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

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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. And depending on the nature and properties of the competing species, the competition may arise as cooperation, 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 sharing becomes easier and clear with resources such as carbon and oxygen (Kreft, 2004; Wintemute and Silver, 2010a). These resources due to their 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 (Pelc et al. 1999; Bull and Harcombe, 2009; Winternute 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 mechanisms, 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 gram-negative 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 (Parsk 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; Magdic et al. 1994; Solomon et al. 1995; Solomon et al. 1996; Steiner et al. 2012), cell differentiation (Horny et al. 2001; Hogan et al. 2004), uptake and exchange of genetic material (Dufflin and Seifert, 2010; Antonova and Hammer, 2011; Snackow et al. 2011; Selza and Bolkesh, 2013) and toxin production (Schenel et al. 2011). In a possible sixth ecological role, 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 different microbial species. Autoinducer-2 (Al-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 (Eglund 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 regulons mechanisms that aids in intra and interspecies interactions remain to be investigated and the expression pattern of these communicating molecules unexplored.

**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 comprehensively elucidated in the pure culture state that most pathogens have 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 potential 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; Schjorrning 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 anoxic 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 Bacteroides fragilis and Bivalvatum both are required for abscess formation by Escherichia coli in mice. Similarly, co-infection of the honeybees jointly by Ascophaera 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. 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 genes of 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 strain into the presence of a competing strain (Even et al. 2009; Tirumalai and Prakash, 2012b). Researchers are now compelled to admit that the expression of specific genes, particularly those of the pathogenicity and virulence, is not the infectious agent alone but rather the environment of the pathogen. The enormous microbial biodiversity of human gut. Although there have also been studies using microarrays for understanding the complex microbial 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 losing its importance 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. Phylogentic Oligonucleotide arrays (POAs), Functional Gene Arrays (FGA), 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 pattern 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-dichlorophenoxycetic acid catalytic 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 these findings drive out interests to Metagenomics. High-throughput microbial genes 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; Brodrosy and Sesslitch, 2004). Besides microarray based biosciences have been developed specifically for screening biogeochemical cycles in the name of ‘GeoChips’ (Beh et al. 2007; Wang et al. 2009; Van Nostrand et al. 2009) and the ‘HiTchip’ (Stojanovic 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 Schnitz, 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 a remains intact. Researchers are now compelled to admit that the 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 (Rob et al. 2010).
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 underlies the complex network of social behavior? Given this discrepancy 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 dynamics (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-fetched 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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