the conditions of low UV stress. The ratio changed in favour of the wild-type strain at higher UV doses tested. This suggests that prodigiosin synthesis causes energy cost for the producing bacteria. Nevertheless, prodigiosin may confer protection against UV in the environment and is overall good for the cell.

Mitomycin C

One of the bacterial responses under stressed conditions is induction of temperate viruses. *Vibrio ruber* harbours several viral genetic elements in its genome, which could be induced by mitomycin C (*32*). Environmental conditions during prophage induction influence the process of induction and replication of different *Vibrio ruber* viruses. The diversity of induced virus-like particles was dependent on salinity, temperature and substrate concentration or composition (*32*). The environmental conditions during virus induction with mitomycin C had a significant effect on the duration of viral latent period, size distribution of complete phages or phage structural parts and the decay rate of *Vibrio ruber.* Upon induction of prophages, less than 0.0001 % of cells survived. Prophage induction dramatically changed host cell metabolic activity. Cells induced with mitomycin C produced more ATP, and decreased the level of nucleotide diphosphate (NDP) sugars, nucleotides and coenzymes, as demonstrated by ^{31}P-NMR (*33*).

Vibrio ruber cells, when lysed by prophage induction, release organic material that can be quickly metabolised by microorganisms in the environment. It is not known how the quality of the released bacterial material affects the flow of carbon in the environment. The results of prophage induction experiments indicate that lysate composition can vary substantially under different environmental conditions. The lysate quality was not the same when it was prepared under high or low salt concentrations or at different temperatures. In this respect, studies of lysate uptake that have been performed with different quality of *Vibrio ruber* lysate are unique. Lysates were prepared either by biological means (virus lysis) or mechanically (sonication, autoclaving) under different growth conditions. The results indicate that environmental conditions affect bacterial cell composition and consequently lysate quality (*15*,*16*,*18*,*32*). Differences in lysate quality influenced the growth rate of *Pseudoalteromonas* sp. as well as its composition. In the simple microbial loop the

initial differences in lysate quality were preserved and were propagated through it (*16*,*18*). This indicates that environmental stress, cell composition and carbon flow in the ecosystem may be coupled. On the other hand, *Vibrio ruber* grown on its own lysate decreased the initial differences in lysate quality, thereby neutralizing the primary effect of environmental conditions on carbon turnover. It has been suggested that low Zn concentration in lysates neutralised the response (*18*).

Nutrients

Nutrient availability is an important parameter that has an effect on bacterial ecophysiology. *Vibrio ruber* can utilise a broad spectrum of organic and inorganic compounds for its growth (*17*,*18*). The uptake of different elements from the growth medium can vary significantly under different environmental conditions. For example, *Vibrio ruber* can accumulate 260 mg per kg of dry cell mass of Fe under optimal salt fractions (3 % NaCl), 349 mg/kg at 0.28 % NaCl and only 100 mg/kg at 10 % NaCl (*18*). Similarly, pH or temperature may affect Fe uptake dramatically. It has been demonstrated that *Vibrio ruber* selectively accumulates elements from the environment (*18*). The following general observations were made: (*i*) an element is accumulated in *Vibrio ruber* cells to a high level (*e.g.* Zn, Fe) or it is only moderately accumulated (*e.g.* Ca, Ni); (*ii*) the level of the element accumulation in the cell is either significantly dependent on the environmental conditions (*e.g.* Fe, Zn, Ti, Pb) or less sensitive to them (*e.g.* Ca, Na, K). The degree of selectivity is therefore substantially modified in different environments. Different carbon sources such as glucose, fructose and glutamic acid, on the other hand, influence prodigiosin production. The highest pigmentation occurred when bacteria were grown in the minimal growth medium with fructose as a sole carbon source. The concentration of carbon source also changed prodigiosin production (*28*). Cells produced maximal prodigiosin concentration at 5 g per L of glucose and the production decreased at both high and low glucose concentrations in the medium.

Temperature

Temperature is one of the most important environmental factors, which determines the growth and survival of bacterial cells, affects enzymatic reaction, and consequently bacterial metabolic activity. *Vibrio ruber* cells changed the composition of minor and trace elements as a response to different temperatures (*18*). Prodigiosin production was dependent on temperature (*28*). There was no pigmentation below 15 °C, and the production was significantly reduced at 43 °C. The maximal prodigiosin production occurred at 28 °C.

Ecophysiological Role of Prodigiosin

Prodigiosin is a red-pigmented secondary metabolite produced by several marine bacterial species including *Serratia* sp., *Streptomyces coelicolor*, *Hahella chejuensis*, *Pseudoalteromonas denitrificans* and *Vibrio* sp. (*10–12*,*20*, *34–37*). Prodigiosin from *Serratia marcescens* was the first isolated and chemically determined prodiginine (*38*,*39*). Prodiginines are a heterogeneous group of molecules, which have a common pyrrolyldipyrromethane core structure and different biological activities such as antibacterial, antifungal, antimalarial, immunosuppressive and anticancer (*9*,*28*,*36*,*40–43*). Biosynthesis of prodigiosin and its regulation in *Streptomyces coelicolor* and *Serratia marcescens* is well documented. Prodigiosin synthesis involves two separated biochemical pathways, which are coupled in the final condensation step of 2-methyl-3-*n*--amyl pyrrole (MAP) and 4-methoxy-2,2′-bipyrrole-5--carbaldehyde (MBC) (*34*,*44–46*). Biosynthesis genes are organised in *red* cluster containing 23 genes in *Streptomyces coelicolor* A3(2) (*47*) and *pig* cluster with 14 genes in *Serratia marcescens* ATCC 274 (*46*). It is known that prodigiosin biosynthesis is regulated by quorum sensing (*48*,*49*). Several studies have demonstrated that environmental factors strongly influence prodigiosin production (*19*,*20*,*28*,*50–52*). As a natural antibacterial colourant, prodigiosin has a dyeing potential for industrial applications. It has been shown that prodigiosin can be used as a dye for polyvinyl chloride (*53*), many fibres such as wool, nylon, silk and cotton (*43*,*54*) as well as rubber latex, polymethyl methacrylate and paper (*55*). In contrast to undisputable biotechnological use and benefit for humanity, the benefit of prodigiosin for the producer cell is a more controversial issue.

During several decades of studies, some putative ecopysiological roles of prodigiosin have been proposed. These include air dispersal of bacteria (*56*), metabolic sink for NAD(P)H or proline (*57*), storage of light energy (*58*), anion exchange (*59*), energy spilling function (*60*), and antimicrobial activity (*28*,*61*). It has been shown that prodigiosin has an antibacterial effect on the growth of non-related bacteria isolated from the same environment, *i.e. Bacillus* sp. (*28*) or other non-related bacteria such as *Pseudoalteromonas* sp., *Escherichia coli*, *Salmonella typhimurium* or *Micrococcus luteus* (*17*). This should give *Vibrio ruber* a competitive advantage in the environment. When *Vibrio ruber* and *Bacillus* sp. were grown together in co-culture experiments, Malthusian fitness (*62*) of *Bacillus* sp. dropped from 4 to –7.6 as compared to the monoculture (*28*). The corresponding decrease in non-pigmented mutant was significantly lower. This indicates that prodigiosin may help *Vibrio ruber* to outcompete non-related bacteria. On the other hand, prodigiosin does not affect the growth of *Serratia marcescens*, which also produces prodigiosin (*34*). A further ecophysiological function of prodigiosin is its ability to absorb UV light. In shallow coastal water environments with high UV stress, the ability to produce UV protective pigment prodigiosin may further give a competitive edge to *Vibrio ruber*. There is a drawback as the results show; as bacteria produce prodigiosin, more UV is absorbed, which in turn reduces penetration depth of UV and consequently enables protection and co-habitation with bacteria that are resistant to prodigiosin antibacterial activity. Prodigiosin production is a luxurious good that *Vibrio ruber* synthesises only under favourable conditions (*19*,*20*,*28*). This implies that bacteria cannot afford to spend a lot of energy on prodigiosin synthesis under stress conditions. The research done so far has raised several questions about the main ecophysiological role of prodigiosin. Most bewildering perhaps is why prodigiosin is produced only at the time of abundance and not under stress condi-

tions, when its antimicrobial and UV protection properties would be most helpful.

Conclusions

In conclusion, *Vibrio ruber* in its short acquaintance with the scientific community (less than ten years) has proven to be an immensely valuable model system for ecophysiological studies of bacteria. Its phenomenological response to different environmental conditions is now rather well characterised. However, there is an urgent need to characterize its molecular makeup and regulation, which may prove as rewarding as the study of ecophysiological response. The first step in this direction has already been done: the genome of *Vibrio ruber* DSM 14379 has recently been sequenced.

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References

1. P.R. Jensen, W. Fenical, Marine bacterial diversity as a resource for novel microbial products, *J. Ind. Microb. Biotechnol. 17* (1996) 346–351.
2. C.C.C.R. de Carvalho, P. Fernandes, Production of metabolites as bacterial responses to the marine environment, *Mar. Drugs, 8* (2010) 705–727.
3. A.L. Demain, A. Fang: The Natural Functions of Secondary Metabolites. In: *Advances in Biochemical Engineering/ Biotechnology: History of Modern Biotechnology I, Vol. 69*, A. Fietcher (Ed.), Springer, Berlin, Germany (2000) pp. 2–39.
4. M.K. Nishiguchi, B.W. Jones: Microbial diversity within the *Vibrionaceae*. In: *Origins, Evolution, and the Biodiversity of Microbial Life*, J. Seckback (Ed.), Kluwer Academic Publishers, Dordrecht, the Netherlands (2004) pp. 529–548.
5. F.L. Thompson, I. Tetsuya, J. Swings, Biodiversity of vibrios, *Microbiol. Mol. Biol. Rev. 68* (2004) 403–431.
6. R.R. Colwell, K.J. Grimes: *Nonculturable Microorganisms in the Environment*, American Society for Microbiology Press, Washington, D.C., USA (2000).
7. J.D. Oliver, The viable but nonculturable state in bacteria, *J. Microbiol. 43* (2005) 93–100.
8. M. Mansson, L. Gram, T.O. Larsen, Production of bioactive secondary metabolites by marine *Vibrionaceae*, *Mar. Drugs, 9* (2011) 1440–1468.
9. J.W. Bennett, R. Bentley, Seeing red: The story of prodigiosin, *Adv. Appl. Microbiol. 47* (2000) 1–32.
10. G.R. Allen, J.L. Reichelt, P.P. Gray, Influence of environmental factors and medium composition on *Vibrio gazogenes* growth and prodigiosin production, *Appl. Environ. Microbiol. 45* (1983) 1727–1732.
11. J.Y. D'Aoust, N.N. Gerber, Isolation and purification of prodigiosin from *Vibrio psychroerythrus*, *J. Bacteriol. 118* (1974) 756–757.
12. W.Y. Shieh, Y.W. Chen, S.M. Chaw, H.H. Chiu, *Vibrio ruber* sp. nov., a red, facultatively anaerobic, marine bacterium isolated from sea water, *Int. J. Syst. Evol. Microbiol. 53* (2003) 479–484.
13. N.R. Kumar, S. Nair, *Vibrio rhizospherae* sp. nov., a red-pigmented bacterium that antagonizes phytopathogenic bacteria, *Int. J. Syst. Evol. Microbiol. 57* (2007) 2241–2246.
14. D. Stopar, A. Cerne, M. Zigman, M. Poljsak-Prijatelj, V. Turk, Viral abundance and a high proportion of lysogens suggest that viruses are important members of the microbial community in the Gulf of Trieste, *Microbial Ecol. 47* (2004) 1–8.
15. T. Danevčič, L. Rilfors, J. Štrancar, G. Lindblom, D. Stopar, Effects of lipid composition on the membrane activity and phase behaviour of *Vibrio* sp. DSM14379 cells grown at various NaCl concentrations, *Biochim. Biophys. Acta, 1712* (2005) 1–8.
16. D. Odić, V. Turk, D. Stopar, Environmental stress determines the quality of bacterial lysate and its utilisation efficiency in a simple microbial loop, *Microbial Ecol. 53* (2007) 639–649.
17. T. Danevčič, D. Stopar: Environmental Quality Determines Physiological Behaviour of Bacteria. In: *Handbook of Environmental Quality*, E.K. Drury, T.S. Pridgen (Eds.), Nova Science Publishers, Inc, New York, NY, USA (2009) pp. 349–364.
18. D. Odić, B. Budič, I. Mandić-Mulec, D. Stopar, Influence of bacterial lysate quality on growth of two bacterioplankton species, *Microbial Ecol. 59* (2010) 246–252.
19. M. Borić, T. Danevčič, D. Stopar, Viscosity dictates metabolic activity of *Vibrio ruber*, *Front. Microbiol. 3* (2012) 255 (doi: 10.3389/fmicb.2012.00255).
20. M. Borić, T. Danevčič, D. Stopar, Prodigiosin from *Vibrio* sp. DSM 14379; A new UV-protective pigment, *Microbial Ecol. 62* (2011) 528–536.
21. K. Hanna, C. Bengis-Garber, D.J. Kushner, The effect of salt concentration on phospholipid and fatty acid composition of the moderate halophile *Vibrio costicola*, *Can. J. Microbiol. 30* (1984) 669–675.
22. C.J.A. van Echteld, B. de Kruijff, J.G. Mandersloot, J. de Gier, Effects of lysophosphatidylcholines on phosphatidylcholine and phosphatidylcholine/cholesterol liposome systems as revealed by ^{31}P-NMR, electron microscopy and permeability studies, *Biochim. Biophys. Acta*, *649* (1981) 211–220.
23. N. Fuller, R.P. Rand, The influence of lyolipids on the spontaneous curvature and bending elasticity of phopholipid membranes, *Biophys. J. 81* (2001) 243–254.
24. C. Ratledge, S.G. Wilkinson: *Microbial Lipids, Vol. 1*, Academic Press, London, UK (1988) pp. 322–327.
25. T. Danevčič, D. Stopar, Asymmetric response of carbon metabolism at high and low salt stress in *Vibrio* sp. DSM 14379, *Microbial Ecol. 62* (2011) 198–204.
26. A. Baich, Proline synthesis in *Escherichia coli*. A proline-inhibitable glutamic acid kinase, *Biochim. Biophys. Acta, 192* (1969) 462–467.
27. V. Créach, A.C. Baudoux, G. Bertru, B. Le Rouzic, Direct estimate of active bacteria: CTC use and limitations, *J. Microbiol. Methods*, *52* (2003) 19–28.
28. N. Starič, T. Danevčič, D. Stopar, *Vibrio* sp. DSM 14379 pigment production – A competitive advantage in the environment?, *Microbial Ecol. 60* (2010) 592–598.
29. S.M. Lewis, W.A. Corpe, Prodigiosin-producing bacteria from marine sources, *Appl. Microbiol. 12* (1964) 13–17.
30. M.S. Melvin, D.C. Ferguson, N. Lindquist, R.A. Manderville, DNA binding by 4-methoxypyrrolic natural products. Preference for intercalation at AT sites by tambjamine E and prodigiosin, *J. Org. Chem. 64* (1999) 6861–6869.
31. M.J. Song, J. Bae, D.S. Lee, C.H. Kim, J.S. Kim, S.W. Kim *et al.*, Purification and characterization of prodigiosin produced by integrated bioreactor from *Serratia* sp. KH-95, *J. Biosci. Bioeng. 101* (2006) 157–161.

32. K. Gnezda Meijer, I. Mahne, M. Poljšak-Prijatelj, D. Stopar, Host physiological status determines phage-like particle distribution in the lysate, *FEMS Microbiol. Ecol. 55* (2006) 136–145.

33. K. Gnezda Meijer, Viral induction influences metabolic homeostasis of the host bacterium, *PhD Thesis*, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia (2005) (in Slovene).

34. R.P. Williams, Biosynthesis of prodigiosin, a secondary metabolite of *Serratia marcescens*, *Appl. Microbiol. 25* (1973) 396–402.

35. S.W. Tsao, B.A. Rudd, X.G. He, C.J. Chang, H.G. Floss, Identification of a red pigment from *Streptomyces coelicolor* A3(2) as a mixture of prodigiosin derivatives, *J. Antibiot.* (Tokyo), *38* (1985) 128–131.

36. K. Kawauchi, K. Shibutani, H. Yagisawa, H. Kamata, S. Nakatsuji, H. Anzai *et al.*, A possible immunosuppressant, cycloprodigiosin hydrochloride, obtained from *Pseudoalteromonas denitrificans*, *Biochem. Biophys. Res. Commun. 237* (1997) 543–547.

37. H. Jeong, J.H. Yim, C. Lee, S.H. Choi, Y.K. Park, S.H. Yoon *et al.*, Genomic blueprint of *Hahella chejunsis*, a marine microbe producing an algicidal agent, *Nucleic Acids Res. 33* (2005) 7066–7073.

38. N.N. Gerber, Prodigiosin-like pigments, *CRC Crit. Rev. Microbiol. 3* (1975) 469–485.

39. R.P. Williams, S.M. Quadri: The Pigments of *Serratia.* In: *The Genus Serratia*, A. Von Graevenitz, S.J. Rubin (Eds.), CRC Press Inc, Boca Raton, FL, USA (1980) pp. 31–75.

40. A.J. Castro, Antimalarial activity of prodigiosin, *Nature, 213* (1967) 903–904.

41. R. Pérez-Tomás, B. Montaner, E. Llagostera, V. Soto-Cerrato, The prodigiosins, proapoptotic drugs with anticancer properties, *Biochem. Pharmacol. 66* (2003) 1447–1452.

42. N.R. Williamson, P.C. Fineran, T. Gristwood, S.R. Chawrai, F.J. Leeper, G.P.C. Salmond, Anticancer and immunosuppressive properties of bacterial prodiginines, *Future Microbiol. 2* (2007) 605–618.

43. F. Alihosseini, K.S. Ju, J. Lango, B.D. Hammock, G. Sun, Antibacterial colorants: Characterization of prodiginines and their applications on textile materials, *Biotechnol. Progr. 24* (2008) 742–747.

44. H. Rapoport, K.G. Holden, The synthesis of prodigiosin, *J. Am. Chem. Soc. 84* (1962) 635–642.

45. D.A. Morrison, Prodigiosin synthesis in mutants of *Serratia marcescens*, *J. Bacteriol. 91* (1966) 1599–1604.

46. A.K.P. Harris, N.R. Williamson, H. Slater, A. Cox, S. Abbasi, I. Foulds *et al.*, The *Serratia* gene cluster encoding biosynthesis of the red antibiotic, prodigiosin, shows species- and strain-dependent genome context variation, *Microbiology, 150* (2004) 3547–3560.

47. A.M. Cerdeño, M.J. Bibb, G.L. Challis, Analysis of the prodiginine biosynthesis gene cluster of *Streptomyces coelicolor* A3(2): New mechanism for chain initiation and termination in modular multienzymes, *Chem. Biol. 8* (2001) 817–829.

48. P.C. Fineran, H. Slater, L. Everson, K. Hughes, G.P.C. Salmond, Biosynthesis of tripyrrole and β-lactam secondary metabolites in *Serratia*: Integration of quorum sensing with multiple new regulatory components in the control of prodigiosin and carbapenem antibiotic production, *Mol. Microbiol. 56* (2005) 1495–1517.

49. N.R. Thomson, M.A. Crow, S.J. McGowan, A. Cox, G.P.C. Salmond, Biosynthesis of carbapenem antibiotic and prodigiosin pigment in *Serratia* under quorum sensing control, *Mol. Microbiol. 36* (2000) 539–556.

50. S. Cang, M. Sanada, O. Johdo, Y. Nagamatsu, A. Yoshimoto, High production of prodigiosin by *Serratia marcescens* grown on ethanol, *Biotechnol. Lett. 22* (2000) 1761–1765.

51. A.V. Giri, N. Anandkumar, G. Muthukumaran, G. Pennathur, A novel medium for the enhanced cell growth and production of prodigiosin from *Serratia marcescens* isolated from soil, *BMC Microbiol. 4* (2004) 11–20.

52. Y.H. Wei, W.C. Chen, Enhanced production of prodigiosin-like pigment from *Serratia marcescens* SMΔR by medium improvement and oil-supplementation strategies, *J. Biosci. Bioeng. 99* (2005) 616–622.

53. N.N. Gerber, D.P. Stahly, Prodiginine (prodigiosin-like) pigments from *Streptoverticillium rubrireticuli*, an organism that causes pink staining of polyvinyl chloride, *Appl. Microbiol. 30* (1975) 807–810.

54. F. Alihosseini, J. Lango, K.S. Ju, B.D. Hammock, G. Sun, Mutations of bacterium *Vibrio gazogenes* for selective preparation of colorants, *Biotehnol. Progr. 26* (2010) 352–360.

55. J.G. Krishna, A. Jacob, P. Kurian, K.K. Elyas, M. Chandrasekaran, Marine bacterial prodigiosin as a dye for rubber latex, polymethyl methacrylate sheets and paper, *Afr. J. Biotechnol. 12* (2013) 2266–2269.

56. S.R. Burger, J.W. Bennett, Droplet enrichment factors of pigmented and nonpigmented *Serratia marcescens*: Possible selective function for prodigiosin, *Appl. Environ. Microbiol. 50* (1985) 487–490.

57. D.W. Hood, R. Heidstra, U.K. Swoboda, D.A. Hodgson, Molecular genetic analysis of proline and tryptophan biosynthesis in *Streptomyces coelicolor* A3(2): Interaction between primary and secondary metabolism – A review, *Gene, 115* (1992) 5–12.

58. I.N. Ryazantseva, I.N. Andreyeva, G.S. Klementyeva, T.I. Ogorodnikova, V.Y. Petrov, Pigment-dependent light influence on the energetics of *Serratia marcescens*, *Thermochim. Acta, 251* (1995) 63–67.

59. J.L. Seganish, J.T. Davis, Prodigiosin is a chloride carrier that can function as an anion exchanger, *Chem. Commun. 46* (2005) 5781–5783.

60. P.L. Haddix, S. Jones, P. Patel, S. Burnham, K. Knights, N.J. Powell *et al.*, Kinetic analysis of growth rate, ATP, and pigmentation suggests an energy-spilling function for the pigment prodigiosin of *Serratia marcescens*, *J. Bacteriol. 190* (2008) 7453–7463.

61. N. Stankovic, V. Radulovic, M. Petkovic, I. Vuckovic, M. Jadranin, B. Vasiljevic *et al.*, *Streptomyces* sp. JS520 produces exceptionally high quantities of undecylprodigiosin with antibacterial, antioxidative, and UV-protective properties, *Appl. Microbiol. Biotechnol. 96* (2012) 1217–1231.

62. H.J. Ahn, H.J. La, J.J. Forney, System for determining the relative fitness of multiple bacterial populations without using selective markers, *Appl. Environ. Microbiol. 72* (2006) 7383–7385.