

9. Bacterial Associations: Antagonism to Symbiosis

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Introduction

*“Nothing in biology makes sense except in the light of evolution.”*Theodosius Dobzhansky

In relationships too, the associations are not static and but are constantly evolving in response to their environment. It could include all degrees of repulsion and antagonisms to affinity, from tight symbiosis of bacteria with their host eukaryotic cells to more loosely bound partnerships between entirely separate organisms. The article examines some examples of interactions at different levels of associations.

The marine environment is the abode of many groups of organisms and also is a complex milieu of interactions among many organisms thriving there. Animals and plants typically exist in association with specific microbial biota (Douglas, 1994; Smith and Crabb, 1981; Bry et al., 1996). The spectrum of direct pair wise interactions of bacteria with marine organisms ranges from mutualism through commensalisms and competition, to antagonism, determined ultimately by balancing the cost of the association against the benefits received (Pianka, 1994). A continuum can be envisioned that spans a dynamic bridge from antagonism to cooperation and these relationships may shift gradually or abruptly along the continuum (Dimijian, 2000).

The process of metazoan evolution took place largely in the sea, the environment in which all known animal phyla first appeared. Only during the last third of this period did relatively few members of these phyla venture onto land and radiate into terrestrial habitats (Ngai and Ruby, 2000). Bacterial associations are certain to have arisen within marine invertebrate taxa. Since marine invertebrates exhibit the greatest phyletic diversity in the biosphere, it is likely that the greatest variety of animal-bacterial interactions still occurs within this group (Brusca et al., 1990). Thus, marine invertebrates and their associated microbiota provide a wealth of material for studies that can reveal much about both the diversity in host-bacterial associations and origin of mechanisms by which symbiosis develops and persists (Saffo, 1992; Douglas, 1994).

The interactions between bacteria and host could be neutral, where one does not influence the other by any means like the several epiphytic surface-dwelling bacteria. Nevertheless depending on the effect of interaction on the host, the relationship can be positive or negative. However, one guest-host relationship is not always sharply distinct from another and even the harmless commensals or useful symbionts under certain conditions, become harmful parasites (Rheinheimer, 1986). Though such a transition can be observed, the relationships can be broadly categorized as neutral, positive and negative.

Neutral interactions

In this kind of relationship, neither of the interacting organisms are affected by the association. Certain marine bacteria attach themselves to bodies of organisms and differentiate to form a complex, multicellular structure called Aufwuch or biofilm. Formations of biofilms under in vitro conditions have been demonstrated by Prochnow et al. (2004) in continuous culture systems. In their studies, the biofilm exhibited a characteristic architecture consisting of differentiated microcolonies surrounded by water channels.

In neutral relationships, bacteria grow and feed partly or wholly on the metabolic products of the host. However, the relationship is strictly unobligatory. Research conducted by Shiba and Taga (1980) point out that the green alga *Enteromorpha linza* produce extracellular products that were absorbed by *Flavobacterium* spp and *Cytophaga* spp which constituted the biofilm on the alga.

Though the formation and interaction in biofilms involve neutral processes, they play active roles in the multicellular biofilm development and subsequent dispersal of surviving cells within the marine environment. Prochnow et al. (2004) observed that the biofilm formed by newly described green-pigmented bacterium *Pseudoalteromonas tunicata* (D2) produces target-specific inhibitory compounds against bacteria, algae, fungi, and invertebrate larvae and is frequently found in association with living

surfaces in the marine environment. Hence, it can be understood that these relationships are not stable but are constantly changing and adapting to their environment.

Positive Interactions

The profitable cooperation by different organisms can either be symbiotic or commensalistic. Symbiosis occurs when two organisms form a relationship, which provides an advantage for both the partners at least temporarily. In commensalisms only one partner derives benefit and the other does not.

Symbiosis

The word, 'symbiosis' is derived from the Greek word, which means 'living together'. Symbiosis could be a close, prolonged or temporary, intimate association between two organisms, which are widely separated phylogenetically. In symbiosis, growth and survival of both the organisms are benefited and neither of them can survive under natural conditions without each other (Odum, 1959). In the association of two organisms the larger one is the host and it harbours the mutual partner- the symbiont, typically providing nourishment and shelter. Thus, microorganisms occur as useful guests in numerous animals and plants. Symbiosis may be divided into two distinct categories, ectosymbiosis and endosymbiosis.

In **Ectosymbiosis** the symbiont lives on the body surface of the host including internal surfaces such as lining of the digestive tube and ducts of glands. Marine invertebrates living closer to the sea bottom, bivalve molluscs have established symbiosis with chemosynthetic bacteria that use inorganic reduced compounds as an electron source, in regions where sulfide and oxygen are present in the water perfusing the sediments. The sulfur-oxidizing epibacteria found on nematodes living in marine sands, at the oxic-anoxic interface; the nematodes appear to meet their carbon requirements directly by feeding on their symbionts, and they move down the chemocline (chemical gradient in the water column) to provide their symbionts with both sulfide and oxygen (Dimijian, 2000).

In **Endosymbiosis**, the symbiont lives within the cells or intracellular space of the host. Such a symbiont is called an endosymbiont. Occurrence of endosymbiosis is well documented in the hydrothermal vent systems (Cary et al., 1993; Feldman et al., 1997; Hurtado et al., 2003). The biodiversity of hydrothermal systems include a large assemblage of invertebrate organisms from large tubeworms to a varied species of crabs (Van Dover

et al., 2001; Guinot and Hurtado, 2003). The deep-sea hydrothermal vent sites are characterized by high concentration of reduced sulfur compounds (Baker, 1999). Here life is supported by the growth of chemolithoautotrophic bacteria, capable of oxidizing hydrogen sulfide to generate energy that is used to fuel carbon dioxide fixation into macromolecules (Compere et al., 2002 and Garcia et al., 2003). An extraordinary animal in these ecosystems is the giant tubeworm, *Riftia pachyptila* (Vestimentifera), a 3-foot-long invertebrate housed in its own 6-foot-tall tube. The adult worms do not have mouth, gut, or anus, and obtain all their energy and food from endosymbiotic chemolithoautotrophic bacteria. These worms have unique hemoglobin in their blood that binds oxygen and sulfide thereby transporting both from the surrounding seawater to the bacteria (Minic and Herve, 2004). Carbon isotope data show that these worms depend on carbon compounds synthesized by the bacteria as the main source of their nutrition to support its large size and high growth rate (Scott, 2003). In *Riftia pachyptila*, the chemoautotrophic bacteria are harbored in a specialized organ called trophosome. Larvae of *Riftia* transiently possess a nascent trophosome from surrounding water. Once a tubeworm is settled, its digestive tract closes off and trophosome enlarges (Goffredi et al., 1997).

Such symbiotic associations are not confined to very special habitats like hydrothermal venting sites. The bacteria-animal symbiosis may vary from transient, nonspecific symbionts in many aquatic organisms to highly specific, non-culturable, intracellular symbionts as in pyrosomes (Nealson et al., 1981). Many of the luminous animals show monospecific associations with bioluminescent bacteria and these are housed in specialized light organs (Nealson and Hastings, 1991). Luminescent bacteria contain a unique and easily detectable enzyme, bacterial luciferase, which allows the detection of the bacteria even when they cannot be cultured from animal tissue. Fishes and urochordates use the symbiont-generated light for their nocturnal feeding habits (McFall – Ngai, 1990). The light also helps in the camouflage and reproduction. In addition, these bacteria also produce certain bioactive secondary metabolites. Rinehart (1992) and Faulkner (2000) reported the occurrence of such metabolites in sponges, molluscs, bryozoans and ascidians. Some investigations by Pennings et al. (1994), Waddell and Pawlik (2000) showed the anti-fouling or anti-infective nature of these secondary metabolites. Feeding studies in ascidians by Paul et al. (1990), Davis (1991), Vervoort et al. (1998), and Pisut and Pawlik (2002) showed that these metabolites conferred anti-predatory protection. In turn, the host supplies adequate nutrition for

proliferation of its endosymbionts. Studies by Graf and Ruby (1998) in *Euprymna scolopes* suggested that it produced free amino acids as well as peptides to support the growth of its endosymbiont, *Vibrio fischeri*.

Even though certain aspects of the establishment, in particular the level of partner integration, nature of metabolic exchange, mechanism of transmission of symbionts to offspring, and identification of the cellular inclusions as modified bacteria are not always known in some cases the outline of what is likely to be a continuous evolutionary sequence is becoming clearer (Nealson et al., 1981).

Researchers worldwide continue to search for genes responsible for the development and regulation of endosymbiosis. Novel computational methods that increase the success of high-throughput screening for vital symbiotic genes have been developed, some of which include the high-throughput EST (Expressed Sequence Tag) which is a tiny portion of an entire gene that can be used to help identify unknown genes and to map their positions within a genome. In this process, small pieces of DNA of usually 200 to 500 nucleotides are generated by sequencing either one or both ends of an expressed gene in certain cells, tissues, or organs from different organisms and use these as "tags" to search a gene out of a portion of chromosomal DNA by matching base pairs. Such methods may equally be applied to understand other symbiotic associations, such as endosymbiosis and antagonism.

Commensalism

'Commensalism' was derived from the Latin term 'com mensa' meaning 'sharing a table'. In Commensalism, one-organism benefits and the other is neither harmed nor helped. Commensalism represents a simple type of positive interaction and perhaps is the first step towards the development of beneficial relations (Rheinheimer, 1986).

The members of this association are the host and the commensal. Bacteria occur as ecto and endocommensals. In this relationship the bacteria do not extract nutrients from the host, but they do utilize the habitat provided by the host. The ocean is a special place for establishment of commensals on the host due to the diversity in the 'habitats'. The commensalistic bacteria present in the intestine of marine animals may not be parasitic or pathogenic but merely resident in the alimentary canal. Several species of *Acinetobacter*, *Alkaligenes*, *Flavobacterium*, *Photobacterium*, *Vibrio*, *Micrococcus* and *Staphylococcus* as well as members of Enterobacteraceae are reported to be endocommensals in the stomach-intestine of soles. Vibrios were found

to be the most dominant among the intestinal commensals and accounted to almost 45% of the total isolates (Mac Donald et al., 1986). Commensalism may be obligatory, in which one organism depends entirely on the other for its habitat or it may be facultative, in which each organism may live independently, but one is enhanced in the presence of the other.

An advantage of commensalism is that through migration of marine animals microorganisms on the surface or in the gut can secure rapid distribution. The luminous bacteria living in the gut of cod can reach the Western Baltic Sea from the North Sea. When the fishes come to rest, the luminous bacteria are excreted with the faeces and then are present in relatively large numbers (Schulz, 1987).

Negative Relationship – Antagonism

Microbial populations have a resilient dynamic stability produced by biological buffering from competition. Microorganisms compete for nutrients, oxygen and favourable ecological niches and are selected for their tolerance towards ambient conditions of pH, carbon dioxide, water and microbial toxins (Baker *et al.*, 1980). Microorganisms secrete metabolites, some of which inhibit other microorganisms (antibiotics), while others stimulate other microorganisms to form essential stages of their life cycle (probiotics). A negative interaction can therefore directly inhibit a pathogen or inhibit a stimulatory microorganism, thereby indirectly inhibiting the pathogen.

Competition

This is a negative relationship in which each population adversely affects the other in the struggle for food, nutrients, living space or other common needs. Interspecific competition is the competition between different species, which adversely affects their growth and survival and intraspecific competition occurs between the same species (Odum, 1959). Competitions for food and space influence quite decidedly the composition of the particular microflora. Microorganisms differ in their growth rates and hence some species multiply more rapidly than others. This leads to the release of some metabolic products, which may, in turn, inhibit or even completely eliminate some of competitors. Competition for nutrients play a subordinate role in extreme conditions as well as at times when the catabolism of the substrate is difficult.

Parasitism

This is an interaction in which one organism adversely affects the other by an obligatory direct attack. These organisms are called parasites and they live in or on their hosts and utilize nutrients and active substances, which they require for their development (Zobell, 1946). Parasitism can cause diseases or even the death of the host. But in most cases equilibrium is maintained between the parasite and the host. Harm to the host is kept within limits. Illness or death can occur only if there is disturbance of the equilibrium harming the host. Parasites could be endo or exo and, obligate or facultative. Many of the marine viruses and rickettsias are strict parasites. Facultative parasites are the ones that can also live saprophytically. Majority of the marine bacteria are facultative parasites.

The impact of parasitism is not restricted to the physiology of the individual host. Studies by Nair et al (1988) substantiate the significance of parasitism with an ecological perspective. *Sagitta* population in the Tokyo Bay may be partially controlled by bacteria that are associated with the animal. There is also a change in percentage composition and diversity of the various bacterial flora when an organism shifts from healthy to deceased state. The percentage *Vibrio* increased from 12.7% in the healthy *Sagitta* to 36.3% in unhealthy *Sagitta*, and in contrast, *Alcaligenes* decreased from 20.6% to 1.3%. Taxonomical analysis clearly revealed that there exists a difference in origin of strains from normal and abnormal *Sagitta*. Most of the *Vibrio* associated with unhealthy *Sagitta* showed high potential for chitinase activity when compared to those from healthy ones.

Antagonism: From Reason to Response

Why should a relationship be antagonistic? Antagonism may occur for space or for a common resource. Nair and Simidu (1987) hypothesized that antagonistic properties are linked to the trophic status of a water body. Studies by the authors show that in shallow areas such as Tokyo Bay, organic nutrients derived from metabolic processes or death and decay of massive phytoplankton population and constant nutritional inputs from external sources assuage the necessity for bacterial populations to produce antibacterial substances to survive competition. Autoinhibition could act as a sort of controlling factor in maintaining species diversity by perhaps allowing the population to partially limit itself and coexist with competitors.

Even within space, the distribution of bacteria showing antagonistic activities is variable. Among different groups of zooplankton, the maximum percentage of strains exhibiting anti-vibrio and anti-staphylococcus activities were obtained from cladocerans, followed by chaetognaths, unlike no activities in deapods (Nair and Simidu, 1987).

Many marine free-living and sediment-inhabiting marine bacteria have been shown to produce secondary metabolites that display antibacterial properties (Burgess et al., 1991). This antibacterial activity has been widely exploited and for the past 50 years antibiotics have revolutionised medicine by providing cures for formerly life-threatening diseases. Despite the immense clinical significance of antibiotics in health care, little is understood on the ecology of the organisms that produce them. The first antibiotic from a marine bacterium was identified and characterized by Burkholder et al. (1966). The marine environment harbours a wide range of microbes capable of exhibiting bacteriolytic and antibiotic activity. Bacteriolytic activities were found to be higher in the zooplankton than in sea water and the major group isolated was the gram negative bacteria, particularly the *Vibrio parahaemolyticus* followed by the gram positive strain, *Staphylococcus aureus* (Nair et al., 1985). There are also reports of such bacteria from nutrient-rich algal surfaces (Jensen and Fenical, 1994; Bernan et al., 1997) sea weeds (Imamura et al., 1997) to large biofilms on the surfaces of marine organisms (Lemos et al., 1986) The bacteriolytic bacteria inhabit mainly in the places where the organic matter is high, and contribute to its decomposition (Nair et al., 1985).

A number of surface-associated marine bacteria have also been found to produce antibiotics. Trischman et al. (1994) isolated a species of *Streptomyces* from the surface of a jellyfish. Though the property of production of antimicrobial compounds is constitutive, Patterson and Bolis (1997) observed that chemical signals received from potential competitor strains elicit an antagonistic response. However, this aspect still remains a little studied phenomenon (Mearns-Spragg et al., 1997; Mearns-Spragg et al., 1998). In contrast, to antibiotics, which promote interspecies antagonism, bacteriocins are responsible for intra specific antagonism. Colicin produced by *E.coli* has been studied extensively. Similarly, there are brevicin, nisin, pediocin produced by different groups. Bacteriocin produced by *Halobacterium mediterranei* ATCC 33500, has been shown to be active against many other halobacteria (Meseguer et al., 1985). Bacteriocin -producing bacteria could be made to change their strategy from anti- to pro-biotic depending on the environment. Thus, a brevicin-producer could be skillfully used as probiotic to ward

off unwanted microbes or to mitigate pathogenesis. A deep sea pigmented *Brevibacterium* spp has been shown to produce linocin-like compound that could be used as probiotic in aquaculture feeds. The extracts of this bacteria have not only been suggested to be useful in prolonging shelf-life of dairy products but the culture *per se* could be used as probiotics and as feed additives in aquaculture (Loka Bharathi et al., 2003).

A predominant response by the bacteria towards antagonism is antibiotic resistance. However, strains of bacteria have recently emerged that are virtually unresponsive to antibiotics. Strains having antibacterial activity are found to be lesser susceptible to inhibitory substances. Resistance of bacteria to antibiotics like chloramphenicol, tetracycline and streptomycin even at higher concentrations were attributed to the pigments present in them (Nair et al., 1991). Although many pre-existing antibiotics have been modified to yield new derivatives, bacteria have the potential to mutate known resistance mechanisms to combat these antimicrobial compounds (Hancock, 1997; Knowles, 1997 ; Levy, 1998).

The evolution of symbiosis

Without symbiosis, life on earth as it is today would not exist. The interdependence could be so tightly woven that such associations could be transferred for succeeding generations. However, with the negative associations like diseases, parasitism there is a tendency to deter. Metabolic cooperation between microorganisms occurs as a result of close proximities between the interacting organisms. Evolution of a consortia or intracellular parasitism are crucial for the establishment of metabolic cooperation. The former is more important as the occurrence of the latter phenomenon is rare. Symbiosis between bacteria and primitive organisms were an absolute necessity for the diversity and evolution of multicelled eukaryotic organisms (Margulis, 1993). Parasitic or pathogenic relationship might evolve into a mutualistic one in the course of time so as to minimize the damage to the host. A pathogen and a mutualist may thus be closely related and utilize similar molecular pathways for infecting the host. Thus the belief that many mutualistic symbioses could have originated as parasitic symbiosis is being accepted. Diseases of animals can be delineated only if the dynamic continuum between pathogenicity and mutualism is understood. In both the cases it can be stated that the host cells are "infected" by intracellular bacteria. At molecular level a single nucleotide substitution could change the interactions from antagonistic to mutualistic. Dynamic environment would require

plasticity in the phenotypic characters of species and these can be delivered through repetitive cycles of gene exchange to produce diversity of genotype and thus this enduring coevolutionary history shared by symbiotic partners has resulted in a major evolutionary transition: a symbiont's loss of independent growth, and possibly also the sexual phase of its life cycle (Ngai and Ruby, 2000).

In bacterial consortia, the natural transfer of plasmids within bacterial species is considered a useful proposition while reconstituting natural populations. Kellog (1984) has proposed a technique called plasmid Assisted Molecular Breeding (PAMB), wherein, under selective environmental pressure, plasmid transfer and natural recombinants could be derived among bacterial species to enhance their competence and degradation efficiency. It has been able to demonstrate PAMB in the consortia developed for hydrocarbon degradation (Mohandass et al., 1996). Such mutually dependent relationship that is accompanied by the exchange of extra-chromosomal exchange of DNA could be more widespread in nature. Ecological studies now get oriented towards understanding bacterial function in consortia than as individual cells *per se* in order to appreciate the whole gamut of symbiotic relationship that drive ecosystems.

Conclusion

Thus while antibiosis and parasitism could shape community structures, bacteriocins could double their role as either as anti or probiotic playing a defensive role for self and protective role to their associates. There is ever growing realization that a mutualist could have evolved from a parasite or a pathogen, as both adopt similar mechanism for infecting their host. With more and more insights into the changing strategies in associations, views of evolution keep changing. As Woese (2004) states, it is perhaps these types of lateral gene transfers that could be more responsible for evolutionary dynamics rather than the vertical inheritance. Most of symbiotic interactions are correlated to an increase in genetic plasticity of an organism that can eventually lead to evolutionary saltations and origin of new forms of life as Provorov (2001) suggests.

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