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UNDERSTANDING SOCIAL INTERACTIONS OF MICROBES USING TRANSCRIPTOMICS : A CODICIL

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ABSTRACT

Sociobiology of microbes is an emerging branch of science that has delivered interesting and intriguing research findings over the last few years. The revelations are such that the research perspective of microbes is gradually drifting from pure culture analysis to community analysis. We review to substantiate the need for microbial socio-bio studies and codicil the decade long research pursuit and their findings using advanced technologies of Microarrays and Next Generation Sequencing, prompting for more lunge in transcriptomic and metatranscriptomic studies.

Keywords: Microbial Ecology, Sociobiology, Transcriptomics, Gene Expression, Microarrays, Next Generation Sequencing

INTRODUCTION

The realm of microbes; the deeper we dive, the farther and wider the bottom is, it appears. Microbiology has been contributing significantly towards understanding biology in general, and prodigious revelations have been made in biology at molecular level. Many of the discoveries made through microorganisms have been the center stage in the development of many genetic-engineering technologies (**Zimmer, 2009)**. Although, it may appear that biological science today has reached the summit, research reports continue to confound our ideas of microbial life and here we discuss the sociobiology of microbes in particular, the least dealt and the most baffling of all and why transcriptomics is of relevance in this context.

Sociobiology of microbes, does that matter ?

For more than a century, ever since the advent of the science of microbiology, microbiologists have been engaged in understanding microbes as pure cultures. In fact, the very basis of classical microbiological methods rests on efficient pure culture techniques. In the pursuit of understanding pure cultures, it was long forgotten that microbes indeed lead a social life and that they seldom exist as pure cultures in natural environment. However, reports in the past decade have given us to believe that the purview of sociobiology of microbes does matter, and it is of relevance.

Like most species, almost everything that a microbe does, has social implications. Even the very basic aspect of life, such as reproduction, could deprive a neighbor of its resources and therefore bring about a conflict (**MacLean, 2006**). Besides, microbes also secrete in abundance, metabolites, toxins (**Pettit, 2009**), and even extracellular DNA (**Johnson *et al.* 2013**), all of which can have an affect on their social neighbours. In this regard, it is of relevance and a necessity to rekindle a view point of ecology, which states that 'the behavior of most organisms living in close proximity will be governed by competition or cooperation' (**Alexander, 1974**). It is understandable that in such social communities, microbes are bound to tradeoff with other community denizens (**Crespi, 2001; Schink, 2002; Velicer, 2003; Mitri *et al.* 2011**). Considering this to be an ecological gospel, it should be agreed upon that such social conditions, not only influence the logarithmic growth but also provide ample opportunities for metabolic interactions within the community. The interactions can and has been radically altering the biochemical phenotypes and or the morphological characteristics of the participating strains, also leading to social evolution (**Foster, 2005; Boyle *et al.* 2013**). This very logical understanding makes it agreeable that the behavioral pattern of a socially living microbe, which is the naturally occurring state of most microbes, is bound to be divergent to the lab grown pure cultures. And it is also understandable that behavioral pattern, be it with respect to metabolism, secretions, the multiplication rate, and the fundamental process of gene expression that governs all of it, is likely to vary. Thus the reasons to discuss transcriptomics with relevance to social psyche are well substantiated and require thorough review and investigation.

Gene Expression with Relevance to Microbial Social Interactions

Discussions on sociobiology of microbes have gained importance in the recent past. Research findings and discussions in this stream have overlapping implications with varied interest, from evolution, ecology to systems science. Reports on varied forms and levels of social interactions have come to light. However, the most intriguing and with immediate interests, in relation to gene expression studies in sociobiology are on Quorum Sensing and Co-existence, Metabolic Synergism in microbial communities and Sociobiology with relevance to pathogenicity and virulence. In order to demonstrate the magnitude of the necessity for gene expression studies in sociobiology, we review and discuss these aspects in brief.

Metabolic Synergism in Microbial communities

Regardless of the locale, a microbial niche is a package of multiple species and strains, wherein interactions govern co-existence and cells compete, co-operate, conflict and if need be, coerce and cheat. This influences the fundamental aspects of microbial progression in a mixed culture community. When in mixed culture, the affiliating strains compete for common resource. The competition may arise not only between different species, but also between strains of the same species. The competition naturally mediates a situation of either co-operation or conflict and depending on the nature and properties of the competing species, the relationship is defined. In such state of competition where in, the competing affiliates share common resources, conflict is evident (**Rainey and Rainey, 2003**). The sharing becomes easier and clear with resources such as carbon and oxygen (**Kreft, 2004; Wintermute and Silver, 2010a**). These resources due to its potential role in growth, when shared, obviously lead to conflict. (**Ratnieks *et al.* 2006**). For instance, in mixed culture, under conditions of oxygen crunch, microbes have the ability to shunt from aerobic respiration to fermentation. (**Pfeiffer *et al.* 2001; Frick and Schuster, 2003; Pfeiffer and Bonhoeffer, 2004; Pfeiffer and Schuster, 2005; Novak *et al.* 2006**). While respiration, owing to efficient resource management, is thought to be a cooperative strategy, fermentation on the other hand in contrast, is observed as a competitive strategy (**Pfeiffer *et al.* 2001**). The phenomenon of shift in the metabolic pathways by microbes in mixed culture therefore, has also brought to focus, the psyche of the competing and co-operating microbes in mixed cultures.

Further, from earlier mixed culture studies (**Christensen*et al.* 2002; Hansen *et al*. 2007; Hibbing *et al.* 2009**) it has been possible to extrapolate that metabolic commensalism exists between species in mixed microbial communities and that the cultures in mixed community, exhibit natural metabolic synergism and they interact (**Pelz *et al.* 1999; Bull and Harcombe, 2009; Wintermute and Silver 2010b**). These interacting partners share metabolites, such as hydrogen (**Hillesland and Stahl 2010**), acetate (**Rozen *et al*. 2009**), amino acids (**Shou et al. 2007**), fixed nitrogen (**Kim *et al.* 2008**) or glucose (**Kim *et al.* 2008; Gore *et al.* 2009**). These sharing features within a community, undoubtedly involves complex molecular mechanism, which at present is an arena, unexplored.

Quorum Sensing and Co-existence

Quorum sensing is machinery for cell-to-cell communication that is exhibited by group living microbes. Through cell-to-cell communication, each cell has the ability to sense the density of neighbours in a community and quorum sensing therefore has a pivotal role in colonization (**Miller and Bassler, 2001; Rutherford and Bassler, 2012**) and biofilm formation (**Parsek and Greenberg, 2005; Moons *et al*. 2006; Waters *et al*. 2008; O'Loughlin *et al*. 2013**). Besides communication, quorum sensing is also known to control diversified biological functions such as spore formation (**LaRossa *et al*. 1983; Magnuson *et al*. 1994; Solomon *et al*. 1995; Solomon *et al*. 1996; Steiner *et al*. 2012**), cell differentiation (**Hornby *et al.* 2001;Hogan *et al.* 2004**), uptake and exchange of genetic material (**Duffin and Seifert, 2010; Antonova and Hammer, 2011; Suckow *et al*. 2011; Seitz and Bolkesch, 2013**) and toxin production (**Schelin *et al*. 2011; Thoendel *et al.* 2011**). In a population of *Staphyloccus* aureus, for instance, secretion of exogenous toxin occurs only in a state of high population density which is regulated by the Agr signal transduction system, a quorum sensing system (**Thoendel *et al*. 2011**).

Microbial colony or a community, as discussed, may comprise of multiple species, and this prompts us to extrapolate that quorum sensing also mediates communication between divergent species. Autoinducer-2 (AI-2), a quorum sensing molecule, produced by diverse set of species that has been inferred to have widespread interspecies communication (**Federle and Bassler, 2003**) is a convincing evidence for this inference. It has also been reported that quorum sensing in a mixed population could also affect the synthesis and secretion of specific proteins of divergent species (**Egland *et al*. 2004; Xavier and Bassler, 2005; Keller and Surette, 2006; Sandoz *et al.* 2007**).

Quorum sensing with such diverse role, is regulated by gene expression. Although different quorum sensing systems have been identified and studied, the network of regulatory mechanisms that aids in intra and interspecies interactions remain to be investigated and the expression pattern of these communicating molecules unearthed.

Microbial Sociobiology with reference to Pathogenicity and Virulence

Pathogenicity and Virulence, the qualities that describe the ability of infectious agents, owing to the magnitude of their role in infection; have been the major target science of study on pathogenic microbes. These qualities of microbes are governed by the expression of specific genes, which in turn is regulated. Although the mechanism of virulence and pathogenicity is well reported and compiled, till recently, it was only in the pure culture state that most pathogens had been studied. In the cases of infections, it is not the infectious agent alone that makes an entry into a host, infectious agent is but one among the plethora of the host intruders. In most cases of microbial intrusion, depending on the source of infection, a consortium of microbes gain entry into the host and the potent of them all establishes as the predominant infectious agent. In fact in some cases it is due to the entry as consortia that pathogenicity is initiated (**Chao *et al.* 2000; Brown *et al.* 2002; Brockhurst *et al.* 2003; Schjorring and Koela, 2003; Thomas *et al.* 2003; Choisy and Roode, 2010; Ben-Ami *et al*. 2011**). For instance, pathogenicity is expressed by *Clostridium tetani* only when the co-inhabiting microbial consortia members cause an anoxygenic state leading to necrosis at the site of infection. And it is this condition of necrosis that triggers the release of the exotoxins by *C.tetani* (**Campbell *et al.* 2009**). As early as the 1980's researchers had already reported that it is only in a group that the state of bacteremia thrives to cause the characteristic clinical symptoms. Vught *et al* (**1986**) reported that *Bacteriodes fragilis* and *B.vulgatus* both are required for abscess formation by *Escherichia coli* in mice. Similarly, co-infection of honeybees jointly by *Ascosphaera apis* and *A.atra* increased the mortality rate of the bees (**Vojvodic et al. 2012**). These evidences indicate that co-infections by multiple species may increase the degree of illness and its effects and therefore the need for understanding the pathogenicity of a microbe in mixed population is significant. On the other hand the suppressing effect on virulence and pathogenicity of an infectious agent in a mixed population have also been reported. For instance, the effect of Lactic Acid Bacilli (LAB) in a mixed population with *Staphylococcus aureus* has been rigorously studied, giving rise to the findings that LAB, *Lactococcus lactis* namely, impairs the expression of virulence by *S.aureus* in mixed culture (**Alomar *et al.* 2008; Charlier *et al.* 2008; Charlier *et al.* 2009; Even *et al.* 2009**). Likewise it has also been reported that the virulence of *Listeria monocytogenes* via the expression of the *prfA*, a virulence regulator gene, is lowered in the presence of *Bacillus subtilis* (**Tirumalai and Prakash, 2012a**). These study have also involved the use microarray technology to investigate the transcriptomic response of apathogenic strainto the presence of a competing strain (**Even *et al.* 2009; Tirumalai and Prakash, 2012b**). Researchers are now compelled to admit that the expressions, genotypic and therefore phenotypic, of a microbial species are different in a social community. And this difference depending on the relatedness of different species in a niche can even alter the expression of Virulence and Antibiotic Resistance (**Foster, 2005**). Microarray and other high throughput technologies for studies on transcriptomic behavioural pattern in a social community have been put to use though, given the magnitude of the implications of gene expression and its regulations in social life of microbes, their applications seems very meager.

Transcriptomics and Microbial Community Analysis

Application of High Throughput Technology

The ultimate object of microbial ecology is to elucidate the governing factors that facilitate a microbial community to exist, in the form they do, and this could be achieved by examining the interactions within the community. The enormous microbial biocomplexity with a multitude of highly complex interactions between different micro-organisms can be understood only using molecular methods. Understanding gene expression in a mixed community therefore came to be an object in sociobiology (**Allen and Banfield, 2005**). Although various gene probes made it possible for studies on species-specific genes in microbial mixed communities, microarray technology radically revolutionized the ability to simultaneously study hundreds or thousands of genes at a time (**Schena *et al.* 1995**). Majority of prokaryotic microarray studies, however, have been used to study the genome or transcriptome of a single organism and therefore for a brief period, was limited from application in understanding the complex microbial ecosystems (**Dennis *et al.* 2003**). However, owing to the enormity of the implications of gene expression studies in microbial ecology, several types of microarrays were developed and successfully applied to microbial ecology research. Phylogenetic Oligonucleotide arrays (POAs), Functional Gene Arrays (FGAs), Community Genome Arrays (CGAs), Metagenomic Arrays (MGA) and Whole Genome Open Reading Frame Arrays (WGA) are to name a few, each of which have been reviewed earlier, in context to microbial ecology research (**Zhou, 2003;Gentry *et al.* 2006**).

Studies involving microarray technology in microbial community analysis have given interesting revelations about gene expression patterns of strains in mixed cultures that are divergent to its pure culture state. Transcriptomics using DNA Microarrays proved to be effective in studies, such as the expression of 2,4-dichlorophenoxyacetic acid catabolic genes and the regulation of resin acid degradative genes by *Ralstonia eutropha* both in mixed microbial communities of industrial effluents (**Dennis *et al.* 2003**.). Expression pattern of the whole transcriptome of *Listeria monocytogenes* in mixed culture broth and biofilm both in the presence of *Bacillus subtilis* has been elucidated using microarrays (data accessible at NCBI GEO database (**Edgar *et al.*2002**), accession GSE27936) and the differential expressions of virulence and antibiotic resistance genes in mixed culture have been reported (**Tirumalai and Prakash, 2012a; Tirumalai and Prakash, 2012b**). All such research explorations also drive our interests to Metagenomics. High-throughput microarray technology has been used for studies of complex microbial communities in various environments and for studies on the diversity functions of genes and gene expressions (**Zhou, 2003; Bodrossy and Sessitsch, 2004**). Besides microarray based biochips have been developed specifically for screening biogeochemical cycles in the name of 'GeoChips' (**He *et al.* 2007; Wang *et al.* 2009; Van Nostrand *et al*. 2009**) and the 'HITchip' (**Stojanović *et al.* 2009**) 'HuGChip' (**Tottey *et al*. 2013**) for determining the microbial diversity of human gut. Although there have also been studies using microarrays for understanding metagenomics, with reference to identification of novel genes or proteins (**Streit and Schmitz, 2004**; **Deutschbauer *et al.* 2006**) and identification of uncultivable microbes in microbial niches (**Wu *et al.* 2001; Sebat *et al*. 2003; Tottey *et al.* 2013**), considering the countless natural microbial niches and the inter and intra-species community interactions that happen, the research focus and reports on metatranscriptomics is negligible.

Microarrays with a decade long history of applications in microbial ecology is gradually being superseded by the Next Generation Sequencing (NGS) technology (**Ledford, 2008**). As compared to microarrays, NGS is more efficient in parallel sequencing of large numbers of DNA fragments, besides being rapid and cost effective (**Roh *et al.* 2010**). Among the different NGS platforms, the pyrosequencing approach is most suited for microbial ecology studies and has been widely used for the purpose (**Angly *et al.* 2006; Brown *et al.* 2009**). NGS pyrosequencing approach holds a better stand to microarrays, for applications in microbial ecology mainly because it reduces issues pertaining to specificity inherent to microarrays in microbial ecological studies (**Roh *et al.* 2010**).

DISCUSSION

Codicil

Sociobiology of microbes has traversed quite a few miles in the last decade and has gathered moss and the prejudiced, misguided opinion that single-celled organisms are asocial, seems to have lost ground. The plethora of reports on microbial interactions, within mixed cultures in particular, brings an intriguing new standpoint. Can we hope to understand gene expression that underlie the complex network of social behavior ? Given this discernment we could anticipate that the expression of gene transcripts in socially living states of microbes is likely to be variable to their well-studied pure culture equivalents. Researchers believe that understanding the social sophistication in microbes can also open new vistas to evolutionary paths and ecological diversities (**Székely *et al.* 2010**). Gene expression studies or transcriptomics therefore, could yield perplexing data on the behavioral pattern concerning various aspects of social existence, from growth rate to competition, co-operation or metabolic interaction between group living strains. Further, analysis of gene expression patterns within a microbial community will allow us to tease out the impact of various biotic and abiotic factors that significantly impact the regulation of metabolic functions. A complete understanding of microbial metabolism is therefore warranted. And this would extend from the properties of individual strains in pure culture to the combinatorial interactions supported by complex communities.

Although the viewpoint of sociobiology from where it stands today, appears to have far-fetching horizon with bewildering complexity, the advancements in the field of transcriptomics, can be envisaged to minuscule the lofty objects of microbial sociobiology in the years to come.

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