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Physiological actions of preservative agents: prospective of use of modern microbiological techniques in assessing microbial behaviour in food preservation

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Abstract

In this mini-review, various aspects of homeostasis of microbial cells and its perturbation by antimicrobial agents will be discussed. First, outlining the position that the physiological studies on microbial behaviour using the modern molecular tools should have in food science sets the scene for the studies. Subsequently, the advent of functional genomics is discussed that allows full coverage of cellular reactions at unprecedented levels. Examples of weak organic acid resistance, the stress response against natural antimicrobial agents and responses against physicochemical factors show how we can now "open the black box" that microbes are, look inside and begin to understand how different cellular signalling cables are wired together. Using the analogy with machines, it will be indicated how the use of various signalling systems depends on the availability of substrates "fuel" to let the systems act in the context of the minimum energetic requirement cells have to let their housekeeping systems run. The outlook illustrates how new insights might be used to device knowledge-based rather than empirical combinations of preservation systems and how risk assessment models might be deviced that link the mechanistic insight to risk distributions of events in food manufacturing.

Keywords: Microorganisms; Preservatives; Antimicrobials; Genomics; Modelling; Systems analysis; Food processing; Food chain

1. Introduction: food preservation demands control of microorganisms throughout the food chain

The food and beverage industry suffers still from significant losses due to problems with food poisoning and spoilage microorganisms. In 2000, figures from the United States Ministry of Agriculture showed that *Escherichia coli* O157:H7 and non-O157:H7 shiga-toxic *E.coli* cost USA yearly >$900 M with >2000 registered cases in 2000 (USDA homepage). Food-related listeriosis showed even twofold higher financial numbers. Table 1 shows the overall percentage of food-related diseases, hospitalisation and deaths per year as recorded in 1999 (Mead et al., 1999). Hence, there is an urgent need to minimize the risk of food

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Table 1
Food-borne illness, hospitalisation and death in the United States (Mead et al., 1999)

Health issues	Illness	Hospitalised	Deaths
Total illness/year	173,000,000	774,000	6800
Food borne illness/year	76,000,000	325,000	5000
Food borne illness caused by known pathogens *Salmonella*, *Listeria*, *Toxoplasma* (related)	14,000,000	60,000	1800, 1500

contamination. In order to ensure that in an optimal way, we need to model the risk on food contamination in a quantitative way. There is a need to predict the behaviour of undesirable microorganisms and thus to get an insight in their cellular functioning under conditions generally encountered under relevant food manufacturing conditions (de Vos, 2001).

Optimal use of the mechanistic knowledge will only be made if these data can be put in the context of single cell versus cellular population behaviour and in the context of the physicochemical parameters that determine food taste, flavor and its nutritional value (Verrips et al., 2001). Such an integrated product design system, our holy grail, is currently being worked on at various levels in the Unilever research laboratories and linked academic and institutional research groups. It needs to cover the translation of mechanistic cellular stress models at the population level to the molecular events and linked cellular physiology occurring in single cells (Sumner and Avery, 2002). In doing so, the stochastic events in terms of modelling, e.g. the amount of transcripts and, hence, molecules of certain transcription factors regulating the onset of stress response routes will play a prime role. The distribution of transcription factors over a population of cells determines the likelihood of a given cell displaying a resistance under a given set of conditions occurring in raw materials and during food processing (McAdams and Arkin, 1997). For example, under conditions of nutrient starvation, as spoilage cells present in the food chain will often experience, *Bacilli* are known to opt for either sporulation, competence development activation of the

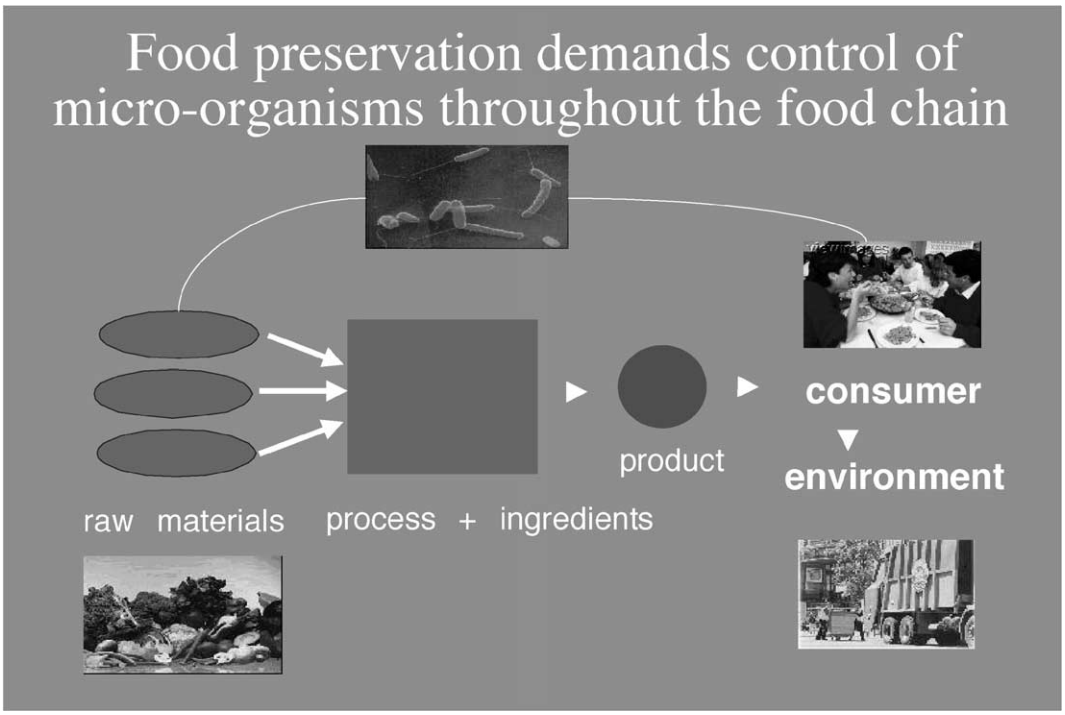

Fig. 1. A schematic illustration of the point that proper food preservation strategies demand a control of microorganisms throughout the entire food chain. From raw materials to the consumer and the disposal of the remains of the product.

general stress response through σ_B, or specific stress responses (Hecker and Volker, 2001).

The results will have to be framed in a risk assessment predicting the occurrence of a given cell with its resistance/sensitivity characteristics in a given (batch of) product(s) or at a given point along the entire food production chain (see, e.g. Marks et al., 1998 and Fig. 1).

Having said that, a first area where genomics then play a major role in food manufacturing is, whichever way we turn the issues, the mechanistic modelling of stress response of the microorganisms of concern. These data will form part of large set of databases of food ingredients and processes where physical, biochemical and nutritional parameters pertaining to the food being produced are all considered. Such databases will form the backbone of systems that will predict in an integrated manner the stability and quality of foods (Jongen, 2001).

2. Physiology of cells

2.1. Genomics

The large-scale sequencing of microbial genomes has opened the way to a full analysis of microbial behaviour. The genomes available at various sites include the model organism for Gram-negative bacteria *E. coli*, the model organism for Gram-positive bacteria *Bacillus subtilis* and the fungal model organism. *Saccharomyces cerevisiae* (Lucchini et al., 2001 and , http://www.tigr.org/tdb/mdb/mdbcomplete.html). *S. cerevisiae* has long been and still is a model (eukaryote) of choice for many different types of cellular physiology studies. It was among the first organisms to be fully sequenced and analysed at the genome-wide level for its gene expression under environmental changes, e.g. leading to the diauxic shift and sporulation (De Risi et al., 1997; Chu et al., 1998).

Subsequently, in bacteria, gene expression of *E. coli* was analysed upon growth in rich and poor media (Tao et al., 1999). The work on *B. subtilis* has lead to the identification of cellular events that take place during carbon catabolite repression and protein secretion (Tobisch et al., 1999; Hirose et al., 2000) at the proteome level. Recently, researchers have also visualized gene expression during early to middle sporulation and growth under anaerobic conditions (Fawcett et al., 2000; Ye et al., 2000). Clearly, the power of a full and relevant description of cellular response to environmental conditions lies in the combination of a transcriptome (completeness) and proteome/metabolome (level of relevance) analysis (see, e.g. Nouwens et al., 2000; Yoshida et al., 2001 and De Nobel et al. (submitted for publication). It is evident that a full understanding of cellular physiology finally depends on a proper integration of the molecular data, the options or buttons to push that microbes (cells in general) have, and the available substrate that cells have at their disposition (or the fuel the motor has to run). This integration of molecular microbiology and classical physiology into what is now often called functional genomics, encompassing also the necessary BioInformatics with it, is what we see as a major challenge and driving force for future research in biology in general and in preservation and fermentation of foods in particular. A more in depth discussion of this is given in Brul and Klis (1999) and Brul (2000).

2.2. Cellular homeostasis

Cells strive to maintain an optimal balance between those metabolic processes needed for growth and those needed for survival stress response. From an application point of view, studies on growth regulatory systems have been the focus particularly of those who aimed at increasing the yield of a fermentation process (see, e.g. van Hoek et al., 1998). Fundamentally, interest in how cells adjust their control of cellular metabolic performance can be found at three levels. Do cells adjust their metabolism, their protein composition and/or even their gene expression pattern upon applying stress (see Fig. 2)? The issue of which steps in cellular metabolism exert most control on the overall performance then complements this type of questions. Important in that respect is the notion that it is virtually never only one "rate" controlling step that is key in this (see, e.g. Fell, 1997; Hofmeyr and Westerhoff, 2001; Stephanopoulos and Kelleher, 2001).

Stress is imposed on cells in various ways. The environment may change in terms of water availability, acidity, temperature profile, presence of antimicrobial

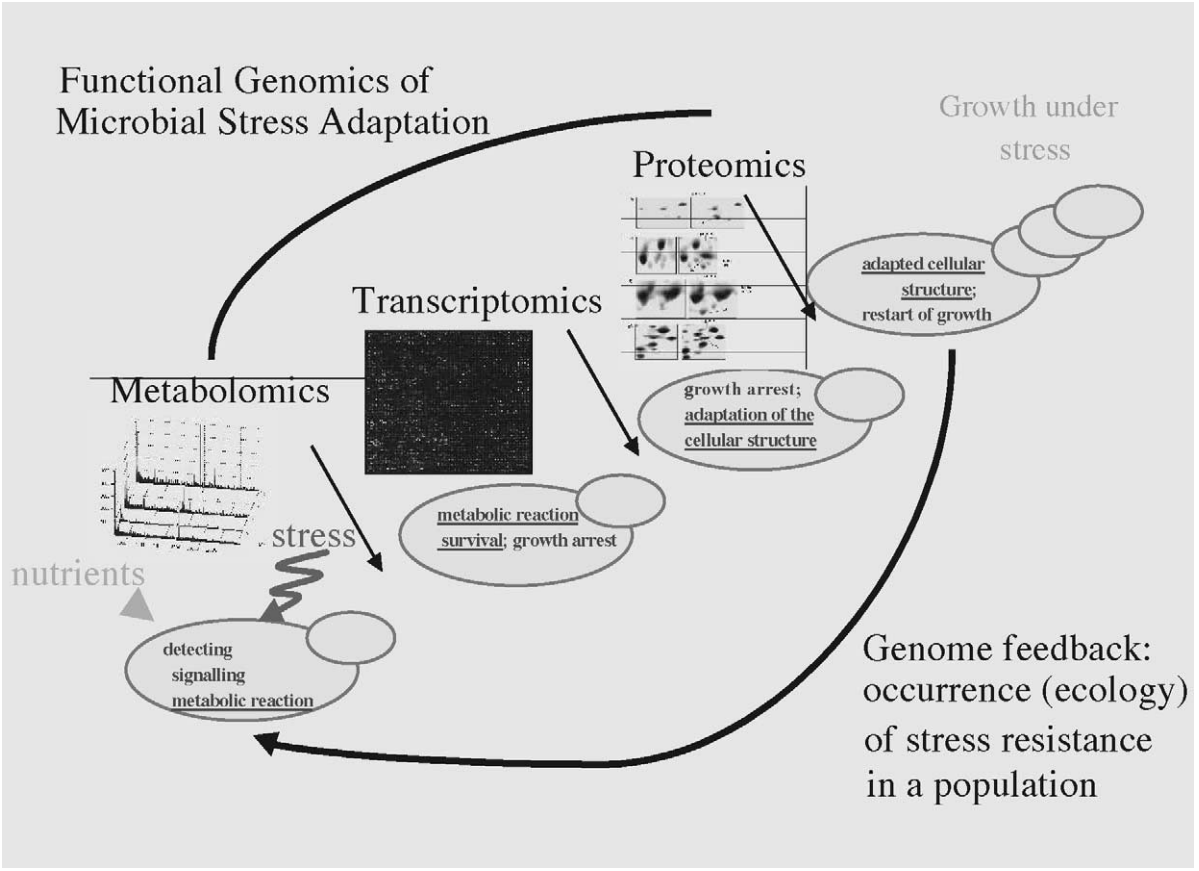

Fig. 2. A schematic outline on the application of metabolomics, transcriptomics and proteomics in a functional genomics (global physiology) study of stress response. Initial stress survival, stress adaptation and stress resistance are indicated. Note the feedback loop from stress resistance and outgrowth to the ecological distribution of stress sensitivity in a given population of cells.

compounds (preservatives), absence of nutrients, etc. In our perspective, the change from a normal physiological situation to a situation of stress is a gradual one. If one takes the analogy with humans walking outdoor in wintertime, it goes from walking outdoors and feeling chilly to walking outdoor and 'freezing to death'. There is a gradual scale. Similar to human populations, microbial cells also generally have two types of defence responses to these events. First, there is an intrinsic distribution of resistance against a particular stress among a population. Indeed in microbial inactivation curves, very often there are shoulders and tails indicative of more resistance of a subpopulation in the isogenic start population. Peleg and Cole (1998) have reviewed the area from a population analysis perspective. They stress that "The presented concept does not take into account the specific mechanisms that are the cause of mortality or inactivation."

They go on stating that "However, it is consistent with the notion that the actual destruction of a critical system or target is a probabilistic process that is due, at least in part, to the natural variability that exists in microbial populations." Studies linking this concept to cellular physiology and molecular biology have been, however, scarce. Recently, Hewitt and Nebe-Von-Caron (2001) have shown that fluorescent staining methods used in their laboratory have allowed a physiological classification of individual cells (*E. coli*, *Rhodococus* sp. and *S. cerevisiae*) based on metabolic activity, reproductive activity and membrane integrity. At least four subpopulations were distinguished based on this analysis. This was brought one step further

recently by Attfield et al. (2001) when they showed that with respect to a resistance to heat stress, there was a varying level of induction of the molecular response in the population analysed. The induction of heat stress response at the molecular level correlated thereby with a low membrane damage as assessed by propidium iodide (PI) uptake. Also, the reverse was true, i.e. a low level of molecular heat stress response induction correlated with a high incidence of membrane stress shown by the significant uptake of PI. In stress-adapted cells, the inactivation curve did not change in shape but was raised a few decimals indicating that both intrinsic and acquired (induced) stress response is distributed heterogeneously in the population (see also McAdams and Arkin, 1997; De Nobel et al., 2000, and reviewed in Sumner and Avery, 2002).

The cellular defence against hostile environments generally consists of a fortification of the cell wall and membrane, the 'outer and inner walls' of microbes where it concerns the action of antimicrobial agents. Membrane adaptations in *Clostridium botulinum* cells resistant to heat and the antimicrobial peptide nisin have been reported (Mazzotta and Montville, 1999). Also, acid-adapted *Listeria monocytogenes* displays enhanced tolerance against the lantibiotics nisin and lacticin 3147, which was presumably at least partially correlated with observed changes in the fatty acid composition of the bacterial membrane (van Schaik et al., 1999). Activity at and induced changes in the bacterial cell wall have also been reported as a response to the presence of antibacterial enzymes and peptides such as nisin (Breukink et al., 1999; Gravesen et al., 2001). In yeast cells, studies on resistance development against membrane active peptides have shown that this generally leads to significant increases in the chitin and cell wall protein levels, specifically of cell wall mannoproteins such as Cwp1p and Cwp2p (Dielbandhoesing et al., 1998; Bom et al., 2001).

Resulting cellular changes as a function of stress adaptation to weak organic acids have been described extensively in yeast. The notion that a proton-pumping ATPase played a crucial role was already established since long. However, these studies received a strong push at the end of the nineties of the previous century as a molecular characterisation of the involvement of a multidrug resistance pump in resistance development against weak organic acids was established (Piper et al., 1998). Coote and collaborators have shown that, indeed, this pump transports preservatives, sorbic acid, benzoic acid, acetic acid from the cytosol to the extracellular environment (Holyoak et al., 1999). Recently, microarray and proteomics analysis of weak organic acid resistant cells has been performed (De Nobel et al., 2001). This has indicated additional important resistant mechanisms such as the activation of heat-shock proteins and indications for the activation of the cell integrity pathway (see below).

3. Cellular stress signalling systems

3.1. Genomic transcript profiling

Genomic transcript profiling combined with cellular physiology has also opened the way to assess the cellular signalling systems involved in transmitting stress and regulating the defence systems. This has a crucial spin-off to food preservation research that we can generate truly predictive models of microbial growth inhibition through hurdle technology.

Of the classical preservatives, weak organic acids, we know little about the initial signal that sets off a cellular response. Besides lowering the internal pH, the acids also have an effect at the cell membrane. Particularly, sorbic acid was thought to exert a large part of its antimicrobial effect in this way (Stratford and Anslow, 1998). Furthermore, in order to prevent the development of a futile ATP-consuming metabolic cycle in which the anions and the protons are extruded from cells while reinflux occurs when the proton and anion reassociate at an extracellular pH equal to the p*K*, adjustments of the cell envelope were thought to take place. Indeed, the recent genome-wide analysis has indicated that cells responding to sorbic acid stress activate their cell integrity pathway signal transduction system (De Nobel et al., 2001). Upon inspection of the set of induced genes and proteins, it is evident that events at the cellular plasma membrane must be the start of the signalling cascade. Indeed, recently, it has been shown that the membrane sensor Wsc2p, belonging to the Wsc sensor family involved in cellular response against membrane perturbation through heat via the PKC1 pathway, regulates the membrane H^+-ATPase. The latter is known to form a crucial part of the sorbic acid stress response output (de la Fuente and Portillo, 2000). Acid stress response in enteric bacteria

involves an orchestrated stress response of partially overlapping arrays of acid stress response proteins (Audia et al., 2001).

Stress response against stressful environmental conditions has also been studied recently genome-wide in *E. coli* as indicated earlier. Such transcription profiling showed that the general stress regulator RpoS, which is normally only induced upon reaching stationary growth phase, is induced in cells experiencing low nutrient levels already in the logarithmic phase of growth (Fig. 3; Tao et al, 1999). In *B. subtilis*, our group recently studied genome-wide the response of cells towards environmental conditions in terms of forming high or low heat resistant spores (Brul et al., 2001). A regulatory role for the small acid soluble spore proteins (Sasps) was proposed. This was corroborated by experiments using knockouts of Sasps and assessment of heat stress resistance in the resulting spores (Setlow et al., 2000).

Stress response against membrane active antimicrobial agents, often of natural origin, has also recently been subject of extensive investigation particularly in yeast and vegetative cells of Gram-positive bacteria. In yeast, it has been shown that the incorporation of different amounts of cell wall proteins 1 and 2 and the increase in cell wall chitin content in response to nisin and synthetically modelled antimicrobial peptides are presumably also regulated at the cellular membrane via the PKC1 pathway (De Nobel et al., unpublished observations). In Gram-positive bacteria, it has been shown by Breukink et al. (1999) that the concentration of cell wall precursor lipid II is crucial in determining nisin resistance. Nisin binds to this molecule and in this way, prevents normal wall biosynthesis next to having a direct membrane perturbing effect (Wiedemann et al., 2001). In fact, recently, Gravesen et al. (2001) have shown that in spontaneous nisin-resistant mutants, the expression of a putative penicillin-binding protein was significantly increased, further indicating the link between cell wall and membrane homeostasis. Finally, the nonproteinaceous molecule carvacrol was studied extensively for its antimicrobial properties by Ultee et al. (1999, 2000). Changes in the membrane phospholipid fatty acid and

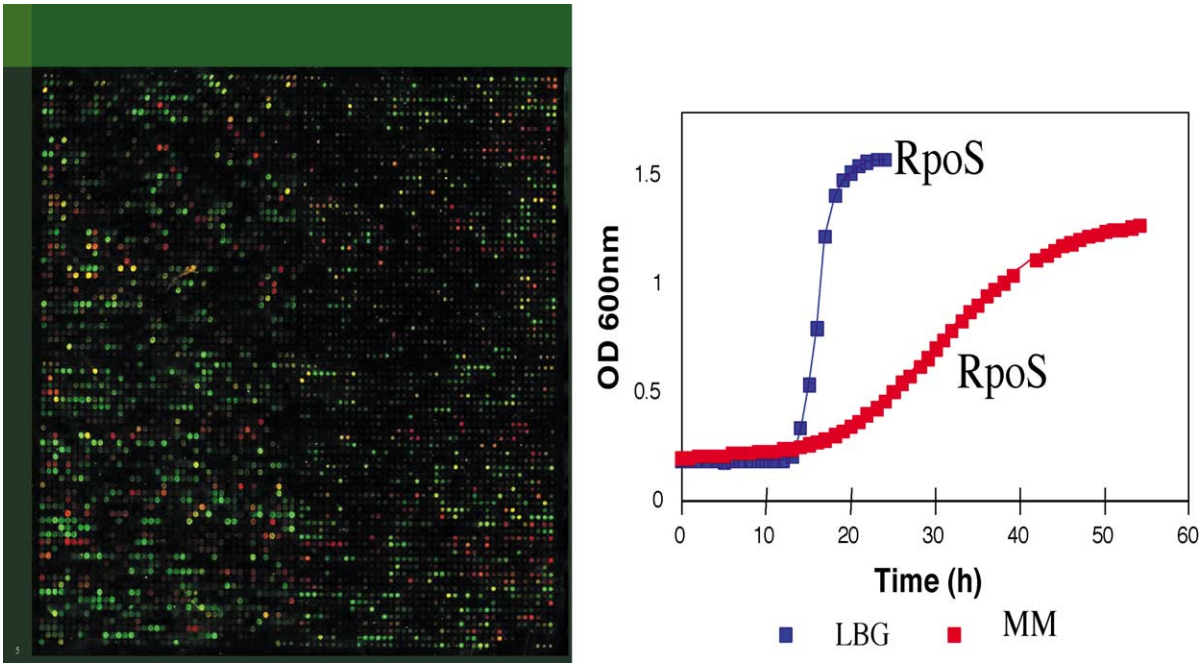

Fig. 3. Genome-wide expression analysis of *E. coli* cells growing in rich (LB) or minimal medium (MM) (Tao et al., 1999). The analysis of the data showed that all genes regulated by the RpoS stress response system were activated in stationary phase cells in *E. coli* grown in Luria Broth Glucose while it is already activated during logarithmic growth in minimal medium.

head-group composition were proven to be crucial in increasing resistance of *B. cereus* against this compound. In quite all cases in bacterial stress response reactions, one or more alternative σ-factors are involved that direct the RNA-polymerase to the specific stress response genes (see, e.g. Abee and Wouters, 1999). The link between cell wall and membrane homeostatis was recently also evident from studies by De Nobel et al. (2000) on the cellular response systems against cell wall degrading enzymes in yeast. Treating cells with wall lytic enzymes resulted in the activation of membrane-localised stretch-sensitive receptors from the Wcs and Mid protein family.

3.2. Integrating physiology with molecular biology

Cells can use like machines so many options, as there is energy available to drive the systems. Recent experiments in yeast aim at documenting the exact energy requirements cells have, when actively growing, initiating stress response systems and when recovering from the growth lag after stress application. First results with a physical stress, heat, in yeast show that applying a continuous temperature stress on wild type cells leads to an arrest of cell growth, a temporal increase in the glucose flux and ethanol production followed by an activation of the cell integrity protein kinase C1 pathway (Mensonides et al., 2002, submitted for approval). The latter alludes to the likelihood that the cells stop cell division and alter their carbon metabolism upon activating their stress response systems. Stress response upon heat stress was shown to activate at the molecular level the transcription factor Slt2 (Mpk1) by directly measuring the protein phosphorylation status using specific antibodies. De Nobel et al. (2001) have meanwhile performed similar studies aimed at preservative agents such as sorbic acid and novel natural alternatives (unpublished observations).

4. Outlook

The understanding of cellular response at the level of the molecular events opens up the way to integrally

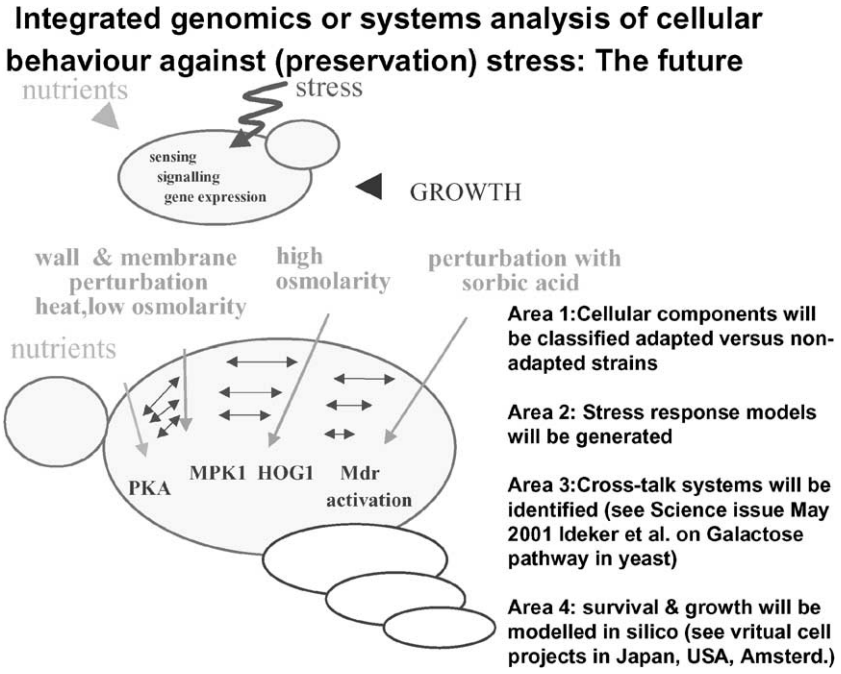

Fig. 4. A schematic outline of cellular signal transduction and response mechanisms in yeast cells towards environmental factors. The combination of a given nutrient availability and a set of environmental (stress) conditions will determine whether a cell can restart growth. Depicted are the response against hyper and hypoosmotic stress, heat stress and stress with the food preservative sorbic acid. A few future scenarios are indicated for the system analysis of cellular behaviour against (food preservation) stresses.

assess microbial response to environmental conditions beyond the level of growth-no growth or survival and death. Thus, this allows for the development of mechanistic growth and inactivation models that will also have a predictive power outside the measured data points. Models that will be able to act as guides in identifying novel combinations of environmental stress conditions through their level of detail at the 'wiring' of the cellular machinery. Models also that will look like chemical engineering tools describing the flow of metabolites through the cellular regulatory systems (see, e.g. Teusink, 2001; Stephanopoulos and Kelleher, 2001) (Fig. 4). With such predictive models at hand, the development of food preservation systems can be approached more and more quantitatively and can be handled integrated with other food processing unit operations in one process model. Recently, this principle was illustrated extensively by Bruin (2000) in his keynote Food Engineering lecture at the annual meeting of the American Society for Food Technologists.

The technology, when applied in an integrated way in food processing, should lead to a significant shortening of the evaluation of novel processing (preservation) systems. This may be achieved in a number of cases through the full identification of relevant cellular targets, but may also be at the level of generating fingerprints of given treatments. In this way, a very fast comparison of cellular response towards a large variety of processing/preservation treatments will be made possible. In the pharmacology area, such a pharmacogenomics approach has been used in characterising novel antifungal compounds (Bammert and Fostel, 2000). In addition, strain comparisons in terms of responses to treatments can easily be made (strain fingerprints). In this way, equivalence of treatments and strains can unequivocally be assessed in a relatively short time frame.

Finally, we will list the advantages of a better understanding of the physiological actions of preservative systems to the food manufacturing industry as we see them over the next 5–10 years:

- improved food quality and wholesomeness through lower thermal treatment;
- possibilities for new products (mildly preserved, organic foods);
- energy savings through better controlled processes;
- less use of waste and cooling water;
- reduction in the use of cleaning and disinfecting agents;
- less waste through better process control.

We are confident that many, if not all, of these promises will be fulfilled. However, only the future can and will tell.

References

Abee, T., Wouters, J.A., 1999. Microbial stress response in minimal processing. Int. J. Food Microbiol. 50, 65–91.

Attfield, P.V., Choi, H.Y., Veal, D.A., Bell, P.J., 2001. Heterogeneity of stress gene expression and stress resistance among individual cells of *Saccharomyces cerevisiae*. Mol. Microbiol. 40, 1000–1009.

Audia, J.P., Webb, C.C., Foster, J.W., 2001. Breaking through the acid barrier: an orchestrated response to proton stress by enteric bacteria. Int. J. Med. Microbiol. 291, 97–106.

Bammert, G.F., Fostel, J.M., 2000. Genome-wide expression patterns in *Saccharomyces cerevisiae*: comparison of drug treatment and genetic alterations affecting biosynthesis of ergosterol. Antimicrob. Agents Chemother. 44, 1255–1265.

Bom, I.J., Nobel, H., Klis, F.M., Brul, S., 2001. A new strategy for inhibition of the spoilage yeast *Saccharomyces cerevisiae* and *Zygosaccharomyces bailii* based on combination of a membrane-active peptide with an oligosaccharide that leads to an impaired GPI-depend wall protein layer. FEMS Yeast Res. 1, 187–194.

Breukink, E., Wiedemann, I., van Kraaij, C., Kuipers, O.P., Sahl, H., de Kruijff, B., 1999. Use of the cell wall precursor lipid II by a pore-forming peptide antibiotic. Science 286, 2361–2364.

Bruin, S., 2000. Food process engineering, the past 25 years and challenges ahead. Abstract seminar at the 2001 annual meeting of the Institute for Food Technologists.

Brul, S., 2000. From cell to molecule and back (Dutch) Inaugural lecture at the acceptance of the chair in Industrial Molecular Microbiology Univ. Amsterdam Vossiuspers AUP, Amsterdam.

Brul, S., Klis, F.M., 1999. Mechanistic and mathematical inactivation studies of food spoilage fungi. Fungal Genet. Biol. 27, 199–208.

Brul, S., Klis, F.M., Coote, P., 2001. Unravelling the mode of action of preservation processes through functional genomics on survivors. Abstract IFT Annual Meeting Proceedings, pp. 8–9.

Chu, S., DeRisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P.O., Herskowitz, I., 1998. The transcriptional program of sporulation in budding yeast. Science 282, 699–705.

de la Fuente, N., Portillo, F., 2000. The cell wall integrity/remodelling MAPK cascade is involved in glucose activation of the yeast plasma membrane H(+)-ATPase. Biochim. Biophys. Acta 1509, 189–194.

De Nobel, H., Ruiz, C., Martin, H., Morris, W., Brul, S., Molina, M., Klis, F.M., 2000. Cell wall perturbation in yeast results in dual phosphorylation of the Slt2/Mpk1 MAP kinase and in an

Slt2-mediated increase in FKS2-LacZ expression, glucanase resistance and thermotolerance. Microbiology 146, 2121–2132.

De Nobel, H., Lawrie, L., Brul, S., Klis, F., Davis, M., Alloush, H., Coote, P., 2001. Parallel and comparative analysis of the proteome and transcriptome of weak-acid adapted *Saccharomyces cerevisiae* reveals a crucial function for the small heat-shock protein Hsp26. Yeast 18, 1413–1428.

De Risi, J.L., Iyer, V.R., Brown, P.O., 1997. Exploring the metabolic and genetic control of gene expression on a genomic scale. Science 278, 680–686.

de Vos, W.M., 2001. Advances in genomics for microbial food fermentations and safety. Curr. Biotechnol. Opin. 12, 493–498.

Dielbandhoesing, S.K., Zhang, H., Caro, L.H., van der Vaart, J.M., Klis, F.M., Verrips, C.T., Brul, S., 1998. Specific cell wall prots. confer resistance to nisin upon yeast cells. Appl. Environ. Microbiol. 64, 4047–4052.

Fawcett, P., Eichenberger, P., Losick, R., Youngman, P., 2000. Global gene expression profiles of *Bacillus subtilis* grown under anaerobic conditions. J. Bacteriol. 182, 8063–8068.

Fell, D.A., 1997. Understanding the Control of Metabolism. Portland, London.

Gravesen, A., Sorensen, K., Aarestrup, F.M., Knochel, S., 2001. Spontaneous nisin resistant *Listeria monocytogenes* mutants with increased expression of a putative penicillin-binding protein and their sensitivity to various antibiotics. Microb. Drug Resist. 7, 127–135.

Hecker, M., Volker, U., 2001. General stress response of *Bacillus subtilis* and other bacteria. Adv. Microb. Physiol. 44, 35–91.

Hewitt, C.J., Nebe-Von-Caron, G., 2001. An industrial application of multiparameter flow cytometry: assessment of cell physiological state and its application to the study of microbial fermentations. Cytometry 44, 179–187.

Hirose, I., Sano, K., Shioda, I., Kumano, M., Nakamura, K., Yamane, K., 2000. Proteome analysis of *Bacillus subtilis* extracellular proteins: a two-dimensional protein electroph. study. Microbiology 146, 65–75.

Hofmeyr, J.H., Westerhoff, H.V., 2001. Building the cellular puzzle: control in multi-level reaction networks. J. Theor. Biol. 208, 261–285.

Holyoak, C.D., Bracey, D., Piper, P.W., Kuchler, K., Coote, P.J., 1999. The *Saccharomyces cerevisiae* weak-acid-inducible ABC transporter Pdr12 transports fluorescein and preservative anions from the cytosol. J. Bacteriol. 181, 4644–4652.

Jongen, T., 2001. e-ntegrated design: where product, process and consumer come together. Unilever Research Prize lecture 2000, Vlaardingen, The Netherlands.

Lucchini, S., Thompson, A., Hinton, J.C.D., 2001. Microarrays for microbiologists. Microbiology 147, 1403–1414.

Marks, H.M., Coleman, M.E., Lin, C.T., Roberts, T., 1998. Topics in microbial risk assessment: dynamic flow tree process. Risk Anal. 18, 309–328.

Mazzotta, A.S., Montville, T.J., 1999. Characterization of fatty acid composition, spore germination, and thermal resistance in a nisin-resistant mutant of *Clostridium botulinum* 169 B and in the wild-type strain. Appl. Environ. Microbiol. 65, 659–664.

McAdams, H.H., Arkin, A., 1997. Stochastic mechanisms in gene expression. Proc. Natl. Acad. Sci. 94, 814–819.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L., Bresee, J.S., Shapiro, C., Griffin, P.M., Tauxe, R.V., 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5, 607–625.

Nouwens, A.S., Cordwell, S.J., Larsen, M.R., Molloy, M.P., Gillings, M., Wilcox, M.D., Walsh, B.J., 2000. Complementing genomics with proteomics: the membrane subproteome of *Pseudomonas aeruginosa* PAO1. Electrophoresis 21, 3797–37809.

Peleg, M., Cole, M., 1998. Reinterpretation of microbial survival curves. Crit. Rev. Food Sci. Nutr. 38, 353–380.

Piper, P., Mahe, Y., Thompson, S., Pandjaitan, R., Holyoak, C., Egner, R., Muhlbauer, M., Coote, P., Kuchler, K., 1998. The pdr 12 ABC transporter is required for the development of weak organic acid resistance in yeast. EMBO J. 15, 4257–4265.

Setlow, B., McGinnis, K.A., Rakousi, K., Setlow, P., 2000. Effects of major spore-specific DNA binding proteins on *Bacillus subtilis* sporulation and spore properties. J. Bacteriol. 182, 6906–6912.

Stephanopoulos, G., Kelleher, J., 2001. How to make a superior cell. Science 292, 2024–2025.

Stratford, M., Anslow, P.A., 1998. Evidence that sorbic acid does not inhibit yeast as a classic 'weak acid preservative'. Lett. Appl. Microbiol. 27, 203–206.

Sumner, E.R., Avery, S.V., 2002. Phenotypic heterogeneity: differential stress resistance among individual cells of the yeast *Saccharomyces cerevisiae*. Microbiology 148, 345–351.

Tao, H., Bausch, C., Richmond, C., Blattner, F.B., Conway, T., 1999. Functional genomics: expression analysis of *Escherichia coli* growing on minimal and rich media. J. Bacteriol. 181, 6425–6440.

Teusink, B., 2001. Exposing a complex metabolic system glycolysis in *Saccharomyces cerevisiae*. PhD thesis, University of Amsterdam.

Tobisch, S., Zühlke, D., Bernhardt, J., Stülke, J., Hecker, M., 1999. Role of Ccp in regulation of the central pathways of carbon metabolism in *Bacillus subtilis*. J. Bacteriol. 181, 6996–7004.

Ultee, A., Kets, E.P., Smid, E.J., 1999. Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. Appl. Environ. Microbiol. 65, 4606–4610.

Ultee, A., Kets, E.P., Alberda, M., Hoekstra, F.A., Smid, E.J., 2000. Adaptation of the food-borne pathogen *Bacillus cereus* to carvacrol. Arch. Microbiol. 174, 233–238.

van Hoek, P., van Dijken, J.P., Pronk, J.T., 1998. Effect of specific growth rate on fermentative capacity of baker's yeast. Appl. Environ. Microbiol. 64, 43–49.

van Schaik, W., Gahan, C.G., Hill, C., 1999. Acid-adapted *Listeria monocytogenes* displays enhanced tolerance against the lantibiotics nisin and lacticin 3147. J. Food Prot. 62, 536–539.

Verrips, C.T., Warmoeskerken, M.M.C.G., Post, J.A., 2001. General introduction to the importance of genomics in food biotechnology and nutrition. Curr. Opin. Biotechnol. 12, 483–487.

Wiedemann, I., Breukink, E., van Kraaij, C., Kuipers, O.P., Bierbaum, G., de Kruijff, B., Sahl, H.G., 2001. Specific binding of nisin to the peptidoglycan precursor lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. J. Biol. Chem. 276, 1772–1779.

Ye, R.W., Tao, W., Bedzyk, L., Young, T., Chen, M., Li, L., 2000. Global gene expression profiles of *Bacillus subtilis* grown under anaerobic conditions. J. Bacteriol. 182, 4458–4465.

Yoshida, K.-I., Kobayashi, K., Miwa, Y., Kang, C.-M., Matsunaga, M., Yamaguchi, H., Tojo, S., Yamamoto, M., Nishi, R., Ogasawara, N., Nakayama, T., Fujita, Y., 2001. Combined transcriptome and proteome analysis as a powerful approach to study genes under glucose repression in *Bacillus subtilis*. Nucleic Acids Res. 29, 683–692.