

Freeze-drying of sol–gel encapsulated recombinant bioluminescent *E. coli* by using lyo-protectants

Dejene A. Tessema^{a,b,1}, Rachel Rosen^a, Rami Pedazur^a, Shimshon Belkin^{a,**},
Jenny Gun^b, Irina Ekeltchik^b, Ovadia Lev^{b,*}

^a The Laboratory of Environmental Microbiology of the Institute of Life sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

^b The Laboratory of Environmental Chemistry, The Casali Institute of Applied Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Available online 10 August 2005

Abstract

Toxicity sensing by live microorganisms has attracted considerable attention during the last decade. These sensors exhibit a non-specific, relatively fast response to changes in water quality or to physiological stress conditions and thus may provide an early warning against intentional or unintentional contamination of water sources. However, maintenance of cell cultures is a demanding task, requiring specialists' attention and careful control of storage conditions, which seriously limits the practical usefulness of such diagnostics for field analysis and distributed monitoring. Encapsulation in hydrogels is a viable way for the construction of user-friendly solid-state sensors. However, the encapsulated cells should still be kept in wet conditions as drying results in immediate and permanent loss of activity. Particularly, the much-researched cellular silicates, biohybrids, lose activity upon drying. Here we introduce a freeze-drying compatible sol–gel process for the encapsulation of *E. coli* reporter cells in silicates. We demonstrate that incorporation of optimized concentrations of trehalose and glycerin in the sol–gel silicate precursors prevents the inactivation of *E. coli* during freeze-drying.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Sol–gel; Freeze-drying; Recombinant bacteria; Reporter; Toxicity sensors

1. Introduction

Recent advances in the field of biotechnology have opened the door to the use of genetically modified reporter microorganisms as whole-cell biosensors. Such biosensors were found to be sensitive and inexpensive analytical tools for laboratory and field assessment of water quality [1,2]. They provide convenient early warning or environmental screening capabilities, which would otherwise require either a large number of different specific sensors or time consuming laboratory analysis [1,2].

Immobilization of the sensing element, be it a molecule, nanoparticle or a whole microorganism solid matrix ful-

fills several tasks: it makes the sensor more user friendly, improves its metrological characteristics, prevents washout of the active reporting element or reagent by the test solution, and may, at times, allow repeated and even continuous use.

Several matrices for encapsulation of live microorganisms have been described, including sol–gel, possibly the most facile and generic immobilization technology available today [3–7]. The technology was optimized by Livage and other research groups [4,8–10] with respect to the encapsulation of *E. coli* cells. In previous reports [11–13], we have demonstrated that sol–gel encapsulated recombinant *E. coli* cells can be 'wet stored' for several months without significant loss of activity [11,12]. However, dry storage complies better with distributed sensing systems, shipment requirements, field analysis and handling by non-experienced personnel.

Straightforward attempts to dry sol–gel encapsulated *E. coli* cells at room temperature were unsuccessful, leading to complete inactivation of the *E. coli* reporters. Therefore,

* Corresponding author. Tel.: +972 2 6594191; fax: +972 2 6586155.

** Corresponding author. Tel.: +972 2 6594192.

E-mail addresses: shimshon@vms.huji.ac.il (S. Belkin),
ovadia@vms.huji.ac.il (O. Lev).

¹ Present address: Chemistry Department, Jimma University, Ethiopia.

and in light of the successful application of freeze-drying of sol–gel enzyme biohybrids [14] we explored the possibility of pursuing this technique for recombinant *E. coli*. As far as we know, the only reports on freeze-drying of sol–gel encapsulated microorganisms pertain to *Bacillus sphaericus* and *Saccharomyces cerevisiae*, which retained approximately 0.1% viability after the freeze-drying process [6,15].

The process of freeze-drying itself, even without the additional complications associated with encapsulation, imposes stress on biological samples and produces undesirable side effects that hamper viability. Consequently, to minimize the perturbation in the native structure of microorganisms during lyophilization, it is often vital that non-specific stabilizers such as sugars be used as protective agents.

Glycerol [4,7], sugars and particularly trehalose [16–23] have been shown to stabilize both isolated membrane vesicles and enzymes, and the membranes and proteins of intact cells during freezing and freeze-drying. Glycerol was particularly useful in preventing membrane damage in *E. coli* during sol–gel encapsulation [4], and it was found to be a useful additive for molding different sol–gel morphologies [7]. Trehalose is a non-reducing disaccharide that may be accumulated at high concentrations by many organisms capable of surviving complete dehydration [24]. It was shown to be an excellent stabilizer of many biomolecules and live cells in the dry state, and appeared to be superior to other sugars [25].

The stabilization effects of trehalose were attributed to several mechanisms/interactions with critical biomolecules or cellular structures such as membranes and proteins. The ability of trehalose to stabilize membranes upon drying was clearly demonstrated [24,25]. The stabilization mechanism of membranes, often referred to as the water replacement hypothesis [24], suggests that trehalose may form hydrogen bonds with membrane phospholipids and proteins and replace water molecules that are removed upon drying. This, in turn, inhibits van der Waal's interactions between adjacent lipids thus preventing the elevation of membrane phase transition temperatures (T_m). Such a T_m change could cause membranes to shift from their native liquid-crystalline state to a gel state under environmentally relevant temperatures [26]. Maintaining membranes in a liquid-crystalline rather than gel state may prevent detrimental phenomena like membrane fusion, lipid, protein and fluid–fluid separation [27,28]. Furthermore, transitions from gel to liquid-crystalline states that may occur upon hydration could cause leakage of cytoplasmic constituents from the cell [26].

Trehalose was also found to stabilize proteins. It was suggested that under freezing or moderate drying conditions, preferential exclusion of solutes from protein surfaces may conserve their native conformational structure, and that under higher dehydration states trehalose may interact by hydrogen bonding of its -OH groups to polar residues in proteins, hence preserving the conformational state that was present prior to the dehydration processes [29].

It was also suggested that the stabilization capacity of trehalose may be related to its high glass transition (T_g) temperature and its ability to vitrify upon drying under environmental temperatures. The formation of a glassy matrix may slow down or even halt all degradative chemical and physical reactions, and the high T_g may enhance stability of the glassy state at ambient temperatures [24]. An additional factor that might contribute to the high stability conferred by trehalose is its ability to form di-hydrate crystals upon wetting, hence isolating the remaining bulk sugar from contact with water and providing increased stability to dried materials that are imbedded in the trehalose matrix [30].

Unlike the extensive studies carried out on the freeze-drying of proteins or of microorganisms in liquid culture, the protective capabilities of trehalose and glycerol during the freeze-drying of sol–gel cellular biohybrids have never been explored. In this report we describe the activity of sol–gel immobilized, genetically engineered *E. coli* cells harboring a *recA::luxCDABE* fusion gene following freeze-drying in the presence of trehalose and/or glycerol.

2. Experimental

2.1. Preparation of bacterial cultures

The bacterial strain used in this study was DPD2794 [2,11]. It contains a multicopy plasmid in which the *recA* promoter was fused to the *Vibrio fischeri luxCDABE* bioluminescence genes. The strain was grown at 37 °C in Luria–Bertani (LB) broth (NaCl 5 g L⁻¹, yeast extract 5 g L⁻¹, Tryptone 10 g L⁻¹) [31] containing 100 µg mL⁻¹ kanamycin to ensure plasmid maintenance. In all cases, luminescence was induced by nalidixic acid (5 mg L⁻¹), and was monitored for 4 h using a microtiter plate luminometer (Victor 2, Wallac, Finland, www.perkinelmer.com). Luminescence values are presented as arbitrary relative light units (RLU).

Before freeze-drying, 1.0 mL of LB medium was inoculated at 0.01% with an overnight culture and incubated at 26 °C to O.D._{600nm} = 0.15 (approximately 1.0 × 10⁷ cells mL⁻¹), which corresponds to the early exponential growth phase. Cells were harvested by centrifugation (4000 rpm, 10 min) and the volume was reduced so that the final concentration was 2.0 × 10⁷ cells mL⁻¹.

2.2. Preparation of biosilicate hybrids

The sol–gel mixtures were prepared by mixing 3.0 mL of tetramethylorthosilane (TMOS, Aldrich, WI, www.sigmaaldrich.com) with 1.5 mL of distilled water, 0.2 mL of 0.1 M HCl and 1.0 mL of phosphate buffer. The mixture was sonicated for 10 min to ensure uniformity and left to age at 4 °C for 1 day.

Each experiment was carried out by transferring 1.0 mL of strain DPD2794, harvested at an early exponential phase, into a 12 mL vial and adding various combinations of tre-

halose and glycerol solutions at different concentrations to a final volume of 3.0 mL. For non-encapsulated assays, aliquots of these mixes were transferred directly into a microtiter plate. To prepare encapsulated samples 300 μL of sol-gel was added to each bacteria, trehalose and glycerol mix and vortexed for 2 min. Then, 50 μL aliquots of the bio-inorganic hybrid were transferred into the wells of an opaque white 96 well microtiter plate and allowed to dry for 5 min before the addition of the nalidixic acid inducer (5 mg L^{-1}) for the luminescence measurement of fresh encapsulated samples. For freeze-drying, 50 μL aliquots were pipetted into a number of glass vials and transferred to a freeze-drier (Advantage, VirTis, USA, www.virtis.com USA). The freeze-drying operation lasted 40 h (2 h, freezing to -40°C at 200 mTorr followed by 38 h drying at -40°C and 25 mTorr). The vials were hermetically sealed under vacuum and stored at either room temperature, 4 or -20°C .

3. Results

Direct assessment of the viability of sol-gel encapsulated bacteria is problematic, since it is impossible to free the cells of their solid confinement and enumerate them by their colony forming ability on a nutrient agar medium. One possible solution may lie in a physical disintegration of the silicate matrix [4]. Here we have used the luminescence of the reporting cells as an indication of both the viability and the activity of the cells, without the need of physically separating them from the silicate.

Estimation of the activity of the encapsulated cells was conducted by exposure of the biogel or the hybrid material to LB medium containing 5 mg L^{-1} nalidixic acid for 4 h and recording the luminescence after a set time span. The luminescence after the 4 h exposure, which in all cases was much higher than the residual luminescence before the exposure, reflects a combined measure for the induced stress, cell viability and cell proliferation during the test. From practical as well as from sensing points of view this is the desired parameter since it reflects sensing sensitivity.

Fig. 1 depicts the effect of various levels of trehalose and glycerol on the induction bioluminescence in a bacterial culture without immobilization and freeze-drying. Glycerol, by itself, had no effect on the luminescence level after 4 h, but the presence of glycerol lowered the induction level when the percentage of trehalose exceeded 10 wt.%. This was clearly not a result of lower viability as confirmed by plate count. We attribute this effect to stabilization of the bacteria by the disaccharide, lowering the physiological stress brought about by the nalidixic acid and consequently the induction of bioluminescence. Interestingly, we observe a significant synergistic stress reduction by the glycerol and the trehalose. The higher the concentration of trehalose the larger was the effect of glycerin. At 3.6% glycerol and 20% trehalose the luminescence was only 30% of the response in the absence of both.

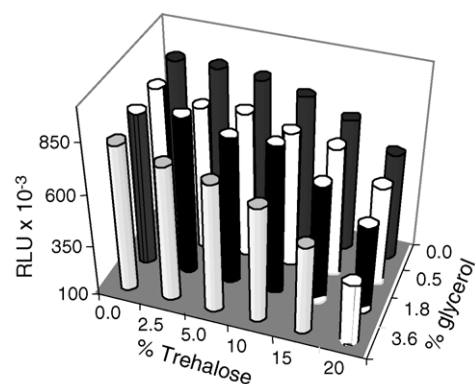


Fig. 1. Luminescence values of non-encapsulated fresh DPD2974 cells at various levels of trehalose and glycerol after induction with 5 ppm NA for 240 min.

Fig. 2 depicts the luminescence response to nalidixic acid (as in Fig. 1) of *E. coli* cells after freeze-drying and storage for 24 h. Without any of the stabilizing agents, the *E. coli* cells were very vulnerable to the freeze-drying/thawing process and were totally inactivated. Maximal activity was obtained for 0.5% trehalose, and a further increase in trehalose concentration resulted in decreased viability and activity of the reporting cells. The addition of trehalose to glycerin free culture resulted in an increased activity peaking at 15% trehalose and going down at higher concentration of the disaccharide. We did not observe any synergistic effect and the addition of glycerin to various levels of trehalose spiked cultures always decreased the response level of the reporting cells rather than enhancing it so, maximal activity was obtained with 0.5% trehalose and glycerin free solution.

Fig. 3 depicts the response of the sol-gel biohybrids containing approximately the same number of reporting cells as in Figs. 1 and 2 after exposure to 5 mg L^{-1} nalidixic acid for 4 h. The addition of trehalose and glycerol did not improve

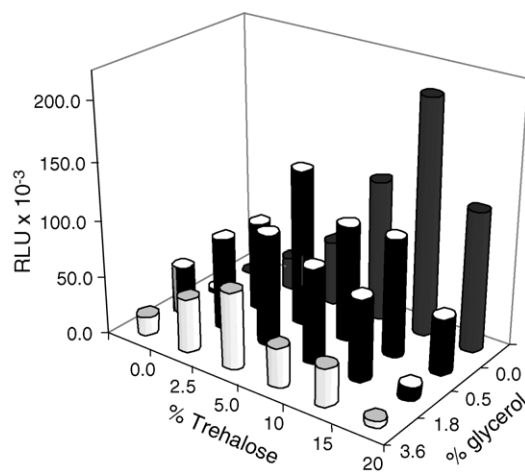


Fig. 2. Luminescence values of non-encapsulated freeze-dried DPD2974 cells at various levels of trehalose and glycerol after induction with 5 mg L^{-1} NA for 240 min.

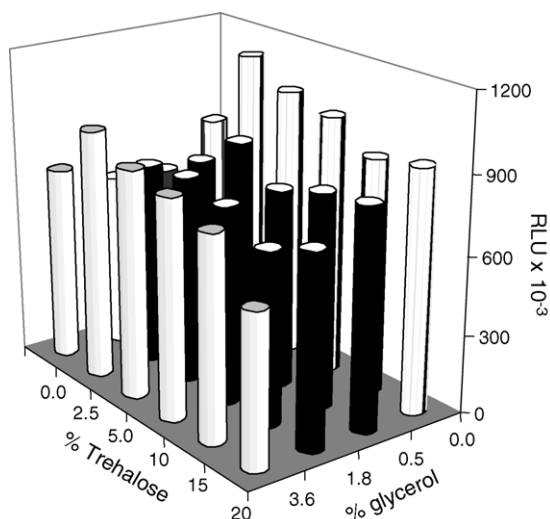


Fig. 3. Luminescence response of encapsulated DPD2974 cells at various levels of glycerol and trehalose after induction with 5 mg L^{-1} NA for 240 min.

significantly the response of the sensing elements. The moderate decrease of activity, which is (arguably) obtained when both trehalose and glycerin were added at high concentrations may be attributed to the reduction of the physiological stress induced by nalidixic acid rather than to the inactivation of the cells by these additives.

Fig. 4 depicts the luminescence response of the biohybrids prepared under identical conditions to Fig. 3 but after freeze-drying, 24 h storage and thawing. The favorable preserving activity of the trehalose is clearly observed. The sensors that contained less than 5% trehalose lost activity irrespective of the concentration of glycerin. All sensors that were prepared with more than 10% trehalose exhibited significant activity. Maximal activity was observed for sensor with 15 or 20% trehalose. The effect of glycerin is more complex. For low trehalose concentrations (i.e. 5–10%) the optimal activity was

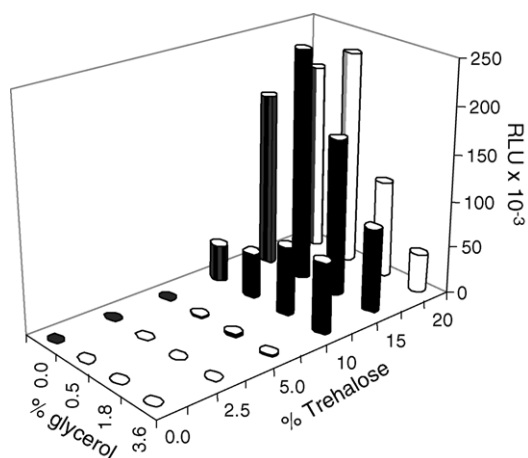


Fig. 4. Luminescence values of encapsulated freeze-dried DPD2974 cells in various levels of trehalose and glycerol after induction with 5 mg L^{-1} NA for 240 min.

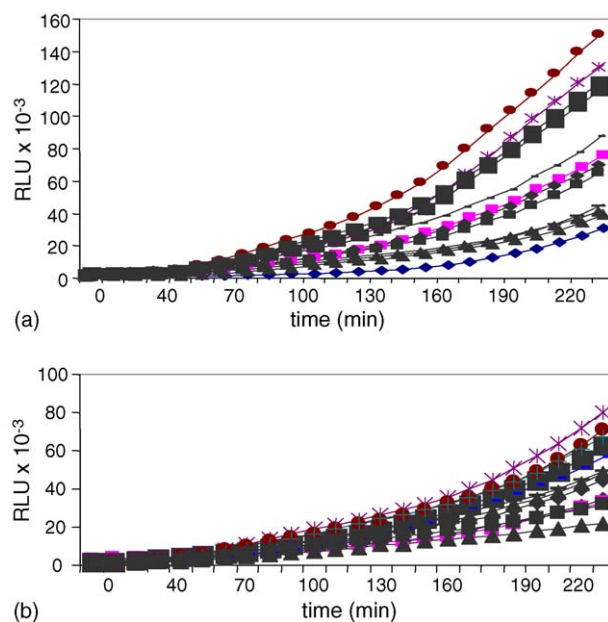


Fig. 5. Kinetics of bioluminescence development of various volumes (same number of cells) of non-encapsulated (a) and encapsulated (b) freeze-dried DPD2974 cell samples. The curves in frame 'a' correspond to (from top) 60, 50, 40, 70, 30, 80, 90, 100, 110, 120, 20 and 10 μL sol-gel samples; curves in frame 'b' correspond to 50, 60, 70, 40, 80, 90, 100, 110, 120, 10, 20 and 30 μL .

obtained at the highest glycerin level. At the high trehalose level maximal sensing sensitivity was obtained at 0.5% glycerin. The maximal activity of the freeze-dried sensors was approximately 25% of the observed response of sensors that were not freeze-dried.

Fig. 5 shows the kinetics of residual luminescence of various volumes of immobilized and non-immobilized DPD2974 cells containing the *lux*-fused *recA* promoter after freeze-drying. In all tests (for both free as well as the encapsulated samples) we used the same amount of cells. All the samples exhibited very similar dynamics irrespective of encapsulation or volume of the freeze-dried material. In both encapsulated and non-encapsulated samples the highest activities were obtained for intermediate volumes between 40 and 80 μL . Activities of lower volumes might have been affected due to excessive drying while higher volumes due to insufficient drying.

Stability tests have been carried out by storing freeze-dried samples at room temperature (approximately 20°C), 4 and -20°C for up to 42 days. Relative luminescence values and percent activity (compared to that measured right after lyophilization, time 0) are given in Tables 1 and 2. Values in Table 1 reveal that sol-gel encapsulated samples are stable for up to 6 weeks at -20°C , fairly stable for about 3 weeks at 0°C and are poorly stable at room temperature. The relative residual activities normalized to the response of non-immobilized freeze-dried samples was 83% for sol-gel immobilized assay at the 6th week in -20°C and 54% at the 3rd week for 4°C , stored samples.

Table 1

Luminescence values (240 min exposure, 5 mg L⁻¹ NA) of freeze-dried encapsulated DPD2794 cells stored for 42 days at different temperatures

Storage duration (day)	Luminescence (RLU) and percent residual luminescence (parenthesis)		
	Room temperature (20 °C)	4 °C	-20 °C
0 (80106) ^a	–	–	–
10	24393 (30)	61118 (76)	79357 (99)
15	6245 (8)	45249 (56)	70161 (88)
21	937 (1)	28279 (35)	68582 (86)
28	48 (0)	7423 (9)	63961 (80)
35	–	3148 (4)	57028 (71)
42	–	2334 (3)	56332 (70)

^a Luminescence right after freeze-drying.

Table 2

Luminescence values (240 min exposure, 5 mg L⁻¹ NA) of freeze-dried non-immobilized DPD2794 cells stored for 42 days

Storage duration (days)	Luminescence (RLU) and percent residual luminescence (in parenthesis)		
	25 °C	4 °C	-20 °C
0 (RLU = 191133) ^a	–	–	–
10	91018 (48)	167471 (88)	188802 (99)
15	67776 (35)	167471 (88)	187113 (98)
21	40333 (21)	124432 (65)	185594 (97)
28	29503 (15)	103896 (54)	171789 (89)
35	14213 (7)	80044 (42)	167937 (88)
42	6664 (3)	67643 (35)	161076 (84)

^a Luminescence right after freeze-drying.

4. Conclusions

The paper addresses only one aspect of the long chain of processes that are required in order to use delicate biological entities for robust and user-friendly sensing elements. The paper describes a way by which sol–gel composition can be tailored in order to increase cellular viability after freeze-drying. We show that incorporation of optimal concentrations of trehalose and glycerin can dramatically increase the compatibility of the biohybrids with freeze-drying. For optimal concentrations of trehalose and glycerin the fraction of lost activity of the encapsulated cells is similar and even lower than the fraction of lost activity of free bacteria undergoing a similar treatment.

Acknowledgements

This research was supported by the Infrastructure Program of the Ministry of Science and Culture, Israel and by a Franco-Israel research grant. Instrumentation was partly funded by the Israel Science Foundation, founded by the Israel Academy of Sciences. We gratefully acknowledge strains provided by T.K. Van Dyk and R.A. LaRossa, the DuPont Company, USA. I.E. thanks the Valazzi-Pikovsky Fellowship Fund.

References

- [1] S. Daunert, G. Barrett, J.S. Feliciano, R.S. Shetty, S. Shrestha, W. Smith-Spenser, Genetically engineered whole-cell sensing systems:

coupling biological recognition with reporter genes, *Chem. Rev.* 100 (2000) 2705–2738.

- [2] S. Kohler, S. Belkin, R.D. Schmid, Reporter gene bioassays in environmental analysis, *Fresen. J. Anal. Chem.* 366 (2000) 769–779.
- [3] E. Bressler, S. Braun, Use of sol–gel entrapment techniques for the resolution of itaconic acid biosynthetic pathway in *Aspergillus terreus*, *J. Sol–gel Sci. Technol.* 7 (1996) 129–133.
- [4] N. Nassif, O. Bouvet, M.N. Rager, C. Roux, T. Cordin, J. Livage, Living bacteria in silica gels, *Nat. Mater.* 1 (2002) 42–44.
- [5] M. Muraca, M.T. Vilei, E. Zanusso, C. Ferraresso, A. Granato, S. Doninsegna, R. Dal Monte, P. Carraroc, G. Carturan, Encapsulation of hepatocytes by SiO₂, *Transplant. P.* 32 (2000) 2713–2714.
- [6] U. Soltmann, H. Bottcher, D. Koch, G. Grathwohl, Freeze gelation: a new option for the production of biological ceramic composites (biocers), *Mater. Lett.* 57 (2003) 2861–2865.
- [7] I. Gill, A. Ballesteros, Bioencapsulation within synthetic polymers (Part 2): non-sol–gel protein–polymer biocomposites, *Trends Biotechnol.* 18 (2000) 282–296.
- [8] A. Coiffier, T. Coradin, C. Roux, O.M.M. Bouvet, J. Livage, Sol–gel encapsulation of bacteria: a comparison between alkoxide and aqueous routes, *J. Mater. Chem.* 11 (2001) 2039–2044.
- [9] L. Bergogne, S. Fennouh, S. Guyon, J. Livage, C. Roux, Bioencapsulation within sol–gel glasses, *Mol. Cryst. Liquid Cryst.* 354 (2000) 667–677.
- [10] N. Nassif, C. Roux, T. Coradin, M.N. Rager, O.M.M. Bouvet, J. Livage, A sol–gel matrix to preserve the viability of encapsulated bacteria, *J. Mater. Chem.* 13 (2003) 203–208.
- [11] J.R. Premkumar, R. Rosen, S. Belkin, O. Lev, Sol–gel luminescence biosensors: Encapsulation of recombinant *E. coli* reporters in thick silicate films, *Anal. Chim. Acta* 462 (2002) 11–23.
- [12] J.R. Premkumar, E. Sagi, R. Rozen, S. Belkin, A.D. Modestov, O. Lev, Fluorescent bacteria encapsulated in sol–gel derived silicate films, *Chem. Mater.* 14 (2002) 2676–2686.
- [13] J.R. Premkumar, O. Lev, R. Rosen, S. Belkin, Encapsulation of luminous recombinant *E. coli* in sol–gel silicate films, *Adv. Mater.* 13 (2001) 1773–1775.

- [14] D. Avnir, S. Braun, O. Lev, M. Ottolenghi, Enzymes and other proteins entrapped in sol-gel materials, *Chem. Mater.* 6 (1994) 1605–1614.
- [15] H. Botcher, U. Soltmann, M. Mertig, W. Pompe, *J. Mater. Chem.* 14 (2004) 2176–2188.
- [16] P.B. Conrad, D.P. Miller, P.R. Cielenski, J.J. de Pablo, Stabilization and preservation of *Lactobacillus acidophilus* in saccharide matrices, *Cryobiology* 41 (2000) 17–24.
- [17] Y. Miyamoto-Shinohara, T. Imaizumi, J. Sukenobe, Y. Murakami, S. Kawamura, Y. Komatsu, Survival rate of microbes after freeze-drying and long-term storage, *Cryobiology* 41 (2000) 251–255.
- [18] Y.H. Liao, M.B. Brown, A. Quader, G.P. Martin, Protective mechanism of stabilizing excipients against dehydration in the freeze-drying of proteins, *Pharm. Res.* 19 (12) (2002) 1854–1861.
- [19] S. Carvalho, J. Silva, P. Ho, P. Teixeira, F. Xavier Malcata, P. Gibbs, Survival of freeze-dried *Lactobacillus plantarum* and *Lactobacillus rhamnosus* during storage in the presence of protectants, *Biotechnol. Lett.* 24 (2002) 1587–1591.
- [20] S.B. Leslie, E. Israeli, B. Lighthart, J.H. Crowe, L.M. Crowe, Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying, *Appl. Environ. Microbiol.* 61 (1995) 3592–3597.
- [21] N. Trsic-Milanovic, A. Kodzic, J. Baras, S. Dimitrijevic-Brankovic, The influence of cryoprotective medium containing glycerol on the lyophilization of lactic acid bacteria, *J. Serb. Chem. Soc.* 66 (2001) 435–441.
- [22] S. Fillinger, M.K. Chaverroche, P. van Dijck, R. de Vries, G. Ruijter, J. Thevelein, C. d'Enfert, Trehalose is required for the acquisition of tolerance to a variety of stresses in the filamentous fungus *Aspergillus nidulans*, *Microbiology* 147 (2001) 1851–1862.
- [23] F.J. Alvarez-Peral, O. Zaragoza, Y. Pedreno, J.C. Arguelles, Protective role of trehalose during severe oxidative stress caused by hydrogen peroxide and the adaptive oxidative stress response in *Candida albicans*, *Microbiology* 148 (2002) 2599–2606.
- [24] J.H. Crowe, J.F. Carpenter, L.M. Crowe, The role of vitrification in anhydrobiosis, *Annu. Rev. Physiol.* 60 (1998) 73–103.
- [25] J.H. Crowe, F. Tablin, W.F. Wolkers, K. Gousset, N.M. Tsvetkova, J. Ricker, Stabilization of membranes in human platelets freeze-dried with trehalose, *J. Chem. Phys. Lipids* 122 (2003) 41–52.
- [26] D. Billi, M. Potts, Life and death of dried prokaryotes, *Res. Microbiol.* 153 (2002) 7–12.
- [27] J. Wolfe, G. Bryant, Freezing, drying and/or vitrification of membrane-solute-water systems, *Cryobiology* 39 (1999) 103–129.
- [28] J.N. Crowe, A.E. Oliver, F. Tablin, Is there a single adaptation to anhydrobiosis? *Integr. Comp. Biol.* 42 (2002) 497–503.
- [29] J.H. Crowe, J.F. Carpenter, L.M. Crowe, T.J. Anchordoguy, Are freezing and dehydration similar stress vectors? A comparison of modes of interaction of stabilizing solutes and biomolecules, *Cryobiology* 27 (1990) 219–231.
- [30] B.J. Aldous, A.D. Auffret, F. Franks, The crystallization of hydrates form amorphous carbohydrates, *Cryo. Lett.* 16 (1995) 181–186.
- [31] J.H. Miller, *Experiments in Molecular Genetics*, Cold Spring Harbor, New York, 1972.

Biographies

Dejene A. Tessema was a UNESCO postdoctoral scholar at the Chemistry and Life Sciences Institutes of the Hebrew University during the years 2002–2003. He is currently a professor at the Chemistry Department of Jimma University, Ethiopia.

Rachel Rosen, a researcher at the Hebrew University Institute of Life Sciences, has received her PhD at the Hebrew University Faculty of Agriculture (1992) and now specializes in environmental microbiology and the genetic engineering of microbial sensor systems.

Rami Pedazur is an environmental microbiologist with an expertise in water and wastewater treatment, water toxicity and whole-cell biosensors. He has a Hebrew University BSc in soil sciences and an MSc (1992) and a PhD (1997) in environmental sciences.

Shimshon Belkin, a professor of environmental sciences, directs the environmental studies program at the Hebrew University, where he received his PhD in oceanography in 1983. He specializes in environmental microbiology and whole-cell biosensor systems.

Jenny Gun received her PhD in 1996 at the Hebrew University of Jerusalem. Since then she is senior researcher at the Laboratory of Environmental Chemistry. She specializes in electrochemistry and advanced materials for analytical chemistry.

Irina Ekeltchik received her PhD in 2003 at the Chemistry Department of Tomsk State University. In the last 2 years she is Golda Meir postdoctoral scholar at the Laboratory of Environmental Chemistry of the Hebrew University. She specializes in electrochemistry, metrology and advanced materials for analytical chemistry.

Ovadia Lev received his PhD at 1986 in the Technion, Haifa. Since 2001 he is full professor and head of the Laboratory of Environmental Chemistry at the Casali Institute of the Institute of Chemistry of the Hebrew University. He specializes in environmental chemistry and advanced materials for analytical chemistry.