Review

Transcriptome Analysis of Organisms with Food Safety Relevance

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Abstract

Transcriptome analysis using microarrays has become a powerful tool to better understand the process of disease and other complex biological processes such as food spoilage and biofilm formation. This review is divided into two basic sections: 1) a short history and description of microarrays and 2) a discussion of studies involving bacterial food safety pathogens that focused on whole genome transcript analysis. Not included are the many studies using microarrays to identify, diagnose, or genetically characterize these organisms. This review focuses on studies involving *Escherichia coli* O157:H7, *Salmonella* spp., *Campylobacter jejuni*, *Listeria monocytogenes*, and *Yersinia enterocolitica*. Many of the studies involve altering the growth environment to simulate stress conditions and the use of host–pathogen model systems to explore virulence mechanisms. Few studies use conditions that might be considered unique to the food industry. Exceptions are studies of biofilm-specific transcriptome changes and analysis following pressure treatment. This review should not be considered as a comprehensive review, and where appropriate, species-specific reviews are cited that are more complete.

Introduction

Definition

The transcriptome can be thought of as the complete population of messenger RNAs of a cell, which may vary under different environmental conditions. Transcriptomics is the study of that transcriptome in a global fashion, most often using high throughput technologies such as microarrays. This is a rapidly evolving field and early manuscripts lack the biological replicates or statistical analyses needed to derive a true picture of variation. Nevertheless, each of the manuscripts discussed here offer insight into the physiology and pathogenesis of organisms that was not previously possible. In this review, we have provided some discussion on the salient features of microarrays and their use in studies of transcriptomics of bacteria of food safety relevance, *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, and *Yersinia enterocolitica*.

History

All groundwork for the beginning of transcriptomics, microarrays, began in the 1960s with the fixation of DNA to nitrocellulose filters followed by detection with radiolabeled probes. In 1975, Southern expanded the technology to include separation of specific fragments prior
to fixation to filters (Southern, 1975). In these experiments, the unknown nucleic acid sequences or “targets” were transferred to membranes and the known labeled sequence or “probe” was allowed to interact. By doing so, Southern was able to detect specific sequences in complex populations of DNA digested with restriction enzymes. The technology is based upon the ability of single-stranded DNA to hybridize to regions of homology forming stable hydrogen-bonded double-stranded molecules. Microarrays are based upon the same technology, but with a much smaller footprint, in an opposite format, where the known probe sequences are depositions on a solid substrate and the unknown nucleic acid targets (i.e., mRNAs, cDNAs) are labeled and interacted with the bound probe sequences. In contrast to Southern technology, however, microarrays offer a massively parallel gene expression and gene discovery approach to complex biological problems. When cDNAs are used as targets, the level of signal intensity can be directly correlated with the steady state levels of mRNAs in the experimental system. Alternatively, DNA has been used to interrogate microarrays to identify nonhomologous sequences. In these cases, fluorescence differences indicate sequence variation (Brown and Botstein, 1999).

The use of microarrays in molecular research is relatively new, with the concept of miniaturized, multianalyte “microspot” immunoassays being initially described by Ekins and coworkers (Ekins, 1989; Ekins et al., 1989). This idea was then carried through to the gene expression world in the mid-1990s with Schena and colleagues publishing the first transcriptomics microarray paper with the differential expression of 45 genes in Arabidopsis using a two-color microarray (Schena et al., 1995) and then further progressing to transcriptome wide coverage in an experiment with Saccharomyces cerevisiae (DeRisi et al., 1997). Since these beginnings, the number of microarray reports on gene expression has been astonishing. The accumulation of genome or cDNA sequence, the reduction in costs of sequencing, the increase in quality of sequencing, and the lower costs of oligonucleotides have been key to this growing field. The commercial availability of glass substrates, target preparation kits, hybridization kits, data acquisition systems, and data analysis software has also helped fuel this research allowing laboratories to study their organism of choice using their own customized DNA microarray.

Types of arrays

Microarrays can be divided into two basic types: oligonucleotide and cDNA or polymerase chain reaction (PCR) products. Oligonucleotide arrays can be constructed by photolithography in situ or they can consist of spotted preformed oligonucleotides. Affymetrix (Santa Clara, CA) has been producing commercially available microarrays called GeneChips® since 1994. Their technology revolves around the use of short oligonucleotides usually 25 nucleotides in length bound to glass slides. The oligonucleotides are produced in situ on glass substrates using photolithography as opposed to spotting pre-synthesized oligonucleotides using a pin-based or ink jet style robot. The use of short oligonucleotides in microarrays presents some specific problems in terms of gene coverage and cross reactivity between genes, but computer algorithms have been developed to account for these adverse reactions.

Spotted arrays consist of DNA sequences that represent individual genes. The spotted DNA, referred to as probes, represent known DNA sequences. The sequence information needed to produce specific probes can be obtained from genome sequencing projects, expressed sequence tag projects, or could be individual cDNA clones spotted as plasmids. The latter could be sequenced later once specific targets have been identified through array analysis. For PCR-based arrays, probe generation must be carefully controlled, taking into consideration things like PCR primer design (for oligonucleotide arrays, oligonucleotide design is the single most important factor), PCR conditions, PCR product purification and purity, product concentration, and spotting buffer composition. The length of the PCR products varies between 100 and 500 base pairs. Long oligonucleotides 40–70 nucleotides long are becoming more common since their cost has decreased significantly and their use eliminates many of the variables associated with PCR product purification, quantitation, etc. They are spotted to
glass substrates using the same technology as with PCR products, but the substrates themselves have a different binding chemistry to enhance their attachment. A more detailed analysis of microarray construction can be obtained from Schena (2003), which describes the dozens of variables involved in array construction from glass substrates and their modification, probe design, manufacturing technologies, detection systems, and informatics.

To generate data, microarrays are hybridized with fluorescently labeled “targets,” such as fragmented genomic DNA, cDNA, RNA, or even proteins. They represent the unknown sequences or sequence quantities of a sample and are the elements of the study under investigation. Ideally, each labeled target is represented in the hybridizing population in the same relative ratio or concentration as in the original sample.

Bacterial systems present unique problems in fluorescent target generation because of the difficulty in identifying bacterial mRNA during the labeling reaction; they have no polyadenylated tails that can be used to purify or selectively label the mRNA molecules. Thus, either total RNA must be labeled (mRNA represents only ~5% of the sample) reducing the signal intensities significantly on the arrays, the ribosomal RNAs must be removed from the total RNA preparations, or gene-specific primers must be used to generate the labeled targets. Procedures and reagents are now available for removing the ribosomal RNAs from many prokaryotic total RNA preparations by using specific primers to bind to ribosomal sequences, which are then removed from the RNA solution using magnetic beads. However, they are not useful for all bacterial species because of the sequence variation in ribosomal sequences and the lack of coverage in the primer sequence pool by the manufacturer (Ambion, Austin, TX).

Following target labeling and hybridization, the fluorescence intensities of each spot should accurately represent the concentration of their corresponding target in the sample. The fluorescence intensities from each spot can then be analyzed for statistical significance of genomic or transcriptional differences. The spotted arrays are usually hybridized with two labeled target samples so that control and experimental samples can be analyzed on the same spot and on the same slide thus allowing background subtraction and normalization to be performed for both control and experimental data from the same array. Short oligonucleotide-based arrays from Affymetrix are hybridized with a single labeled sample, but other companies offer custom-synthesized sets of oligonucleotides that can be spotted or supply spotted arrays that can be used with either one or two color analyses.

Informatics

The development of microarray technology has brought additional needs for data management and analyses. Software development alone has been extensive and includes primer design, image acquisition and quantitation, database design, and statistical analysis.

The processing of a scanned image requires three separate tasks: addressing or gridding, segmentation, and intensity extraction (Schena, 2003). Addressing is assigning the coordinates to each of the spots on the image. Segmentation classifies the pixels as foreground (within the spot) or background (outside the spot). Intensity extraction involves both summing the pixel intensities within the spot mask and the calculation of local background intensities that arise from nonspecific hybridization and fluorescence emitted from other chemicals on the glass.

Presentation and analysis of microarray data are continuing to evolve. Early studies presented false color images (Schena et al., 1996; Welford et al., 1998), scatter plots (Hilsenbeck et al., 1999), and cluster diagrams (Eisen et al., 1998). These methods of analysis give an overview of the data, but do not provide critical evaluation of the data or its significance. To statistically test and evaluate data of such magnitude, new methods of analysis needed to be applied.

Analysis of microarray data requires multiple steps (Schena, 2003). Microarray data is not normally distributed and therefore it is necessary to treat the data by log transformation. Normalization then adjusts for systematic differences in the relative intensity of each signal channel, and correct for differences in intensities.
within the same slide or between slides that are array dependent and not due to true biological variation. A number of approaches have been defined for normalization and are discussed by Schena (2003).

Many aspects of array construction and design can help to ensure the quality of the array and hence the value of statistics resulting from an experimental study. Increasing the number of biological replicates is favored over increasing technical replicates from limited biological samples (Churchill, 2002). Normalizing data compensates for the variation introduced by the technical aspects, such as unequal fluorescent dye incorporation and the array-to-array variation, but not variation due to biological differences (Smyth and Speed, 2003). Normalization in two-color systems is almost always necessary due to the varied incorporation rates and stability of the fluorescent dyes and is completed before statistical analysis.

A two-color array design is powerful because it has the ability to directly compare two samples (Churchill, 2002). Factors included in the statistical model are the arrays, experimental treatments, genes, array by gene interaction, and the most interesting effect, treatment by gene (Kerr et al., 2000). Kerr et al. (2000) describe the ease with which data is normalized and then analyzed to reflect the significant effects of treatment upon a specific gene. Raw data should be transformed using a logarithmic scale to provide a more uniform distribution of the data for analyses using parametric procedures (Schena, 2003). Using a logarithmic scale to transform the raw data and not the log ratio values as is often reported also allows for ease of representing the data as a fold-change in gene expression with statistical significance associated with that change in expression. However, relying strictly on fold-change values results in numerous false positives and false negatives (Tanaka et al., 2000). Tanaka et al. (2000) found that differentially expressed genes in the 1.2- to 2-fold change can often be overlooked as nonsignificant, when they have actual biological and statistical significance in replicated data sets.

There are numerous options for data analysis including freeware open source software and commercial products. Examples of freeware packages include Significance Analysis of Microarrays (SAM; www-stat.stanford.edu/~tibs/SAM/), Cyber-T (cybert.microarray.ics.uci.edu/), ScanAnalyze (rana.lbl.gov/EisenSoftware.htm), and the TM4 microarray software suite (www.tm4.org). Commercial software products are also available from Agilent (www.agilent.com), Nimblegen (www.nimblegen.com), BioDiscovery ImaGene (www.biodiscovery.com), and Molecular Devices (www.moleculardevices.com) among others. The microarray software continues to improve, but it has been the experience of these authors that a good spot finding software package coupled with the statistical packages SAS (www.sas.com) and R (www.r-project.org) excels in data analysis while providing a better understanding and control of each step of the analysis process.

Applications

Microarrays are used to elicit information about genetic or transcription differences represented on the array. For example, genetic variation in clinical or field isolates of pathogens, transcriptional variation between isolates, and effects of environmental changes on transcriptional variation in pathogens are all suitable topics for investigation using microarrays. Although too many to list, the number of publications utilizing microarray technology is growing rapidly and the possible experiments seem limitless.

The examination of the genetic content of bacteria is not discussed here, but genetic variations often indicate the emergence of new strains and adaptations to new environmental niches. Genetic comparisons allow the medical field to track how bacterial species may acquire genes, increasing the occurrence of infections in human populations (Tettelin et al., 2002; Wick et al., 2005). Additional studies outline the similarity of laboratory and clinical strains giving validity to in vitro assays using laboratory strains (Snyder et al., 2004). These are all important uses for microarrays.

**Escherichia coli O157:H7**

*Escherichia coli* O157:H7 has captured the majority of the public awareness as the agent of food safety importance because of the massive product recalls, its contamination of seemingly
unrelated products, and the severe nature of the disease in children (hemolytic uremic syndrome). Despite the public interest and the large numbers of scientists addressing this problem, transcriptome analysis of *E. coli* O157:H7 using microarrays has been limited. The bulk of *E. coli* O157:H7 studies are geared towards rapid detection while only a handful of studies have used whole genome arrays. The few transcriptional studies have mainly focused on gene expression changes due to an environmental stressor such as high pressure or conditions that are meant to mimic *in vivo* environments.

Ultra high pressure is used in food processing as a method of preserving food by reducing or eliminating bacterial populations. The effect of high pressure on bacterial gene expression was studied by comparing normal and high pressure resistant *E. coli* O157:H7 strains at normal pressure to high pressure treatment (Malone et al., 2006). In this study, genes involved in spontaneous mutation, thiol-disulfide redox, Fe-S cluster assembly, and stress response genes were induced. Since the *E. coli* K-12 Affymetrix gene chip was used in these studies, no *E. coli* O157:H7–specific genes would have been measured. This would include genes encoded on O-islands or in other *E. coli* O157:H7–specific gene regions (Perna et al., 2001).

While in the intestinal tract of humans, *E. coli* O157:H7 is exposed to many different compounds including molecules that act as cellular signals. *Escherichia coli* Affymetrix 2.0 chips, which covers strains K-12, O157:H7-EDL933, O157:H7-Sakai, and CFT073, were used to investigate the effects of such molecules including epinephrine, norepinephrine, and indole on *E. coli* O157:H7–specific gene regions (Bansal et al., 2007). Results indicated that epinephrine and norepinephrine up-regulated genes involved in virulence and surface colonization while indole down-regulated genes involved in these processes. Similarly, the effects of 7-hydroxyindole and isatin derived from the oxidation of indole on gene expression during biofilm growth as compared to planktonic growth was measured (Lee et al., 2007). During biofilm growth 20 genes in the locus of enterocyte effacement (LEE) were repressed as well as genes previously shown to be involved in biofilm growth and colonic acid biosynthesis in other systems. 7-Hydroxyindole repressed genes involved in purine nucleotide biosynthesis, cysteine biosynthesis, and carbamoyl phosphate synthetase. Isatin repressed genes involved in AI-2 transport and indole biosynthesis while inducing flagellar genes and various transport genes.

A family of non–LEE-encoded (Nle) effector proteins was studied by Roe et al. (2007). Using a combination of microarrays, reporter fusions, and proteomics, they showed that only NleA is expressed coordinately with LEE under control of Ler. None of the other Nle proteins, NleB-F, were transported to the culture supernatant under secretion-permissive conditions (Roe et al., 2007).

In some cases, microarrays have been used to study transcriptional differences due to specific mutations. Campellone et al. (2007) studied transcript levels in an *E. coli* O157:H7 dam mutant finding, that despite increased levels of Tir in the cell, *tir* transcript levels showed little elevation. They reasoned that *dam* methylation must affect protein expression independent of transcription. Fitzhenry et al. (2006) studied a mutation in long polar fimbriae showing an altered tissue tropism for human intestinal tissues changing from specificity for follicle-associated epithelium to also include small intestinal mucosa, causing attaching and effacing lesions on both tissues.

During infections, *E. coli* O157:H7 comes in close contact with eukaryotic cellular membranes when colonizing the intestinal tract of humans and ruminants. Microarray analysis of *E. coli* O157:H7 adhered to rabbit red cell membranes for 5 hours revealed that stress response genes are induced, and genes responsible for attachment and translation are repressed (Dahan et al., 2004).

**Salmonella Spp.**

*Salmonella* species are gram-negative, facultative, motile rods that are foodborne human pathogens. *Salmonella enterica* serovars Enteritidis and Typhimurium comprise the majority of *Salmonella* infections (CDC). *Salmonella* is one of the most common causes of diarrhea and is transmitted primarily by contaminated poultry meat or eggs. The majority of gene expression microarrays experiments conducted
on *Salmonella* fall into two major categories; comparisons of mutant vs. wild-type and treatments that mimic conditions found *in vivo*.

The pathogenesis of *S. typhimurium*, like most intracellular pathogens, is complex, requiring regulation of gene expression to survive in the gastrointestinal tract or in intracellular locations. Several genetic regulators involved with these processes have been identified and characterized. An excellent recent review of transcriptomics with *Salmonella* has been published and should be read by those interested in this pathogen (Thompson et al., 2006).

Microarray studies comparing gene expression in wild-type vs. mutant allow for the elucidation of additional genes that may be involved in certain phenotypes. For instance, a *S. typhimurium* polynucleotide phosphorylase mutant that demonstrated a chronic infection in the BALB/c murine model was compared to its wild-type using microarrays (Clements et al., 2002). Genes located within the two major *S. typhimurium* pathogenicity islands SPI-1 and SPI-2 were up-regulated in the mutant suggesting that this gene may play a role in the establishment of persistent infection in the mouse model of infection. In a similar experiment, a mutant in the known regulator of *S. typhimurium* invasion genes, *csrA*, was compared to its wild-type using microarrays (Lawhon et al., 2003). The results of Lawhon et al. (2003) demonstrated that genes within SPI-1 and the effector proteins that are translocated by the type III secretion systems were repressed in the *csrA* mutant. Another gene whose product is involved in nucleoid organization, *fis*, has been shown to up-regulate genes within multiple SPIs as well as metabolism genes associated with life inside the host gut (Kelly et al., 2004). In a microarray study of the two-component regulatory system *phoPQ*, regulon mutants were compared to wild-type by Monsieurs et al. (2005) showing that this regulon in *S. typhimurium* has diverged from *E. coli* K-12 both in regulon members and in regulatory recognition sequences. Quorum sensing in *S. typhimurium* was examined using a *luxS* mutant (Widmer et al., 2007). In this study, AI-2 was added exogenously resulting in the differential regulation of 23 genes. In the presence of AI-2 several virulence-associated genes were down-regulated.

While inside the host, *S. typhimurium* encounters numerous environments that it must endure and overcome in order to ultimately cause disease. Understanding the response that pathogens have to these environments can give us a better sense of the biology within the host and what measures can be taken to prevent infection. Measuring global transcript levels using microarrays during exposure to specific conditions gives us insight into how pathogens regulate genes to cope with the *in vivo* environment. A series of experiments looking at microgravity, a condition meant to mimic low-shear conditions *in vivo*, have given some interesting insight into what may be happening in host tissues (Wilson et al., 2002a, 2002b; Wilson et al., 2007). In response to microgravity, *S. typhimurium* differentially expressed 163 genes; genes involved in virulence, acid stress, and iron acquisition among others were up-regulated (Wilson et al., 2002a, 2002b).

While inside the host, *S. typhimurium* comes in contact with a variety of compounds. Bile salts are an integral part of digestion in the human gut and can be toxic to bacteria. Treatment of *S. typhimurium* with the bile salt deoxycholate induces expression of the multiple antibiotic resistance locus *marRAB* along with *acrAB* which encode members of the AcrAB-TolC efflux pump (Prouty et al., 2004a). Altogether 230 genes were differentially expressed due to bile treatment including repression of flagellar genes and genes associated with type III secretion system–mediated invasion of host cells (Prouty et al., 2004b).

*Salmonella* also encounters host microflora and their products. To determine the effect of probiotic bacteria on gene expression of *S. typhimurium*, researchers exposed cells to spent culture supernatants of *Lactobacillus rhamnosus* (De Keersmaecker et al., 2005). It was found that the virulence regulator *hilA* was repressed, showing insight into how probiotic bacteria may antagonize a pathogen other than by competing for attachment sites and preventing colonization.

Wang et al. (2004) used microarrays to interrogate transcript levels of *S. typhimurium* while engaging in the surface motility phenomenon called swarming (Wang et al., 2004). An 8-hour time course experiment indicated
that genes involved in LPS synthesis, the type III secretion system, and iron metabolism were all up-regulated during swarming. Maximum differences occurred at 3 hours coinciding with maximal swarming activity. As the cells entered stationary phase, genes were generally down-regulated in the swarming cultures compared to broth grown organisms. The authors suggest that this may be able to serve as a model of host infection since many genes previously identified as virulence factors were up-regulated. In another study, Wang et al. (2007) used microarrays to determine the effect of an igaA mutation on maximal activation of the Rcs phosphorelay signaling system on global gene expression. Their results showed a large number of regulated genes, potentially as many as one-fifth of the genome. The key to this study was the identification of an igaA mutation that maximally expressed the Rcs system so by comparing that strain to a ΔrcsB strain, they were able to identify the whole range of negatively and positively regulated genes under RcsB control (Wang et al., 2007).

Salmonella has been the test organism for several publications involving new software or techniques using microarrays. In a demonstration of a bioinformatics tool named LACK the effects of the iron chelator dipyridyl on S. typhimurium was studied using microarrays. It was found that iron deprivation up-regulates genes located within SPI-2 (Kim and Falkow, 2003). Another study combined the knowledge of operon transcriptional coupling with expression response data to improve the analyses of Salmonella exposure to 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), the primary mutagen in drinking water. The authors considered all genes in an operon as changed if one member showed significant change in response to MX as well as those genes that showed monotonic change with increasing MX concentration as a way to enhance identification of differentially expressed genes beyond the standard Bayesian $t$ test (Ward et al., 2007). Ward et al. (2007) found that DNA damage–responsive operons were induced along with porphyrin metabolism and membrane transport genes.

The power of microarray analysis was illustrated by a study by Frye et al. (2006). In this study, RNA levels of flagella genes in eight different genetic backgrounds were measured. By combining directed mutational analysis with microarray data, they were able to identify new flagella genes and delineate control mechanisms through three putative methyl-accepting chemotaxis proteins.

**Campylobacter jejuni**

As the leading cause of bacterial gastroenteritis, the focus on eliminating *C. jejuni* from our post-harvest food supply is essential. Sources of infection are improperly prepared poultry and meat, and unpasteurized milk products. Although rarely fatal, the symptoms of enteritis can be debilitating lasting 24 hours to 1 week.

Microarrays have allowed a global view of both the genomic make-up of various strains of *Campylobacter* and the transcription regulation that occurs. The genetic analysis has been able to identify and study antibiotic resistance (Friis et al., 2007; Guo et al., 2008), clinical differentiation of species (Volokhov et al., 2003), and genetic variability within the species (Taboada et al., 2004). Current research has focused on the mechanisms of colonization and pathogenesis. Transcriptional studies using microarrays have examined *hspR* mutants, *cgb* expression, multidrug resistance, and interactions with human epithelial cells. Additional studies have been reported on iron deprivation (Palyada et al., 2004), heat shock (Stintzi, 2003), exposure to bile acids (Malik-Kale et al., 2008), phosphate limitation control (Wosten et al., 2006), and regulation by the DccRS two component sensory system (MacKichan et al., 2004).

HspR is thought to participate in the heat shock response of other bacteria, but it seems to have additional function in *C. jejuni* (Andersen et al., 2005). The transcriptional analysis indicates many of the heat shock chaperone genes are up-regulated, and the organism loses the ability to form colonies at the higher incubation temperature of 44°C. Additionally, the flagellar apparatus is also affected in *hspR* mutants. This data implies that *C. jejuni* *hspR* mutants down-regulate eight genes associated with the flagella formation. *In vitro* studies also demonstrate that *hspR* mutants are less able to invade human epithelial INT-407 cells. Andersen et al. (2005) hypothesized that alteration of HspR
function could decrease motility and alter the heat shock response affecting the ability of C. jejuni to survive during post-harvest food preparation and decrease possible human infections.

Nitric oxide (NO) is an important part of the immune response. The ability of C. jejuni to respond to nitrosative stress is one means by which C. jejuni can evade the immune system and potentiate disease. The cgb gene is induced following exposure to nitric oxide and is regulated by NssR. Microarray studies of a nssR-deficient mutant have identified a unique regulon that responds to NO stress (Elvers et al., 2005). By identifying the pathways and genes involved with evasion of the immune response, more effective treatments may become available.

The multidrug efflux pump, CmeABC, is regulated by CmeR in C. jejuni. By combining mutational analysis with transcriptional response studies, Guo et al. (2008) showed that 28 genes with diverse functions were differentially regulated in a cmeR mutant. The cmeR mutant was also less effective in colonizing chickens.

Gaynor et al. (2005) studied the interaction of C. jejuni with human intestinal epithelial cells. Among several virulence-related genes that were up-regulated during cell interactions was spoT, which controls the stringent response during nutrient deprivation in other bacteria. This was the first report of a stringent response in C. jejuni.

A rabbit ileal loop model of infection was used to study the transcriptome of C. jejuni (Stintzi et al., 2005). Stintzi et al. (2005) showed that differentially regulated genes reflected the oxygen-limited, nutrient-poor, and hyperosmotic environmental condition as expected. However, they also showed that C. jejuni differentially expressed its membrane proteins and altered its peptidoglycan and glycosylation patterns in individual rabbits. Further analysis also showed important contributions by the heat shock and stringent responses to gut colonization (Stintzi et al., 2005).

Listeria monocytogenes

Listeria monocytogenes is a gram-positive, facultative intracellular bacterium and is a significant foodborne human and animal pathogen. Listeriosis is a serious disease for humans; the overt form of the disease has a mortality of about 25%. In the United States, humans listeriosis occurs in 2500 persons with 500 deaths each year (Mead et al., 1999). Transcriptome analysis has been widely used to study stress response in L. monocytogenes.

Listeria monocytogenes alternates between free-living and host-associated environments whose physico-chemical parameters are very different, exerting different demands and stresses on the bacterial cell. This organism has the ability to survive these extreme environmental conditions due to an extensive interacting network of stress responses (Kallipolitis and Ingmer, 2001). Variation in temperature is a stress that is commonly encountered in nature and during the processing of foods. Listeria monocytogenes uses heat-shock–response mechanisms defined as class I, which includes genes dnaK, dnaJ, groES and groEL; class III, whose heat-shock genes are regulated by CtsR and includes Clp proteases and additional chaperones; and class II, a general stress-response mechanism regulated by SigB (van der Veen et al., 2007).

The complete heat-shock regulon of L. monocytogenes has been investigated using microarrays by measuring whole-genome expression profiles of cells that were grown at 37°C and then exposed to 48°C, sampling at four time points after temperature up-shift (3, 10, 20, and 40 minutes) (van der Veen et al., 2007). A total of 427 genes showed up-regulation and 287 showed down-regulation in one or more time points compared to the zero control time point. For many differentially expressed genes, a transient expression pattern was observed between 3 and 40 minutes after temperature increase. This study showed that heat shock triggers the classical heat-shock, cell replication machinery, and SOS response genes in L. monocytogenes.

Microarrays combined with other biocomputing and molecular techniques can be used very efficiently for studying global regulators (Kazmierczak et al., 2003; Hu et al., 2007a, 2007b; Hain et al., 2008). To study the genes regulated by σB (a positive regulator of class II stress response genes) and to decipher the role of σB in stress responses and virulence in L. monocytogenes, Kazmierczak et al. (2003) used
a combination of DNA microarrays and promoter searches identified with Hidden Markov model approach. They developed a customized 208 gene microarray comprising 166 genes that contain predicted $\sigma^B$-dependent promoters identified by the Hidden Markov model and other virulence and stress response genes. The transcriptome analysis using these arrays identified a total of 55 genes with $\sigma^B$-dependent expression. Hain et al. (2008) used whole genome–based transcriptome analysis with a sigB deletion mutant to study the $\sigma^B$ regulon in detail. They identified 216 genes as being regulated directly or controlled by $\sigma^B$ (105 positively regulated and 111 negatively regulated by $\sigma^B$).

In L. monocytogenes, $\sigma^B$ positively regulates the transcription of class II stress response genes; CtsR and HrcA negatively regulate class III and class I stress response genes, respectively. Hu et al. (2007a, 2007b) combined transcriptomic analysis with phenotypic analyses to understand the role of CtsR and HrcA in the stress response and the interactions between $\sigma^B$, CtsR, and HrcA. These studies of microarray-based transcriptome analyses and promoter searches identified 64 CtsR-dependent genes (42 genes negatively regulated, 22 genes indirectly but positively regulated), 25 HrcA repressed genes, and 36 genes indirectly up-regulated by HrcA. They also identified several co-regulated genes, 40 genes by CtsR and $\sigma^B$, two genes by HrcA and CtsR, 31 genes by HrcA and $\sigma^B$, and five genes by all three regulators. This study showed that HrcA, $\sigma^B$, and CtsR together form a regulatory network that is critical for expression of a number of virulence and stress response.

One property of L. monocytogenes is the ability to multiply at low temperatures. The bacteria can grow and accumulate in contaminated food stored in the refrigerator. Whole genome microarrays were used to identify L. monocytogenes cold-induced genes at 4°C compared with 37°C both in log phase and stationary phase growth. (Chan et al., 2007). The results showed that 245 out of 2857 open reading frames (ORFs) tested have higher transcript levels and 166 genes have lower transcript levels at 4°C than at 37°C. The up-regulated genes of the cold regulon include genes that encode the cold shock protein (CspL), two RNA helicases (lmo0866, lmo1722), a two-component response regulator (lmo0287), a flagellar biosynthesis protein (lmo0679), and several hypothetical genes. The down-regulated genes include genes encoding class I heat shock proteins (groES, groEL), virulence-associated genes (hly, plcA, plcB, prfA) along with several other genes with unknown function.

Hydrostatic pressure processing is used in the food industry to control foodborne pathogens. Bowman et al. (2008) treated L. monocytogenes at 400 and 600 MPa for 5 minutes and then measured whole genome differential transcription. Their results indicated that the treatment induced genes associated with DNA repair, transcription/translation, cell division, protein secretion, flagella assembly, and lipid and peptidoglycan biosynthesis (Bowman et al., 2008). Down-regulated were genes associated with energy production, carbohydrate metabolism, and virulence-associated genes including SigB and PrfA regulons.

Apart from studying stress response, L. monocytogenes transcriptome analysis using whole genome microarrays has been used to understand the gene expression profile of the bacterium in the cytosol of epithelial cells (Joseph et al., 2006) and in the vacuolar and cytosolic compartments of infected macrophages (Chatterjee et al., 2006). Microarrays were also used in investigation of global transcriptional regulators such as the DegU response regulator that regulates expression of flagella specific genes (Williams et al., 2005) and MogR, a transcriptional repressor that regulates flagellar motility genes and virulence (Shen and Higgins, 2006).

Yersinia enterocolitica

Yersinia enterocolitica is a gram-negative, facultative anaerobe, human foodborne enteropathogen that can cause enterocolitis accompanied by diarrhea, fever, and abdominal pain (Bottone, 1999). Not all strains of Y. enterocolitica are pathogenic. There are six biotypes (1A, 1B, 2, 3, 4, and 5). Biotype 1B is highly pathogenic; biotypes 2, 3, 4, and 5 are moderately pathogenic; and 1A is considered nonpathogenic but some strains are potentially pathogenic (Wauters et al., 1987; Burnens et al., 1996). Thus, it is important to differentiate pathogenic strains from nonpathogenic regardless of biotypes.
Most of the reported studies have focused on genetic characterization or detection and will not be discussed here. These include identification of the organism in contaminated foods (Myers et al., 2006), comparative analysis of biotypes (Thomson et al., 2006), and species-specific genes (Wauters et al., 1987).

Analysis of transcriptional responses of Y. enterocolitica using microarrays has been limited, focusing on master regulators of virulence, flagellar synthesis, and stress-response genes (Kapatral et al., 2004; Horne and Pruss, 2006; Seo et al., 2007). The studies on global gene regulation in Y. enterocolitica include the analysis of FliA, which can positively affect expression levels of eight class III flagellar operons (fliz, flgK, flnD, motA, tar, flaE, flaB, flaC). FliA can negatively regulate, possibly indirectly, the expression of four virulence operons (yadA, virC, yopQ, ISYen1) (Horne and Pruss, 2006). Kapatral et al. (2004) showed that the genes encoding enzymes involved in purine/pyrimidine biosynthesis via carbamoylphosphate and carbamoylaspartate (hutU, hutI, carAB, pyrBI) were regulated by FlhD/FlhC (Kapatral et al., 2004). Seo and Savitzky studied the role of secretins in the stress response (Seo et al., 2007). In addition to traditional transposon mutagenesis, they also employed microarrays to better understand the regulation of the phage-shock-protein (PsP). Their results indicated that unlike E. coli, PsPs in Y. enterocolitica were regulated directly by some unknown sensing trigger and not through ArcB, the redox sensor activating anaerobic metabolism.

Summary

Microarrays have become an important tool in the arsenal of the study of pathogenesis. They are often considered as a hypothesis-generating tool, and if possible, should be combined with other more defined or focused analyses, i.e., mutant generation and characterization. Surprisingly, few studies focused on areas directly relevant to food safety have been published, subjects like treatments with sanitizers or inhibitors, or biofilms on foods or food processing surfaces. New innovative ways to generate sufficient quantities of RNA for microarray analyses will be necessary. The unbiased, global approach used by microarrays will provide important keys to better control these agents in the pre- and post-harvest food industries.

References


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