

It’s catching: the hologenomic bug

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*Bachelor Thesis*

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Submitted – Monday 8th May, 2017

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Traditionally, scientists have viewed eukaryotic organisms as autonomous entities, defined by their nuclear genome. Up to now, an individual’s sequence of DNA was viewed as the supreme code, stipulating phenotype and determining disease profiles. The advent of genotypic profiling has been demonstrably useful in both uncovering information about *Homo sapien*s evolution and allowing us to identify genotypes behind disease profiles (McFall-Ngai et al., 2013). It is now clear, however, that in focusing wholly on the nuclear genome we have excluded another very important aspect of human disease and force of evolution, the hologenome.

In their ancient past, eukaryote’s predecessors acquired mitochondrial organelles via a mutually advantageous endosymbiosis with bacteria, resulting in a eukaryote cell that carried DNA of both bacterial and archaeal origin (Ettema, 2016). After this first endosymbiotic event, the cells that would eventually result in human life continued to evolve and thrive in symbiosis with miniscule organisms. This association has never been broken, and out internal microbes not only outnumber the amount of somatic cells that make up the human body, in orders of magnitude, but they also outnumber any other organism on earth in terms of sheer population numbers (Singh et al., 2013, Donia & Fischbach, 2015, Knights et al. 2011, Brucker & Bordenstein, 2013). These vast collections of bacteria, eukarya, viruses, protists, and fungi are housed under the broader term of ‘microbiota’ or ‘the microbiome’ (Lederberg, McCray, 2001, Mustata and Mustata, 2014). The microbes live in huge communities within their host, in what is called a symbiosis, or the ‘living together of separate species’ (Goff, 1982). It seems only reasonable, given the collective sheer magnitude, ubiquity and diversity of these micro-organisms residing within the human body, that we devote proper analysis to their influence.

Up until now, the autonomous view of mankind has prevailed, namely that humans are entities with a phenotype defined by their nuclear genome. Darwinian and Mendelian theories, as well as those of the modern synthesis, now need to be assimilated with hologenomic principles that consider the interaction of microbes (Bordenstein & Theis 2015). Hologenomic theory was originally described by Zilber-Rosenburg & Rosenburg in 2008, as an evolutionary concept centred around the definition of an organism as a sum of both its nuclear and microbial genome, forming what is called ‘the holobiont’ (Zilber-Rosenberg and Rosenberg, 2008, Van Leuven, et al., 2014, Bordenstein & Theis 2015).

The principles behind hologenomic theory are comprised of four assumptions: 1) symbiotic relationships between host and microbe are ubiquitous among all species of organism, 2) symbiotic microbes are transmitted vertically to offspring, 3) these symbiosis’s affect fitness of the holobiont, and 4) the holobiont phenotype is characterised by both the nuclear and microbial genome (Zilber-Rosenberg and Rosenberg, 2008).

This essay will show how notions of human phenotype and theories of evolution have been impacted by the conception of the hologenomic theory. It will also examine how both medical practice and modern scientific pursuit will be revitalised by its principles. It is important to prove that the holobiont does not obviate any basic tenets of traditional theory. Instead, it brings new queries to the forefront and enables assimilation of aspects of Darwinism, Mendelian inheritance and the modern synthesis, with that of the human relationship with their microbiome (Bordenstein & Theis 2015). This discussion will be followed by an examination of human phenotype changes mediated by microbiota, as well as the role of the microbiome in the evolution of *Homo sapiens.* After acquiring this knowledge that the holistic view of the human ‘holobiont’ provides, I will put forward some examples of our ability to steer our own contemporary evolution through medical practice.

**Hologenomic theory: integrating into the traditional**

Hologenomic theory will induce a new integration and assimilation of microbial relationships into many of the principles that biology holds dear, especially those of Darwinian natural selection, Mendelian inheritance and the variation in modern synthesis. For example, Darwin’s theory designated the nuclear genome as the target of natural selection. This will have to expand to include the microbial genome as a consideration with fitness consequences in natural selection. This selection, as well as resulting in nuclear inheritance through Mendelian mechanisms, also needs to include non-Mendelian transmission of the microbial genome. The modern synthesis that united Darwinism and Mendelian theories will also have to include the microbiome as a source as variation, in addition to the nuclear genome (Bordenstein and Theis, 2015). All three of these theories were envisioned in times of eukaryocentricism and nucleocentrisicm where very little consideration was devoted to microbiology, due to both a lack of perspective and the appropriate technology to investigate the microbial world (Bordenstein and Theis, 2015). With the advent of hologenomic theory these three philosophies will undergo the amalgamation of their principles in combination with those pertaining to the microbiome.

This essay will discuss how hologenomic thought adds onto these traditional theories by introducing the importance of microbial consideration. This will occur first through discussion of mechanisms of non-Mendelian inheritance of microbes and then through an analysis of how phenotype is influenced by our microbiomes. The microbe mediated evolution behind hologenomic theory is discussed, using the same mechanisms of Darwinian natural selection, with influences from Lamarckian thought (Bordenstein and Theis, 2015). This essay will demonstrate that hologenomic evolution does not eclipse or invalidate the traditional biological theories, but instead enhances the principles that they rest on with the inclusion of microbiomes and the influence that they too exert (Bordenstein and Theis, 2015).

 The hologenomic premise as an ‘evolutionary theory’ rests on its heritability, detailed in clause 2 of Zilber-Rosenberg and Rosenbergs description (Zilber-Rosenberg and Rosenberg, 2008). For this a mechanism of the transmission of microbiota to successive generations is required, as the heritability of the nuclear genome is already well established. An individual’s microbiota, the inheritability of which will be discussed, is susceptible to profound alterations during the hosts lifetime in response to host behaviours such as medications, diet, exercise, and levels of stress (Coburn and Guttman, 2015, Soen et al., 2015). Hologenomic theory suggests that these microbial changes result in altered phenotypes that might ultimately be assimilated into the genome and will be inherited by further generations (West-Eberhard, 2005, Waddington, 1953). This suggests that acquired characteristics or microbes could feasibly then be passed on to a host’s offspring, a mechanism that is similar to that posited previously by Lamarck (Rosenberg et al., 2010). Lamarckian theory asserted the development of advantageous attributes during the individual’s lifetime, the characteristics of which could then be inherited by offspring (Rosenberg et al., 2010).

The consideration of such an intersect between Lamarckism and modern Darwinian theory needs to be properly analysed by exploring microbial inheritability and transmission. Microbes need to be inheritable for the phenotypic influence they impart to be passed onto any host offspring (Bordenstein and Theis 2015). While in insects and plants, precise and effective methods of vertical transmission have been demonstrated, in humans, such mechanisms for microbial heritance are less clear (Rosenberg et al., 2010). Studies have suggested that an assimilation of the initial microbiome occurs prenatally, a finding that goes against the previously assumed vertebrate ‘sterile womb’ theory (Perez-Muñoz et al., 2017, Funkhouser and Bordenstein, 2013, McFall-Ngai et al., 2013). A human baby’s first postpartum bowel movement contains populations of microbes, suggesting that maternal inheritance of microbiota occurs at least twenty-four hours before birth (Jiménez et al., 2008). The genetic labelling of mice has also shown that maternal bacteria can be passed on to offspring whilst in the uterus, as the tagged maternal microbes were located in the first meconium of the babies after the offspring had been delivered by caesarean section (Jiménez et al., 2008). These two studies provide strong evidence for maternal microbial transmission in mammals (Perez-Muñoz et al., 2017, Jiménez et al., 2008, Funkhouser and Bordenstein, 2013).

In addition to prenatal transmission, it is known that that the most significant vertical transmission of microbes occurs uni-parentally during the baby’s passage through the mother’s birth canal (Gilbert et al., 2015, Funkhouser and Bordenstein, 2013). Studies have shown that those babies delivered by Caesarean birth have significantly lower microbe diversity and delayed colonisation of normal infant microbiota (Makino et al., 2013). Not to be forgotten are more external modes of transmission, such as close personal contact with family members after birth or the transferal of organisms through the breast milk (Gilbert et al., 2015, Funkhouser and Bordenstein, 2013).

Although very little research has been done on the foetal microbiome transmission due to the ethics surrounding experimentation on human embryos, evidence has suggested vertical transmission is plausible, therefore confirming non-genetic, non Mendelian inheritance of microbes (Jiménez et al., 2008, Funkhouser and Bordenstein, 2013, Soen et. al., 2015).

Confirmation of the inheritance of microbes through both prenatal and postnatal mechanisms suggests that hologenomic theory could therefore promote a new ‘blending of Darwinism and Lamarkism’ in evolutionary theory (Singh, Ahmad, Musarrat, Ehtesham, & Hasnain, 2013). The mechanism behind the acquisition of such transmissible traits or microbes in the original host has been called ‘adaptive improvisation’ (Soen et al., 2015). Adaptive improvisation is composed of traditional Darwinian evolution with the addition of a continually adapting individual microbial evolution (Soen et al., 2015). The changes to the microbiome have been hypothesised to act as an ‘adaptive buffer’ in response to stress exerted by the environment (Soen et al., 2015). In many cases, microbes can respond more rapidly with phenotype alterations than the host’s own somatic cells could produce (Soen et al., 2015). If these phenotypic adaptations are advantageous to a persisting stressful external environment then this novel microbiome becomes permanent, and is inherited by subsequent generations (Soen et al., 2015). Microbiome adaptations could be quicker, short term reactions that allow assimilation to an environment until the traditional long term Darwinian evolutionary alterations allow genotypic adaptation (Rosenberg et.al. 2010). As the phylogeny spreads its branches, the composition and function of the microbiome will also change as the host encounters heterogeneous situations. Darwinian natural selection chooses the most advantageous ‘teams’ from both the nuclear genome and the microbial genome for that environment, leading to additions and alterations of beneficial microbes or genetic traits (Gilbert et al., 2015, Soen et al., 2015). These changes are then passed on to successive generations. In this way, the microbiome and the nuclear genome are combined into the holobiont, a single unit of natural selection.

‘Holo’ itself means whole or entire, suggesting that hologenomic theory is more holistic in approach than the traditional principles (Bordenstein and Theis, 2015). This holistic view is important as the microbiome is an internal ecosystem, and therefore exerts influence as a sum of its parts. In disciplines, like ecology, while it is useful to decipher the complicated systems and interactions of organisms to their most basic levels, eventually unification is needed to account for the influence of the microbiome as a whole (Li, 2000). The hologenomic view enables this, replacing what Carl Woese says is the 20th century ‘reductionist “eyes down” molecular perspective with a new and genuinely holistic, eyes-up, view of the living world’. (Woese, 2004).

A host’s physiology, evolution or development, therefore, cannot be understood without the holistic combination of nuclear genome and microbial symbionts (Gilbert et al., 2015). This essay, however, will separate these vital constituents to explore the differentiated roles. First microbiota will be investigated for its influence on human phenotype. This knowledge can be used to examine the rarely explored role of microbiota in human evolution. The microbial and nuclear genome will then be amalgamated back into the true hologenomic form to investigate how humans can utilise this new knowledge of microbes to further develop modern medicine.

**The role of microbiota in human phenotype**

While a great amount of research has been conducted on the influence of external environment on the internal microbiome, very little exists on the opposite impression: that of the microbiome on *Homo sapiens* phenotype. Studies have concluded that host behaviour and environment are factors that exert great influence on the microbiome, with the capacity to alter both quantity and quality of microbiota (Phillips, 2009, David et al., 2014, Flint, 2012). The future of microbial scientific exploration will hopefully see rapid expansion of this area as we move from ‘what is there and how can we alter it?’, to queries of ‘how are they altering us?’. Eventually this could result in comprehensive databases of the impact of differing species and quantities of microbes on human phenotype, in combination with the influence of the nuclear genome (Donia and Fischbach, 2015).

The microbiome can drive host phenotypic change through processes related to its own microbial natural selection. To explain, the gut microbe composition is governed by two main components: lifestyle choices such as growth and diet, and the effect of their microbial function on their host fitness (Scarpellini et al., 2008, Gilmore & Ferretti, 2003). This latter selection component is reflected in human phenotype as the functional microbiome undergoes selection.

Animal studies have indicated that phenotypic influences of microbial symbionts are ubiquitous in the animal kingdom (Muscatine & Porter, 1977, Dedeine et al., 2001, Rigaud et al., 2001, Mustata and Mustata, 2014, Ross et al., 2013). It is only rational to suppose, therefore, that humans experience these same microbial phenotypic effects. Research on organisms such as coral and insects, as well as the more closely related cows and monkeys have indicated that microbiota is integrally important in host fitness. Such research has shown microbiomes are implicated in bodily functions as diverse as digestion, immunity, olfaction, and organ and neural development (Muscatine and Porter, 1977, Dedeine et al., 2001, Rigaud et al., 2001, Mustata and Mustata, 2014, Ross et al., 2013, Brucker and Bordenstein, 2013, Li et al., 2013).

Faecal microbiota transplant (FMT) is a medical practice that directly demonstrates how alteration of quality and quantity of gut microbiota can alter host phenotype. Evidence for FMT’s therapeutic use in humans has been observed in the treatment of conditions as diverse as obesity (Jayasinghe et al., 2016), chronic colitis (Mattner et al., 2016), and autism (Kang et al., 2017).

A mouse model demonstrated with FMT that combinations of microbes could be linked to the development of obesity due to their increased capacity for energy harvest. The mice that received transplants of ‘obese’ gut bacteria experienced a significant increase in total body fat than those that received microbiota from normal or lean mice (Turnbaugh et al., 2006). The use of similar FMT methods in treatment of obesity in humans is in its experimental phase, however initial results suggest that colonisation of preferential microbiota might be of use in management of severe human obesity and obesity related conditions (Jayasinghe et al., 2016). It is not just colonisation of specific microbes that can increase the likelihood of obesity. Germ free mammals, living in states of gnothosis, have been shown to gain more body fat, showing it is not just the diversity but the volume of microbes that influences the development of metabolic disorders (Bäckhed et al., 2004). It is not surprising, given that the largest amount of microbiota is found in the human gut, that there is a significant influence between microbes and metabolic disorders (Turnbaugh et al., 2007, Burcelin, 2012). From these studies, a conclusion can be obtained that a lack of microbes or the colonisation of obesity associated gut microbiota has an increased and transmissible capacity to promote fat deposition and elevate the risk factors associated with conditions of obesity (Turnbaugh et al., 2006, Qin et al., 2012).

In addition, trials are being conducted in both animals and humans to investigate diseases such as chronic ulcerative colitis and *Clostridium difficile* colitis. These have been successfully treated with FMT, supporting the correlation between the transfer of microbial communities and changes in phenotype, in this case a transition from an ailing phenotype to that of a healthier profile (van Nood et al., 2013, Mattner et al., 2016). Interestingly, FMT has also proved therapeutic with neurobiological disorders, especially for those on the autism spectrum, who often find their condition worsened with intestinal discomfit (Kang et al., 2017). The success of FMT in individuals with conditions such as obesity, colitis and autism have shown that phenotype can be conferred via the colonisation of certain microbiota. This demonstrates the importance of the consideration of hologenomic theory in medicine, especially in understanding the relationship between microbiome, phenotype, and disease profile. Now that this influencing capacity on phenotype has been established, the role of microbiomes in the evolution of *Homo sapiens* should be considered.

# The role of microbiota in human evolution

As with any newly emerging scientific concept, many areas of hologenomic theory are lacking. As well as the deficiency of information of the influence of microbes on human phenotype, there is also a lack of evidence for how this phenotypic influence has translated in evolutionary terms. A plethora of literature describes how the evolution of humans has been investigated with microbial archaeological evidence, but very rarely is this evidence considered as a driving factor of the evolution itself. For example, paleobiology has been utilised in examining the microbiota found on the teeth of ancient skulls, enabling the compilation of a history of human diet (Warinner et al., 2015 Sperber, 2013). Similar investigation of ancient microbes has revealed the health status of the communities of our ancestors (Metcalfe, 2007, Warinner et al., 2015). More individually, microbes found in tooth calculus provide information about life history, the cause and location of death, and the method of mummification (Rollo et al., 2000, Metcalfe, 2007). These ancient remains and their microbes, however, also present interesting opportunities for exploration of any causal link between microbiota and evolution (Warinner et al., 2015).

In a similar way to genes, microbes can be classified as advantageous, neutral, or deleterious variations, with natural selection retaining the most favourable combination for that environment (Bordenstein and Theis 2015).The potential for phenotypic and trait variation, therefore, can be considered as a function of both the number of symbiont genomes as well as that of the genome of the host (Singh et. al. 2013). The holobiont, therefore, can be considered a unit of natural selection, with the retention of the most favourable genes or microbes for that environment, as specified under Darwinian theory (Bordenstein and Theis 2015, Brucker & Bordenstein, 2013).

Since human’s humble cellular beginnings, microbes have been instrumental in our composition. As organisms, we have been constructed, cell by cell by interactions between host and symbiotic microbes (Gilbert et al., 2015). Our own mitochondria are a product of an association that occurred almost two billion years ago (Sapp, 1994). Intracellular organelles such as cilia are posited to have bacterial origination via other endosymbiotic mechanisms (Chapman et al., 2000). In addition, multicellularity itself is a result of yet more intimate relationships between bacteria and the ancient unicellular eukaryotic protists (Margulis 1981, Alegado et al., 2012). Further down the timeline, the origination of the mammalian placenta is thought to be facilitated by microbial interaction (Chuong, 2013). These developments in our earliest origins as multicellular, mitochondrial, vertebrate and eukaryotic organisms were enabled by the many and varied states of symbiosis between microbes and the cell host.

Microbes also have been important evolutionary elements during times of necessary *Homo sapiens* diet change. Our microbiota has allowed a plasticity in diet, due to the ability to acquire microbial ‘traits’ fairly rapidly over our evolution. A healthy microbiome allows increased resilience to unfamiliar nutrition sources through ease of change and acquisition of useful microbes (McFall-Ngai et al., 2013). These organisms can also transform materials that previously would have had little nutritional value into useful sustenance for our ancestors, producing digestible molecules from indigestible substrates (McFall-Ngai et al., 2013). This ability of microbes to allow humans to radically change their feeding patterns has assisted in two main nutritional shifts in *Homo sapiens* history. The first occurred ten thousand years ago, as Neolithic man adopted the carbohydrate rich diet stimulated by their recent adoption of farming from the previous hunter-gatherer life style (Adler et al., 2013). A cooperative microbial process degraded the starch polysaccharide in the colon, resulting in butyrate, a molecule that *Homo sapiens* can digest (Duncan et al., 2002). This allowed the human body to adapt and transfer starch components into the colon, where these indigestible parts could be transformed into utilisable nutrient particles by the microbiota (Walter & Ley, 2011). Included in this adoption of pastoral life was the need to digest milk, which was helped by lactate fermenters, such as *Eubacterium hallii,* that also turned this otherwise indigestible substance into butyrate (Duncan et al., 2004, Walter & Ley, 2011). These microbes improved nutrient acquisition from both grain and milk sources that otherwise would have been indigestible and negligibly nutritional. The help of microbes in assimilating these novel substrates efficiently provided clear advantage in terms of natural selection, for both the microbe and host. The former would receive sustenance in the colon from the human diet, while the latter would be able to digest the material most readily available to them, therefore maximising the fitness of the complete holobiont that possessed such organisms (Walter & Ley, 2011).

The second dietary evolution occurred as humans continued to colonise different areas of the world and communities evolved to digest the nutrients that were most available to them, in a similar way to the New Age Man adjusting to a pastoral lifestyle. The microbes found in US citizens today are more suited to the digestion of high fat, high protein diets that were, and still are, available. More isolated populations such as those sampled in rural Malawi and the Amazonas of Venezuela have been shown to have gut microbiomes optimised for breaking down the complex carbs that can be grown in these areas (Yatsunenko et al., 2012). In addition, Japanese people evolved the ability through the association of marine bacterium to digest a seaweed polysaccharide available to them (Hehemann et al., 2010). This diet specialisation enabled humans to evolve within their environment and extract the most advantageous nutrients from their surroundings. Without these changes, there would have been intense conflict between the human digestive system, microbiota and the novel materials, correlated in a fitness depreciation in the individual (Walter and Ley, 2011). Microbial genomes can be envisaged as traits that can be selected for in respect to benefit for the surrounding environment, and therefore can drive the process of natural selection and spur on the unique evolution of *Homo sapiens* in a diversity of settings*.*

These two nutritional shifts highlight the important consideration that must be given to microbes and their role in adjusting humans to changes in diet, nutrition, and environment. After the industrial revolution, the human mouth lost much of its microbial diversity, due to improvements in oral hygiene and increased use of disinfectant agents (Adler et al., 2013, Kilian et al., 2016). While improved dental hygiene is widely promoted, it also has a role in the elimination of excessive microbiota from the mouth, making it vulnerable to change. This is important, as resilience of our microbiota is required for humans to adjust to further changes. The lack of diversity of microbes in the mouth could very well be reflect the status of other microbial colonies within the human body. With the current shift towards a Westernisation and ubiquity of worldwide diet, a lack of overall microbiome diversity could cause a standstill in nutritional evolution towards a new fitness level that suits this changing diet. This lack of evolution, in fact, could be a driving factor in the global epidemic of metabolic disorders (Walter and Ley, 2011). Considering their role in our past evolution and natural selection, microbes are greatly important in facilitating adjustment and selection to suit new modes of human diet.

Microbes also produced increased resilience in individual evolutionary terms to changing situations, as well as diet. Microbiomes may have increased rates of survival for our ancestors during the environmental fluctuations so characteristic of early *Homo sapiens* history in the Pleistocene glacial and interglacial periods (Butzer, 1977). The flexible hologenome can quickly respond to change with alterations and acquisition of microbes and the consequential phenotypic adaptations (Rosenberg et.al. 2010). Similarly to modern day, in ancient times a more diverse microbiome would be preferential, as the larger the biodiversity of organisms, the increased stability of the microbial ecosystem (Tilman et al., 2006). *Homo sapiens* ancestor’s microbiomes, therefore, were integrally important in allowing them to respond to changes in their environments and situations (Rosenberg et.al. 2010).

In addition to the resilience to diet changes and fluctuating environments that microbes imparted in *Homo sapiens* ancestors, they also held a significant role in the development of the vertebrate, and therefore human, immune system (Lee and Mazmanian, 2010). The microbiome poses a challenge for an immune system to overcome as it contains so many organisms of foreign origin, emitting similarly foreign particles. The host immune system has evolved, therefore, to function adequately, without causing unnecessary inflammation in response to the symbiotic microbiota residing in the host (Lee and Mazmanian, 2010). One of the proposed methods that microbes helped in the evolution of the adaptive immune system is by communicating with undifferentiated CD4+T cells, who could receive such microbial environmental signals (Lee and Mazmanian, 2010). Evidence for this is provided by mouse studies such as one conducted by O’Mahony et.al. in 2008 that demonstrated that Bifidobacterium infantis was able to trigger the differentiation of the TREG regulatory T cells from the CD4+T. These TREG’s are able to control or dampen any unwanted immune system activation (O’Mahony et al., 2008). The sensitivity of the undifferentiated CD4+ cells to the microbial signals, over time allowed evolution of the immune system, by designating the correct reactions to have to certain organisms, depending on if they were pathogenic or symbiotic (Lee and Mazmanian, 2010). The microbes that were able to facilitate this communication to CD4+ cells would be selected for in the evolution process as this recognition and consequential suppression of inflammation would be needed for successful colonisation (Sprinkle, 2006, Lee and Mazmanian, 2010). Through communication between microbes and CD4+T cells, a functioning adaptive immune system was created, that resulted in beneficial outcomes and fitness levels for symbiotic microbes, by letting them live, feed and reproduce within the host, and for humans, by recognising appropriate reactions to pathogenic or symbiotic organisms (Lee and Mazmanian, 2010).

Microbiota undeniably had an important role in human evolution, essential from the earliest days of multicellularity. Microbes aided in times of enormous changes in both human nutrition and environmental fluctuation. The human microbiome was one of the factors that allowed *Homo sapiens* to defy the environmental constraints exerted upon then and slowly colonise almost every continent on earth (Butzer, 1977). Microbes also facilitated the evolution of the vertebrate adaptive immune system, ensuring that pathogenic organisms did not colonise them.

Often evolution and microbial change are so interwoven that it is hard to distinguish which is the main driver. In circumstances such as diet, environmental change, the microbiome is altered in order to allow the evolution of digestion of available food. The microbiome also has the capacity to influence immune cell differentiation and therefore drive human evolution by facilitating the beginnings of adaptive immunity.

The latter ability to evolve a phenotype through microbial alteration is important, as it suggests that we can manipulate our own microbiomes to acquire advantageous traits in our lifetime, and possibly even influence the microbiomes of future generations. We know that our microbiome has an ability to ultimately impact our phenotype and that we can ourselves edit these microbes, therefore can harness hologenomic theory in the practice of modern medicine to shape the future of human kind.

**Applications in medicine**

The microbiota portion of the complete human holobiont can alter the host’s phenotype, and has applied this capability many times over in *Homo sapiens* evolution. It has been discussed with regard to alterations in fitness with treatments and conditions such as autism, ulcerative colitis and obesity, and increases in fitness involved in the evolution of cellular composition, diet change, and population structure. With this knowledge, human beings can utilise methods of microbiome manipulation to both their detriment or advantage (Mustata and Mustata, 2014). I will describe antibiotics and probiotic use can drastically change our internal microbial environments and result in elimination and diversification of microbial species respectively (Mustata and Mustata, 2014).

An example of medical intervention detrimental for our microbiome, is the use of antibiotics. Since the discovery of the ‘magic bullet’ that could rid the twentieth century of the plethora of infectious diseases, antibiotics have been applied liberally and ubiquitously across mankind’s ailments (Zaffiri et al., 2012). Previous generations however, unknowingly sacrificed their own healthy microbiome as collateral in the battle against infection (Modi et al., 2014). Even the name: anti-biotics, suggests some excessive harm to the internal biota. The anti- mechanism unfortunately does not discriminate, and in attempting to destroy an infectious agent, antibiotics also depopulate our healthy microbiota, causing microbial destabilisation and possibly acute or chronic disease (Dethlefsen et al., 2008). The human microbiome is profoundly altered in diversity and quantity of microbes with antibiotic use, transformed into a state of unbalance or dysbiosis. In the short term dysbiosis can cause the selection of resistance microbes, and in the long term can be associated with health problems related to the loss of beneficial bacteria (Francino, 2016). Countless studies of the effects of antibiotics on the microbiota of humans show that antibiotic use, whether it be long or short term, severely impact the microbial community composition, function and response (Francino, 2016, Jernberg et al., 2010).

Dysbiosis occurs as the antibiotics eliminate not just the infectious agents but the vital microbes important in the health of the host. The ‘good’ microbes are often paramount in preventing pathogenic microbe settlement (Acheson and Luccioli, 2004). The risks of disease resulting from this dysbiosis range from diarrhoea to pseudomembranous colitis (Wilcox, 2003). The side effects of antibiotics have been shown to last for years, meaning one relatively short antibiotic course could be long term in its consequences (Jernberg et al., 2010, Dethlefsen et al., 2008). Additionally, long term use of antibiotics can supply more opportunities for invasion of pathogenic bacterial strains into areas where antibiotic sensitive indigenous bacterial originally inhabited (Wilcox, 2003, Acheson and Luccioli, 2004,Modi et al., 2014). Here they can thrive, and utilise horizontal gene transfer with additional strains to increase their pathogenicity (Modi et al., 2014).

A perfect way to study the effect of antibiotics is by examining isolated populations that have not been exposed to the almost ubiquitous pharmacologic doses of antibiotics found in western populations (Clemente et al., 2015). The Yanomami hunter-gathers are a group of previously uncontacted Amerindians, whose microbiomes were analysed and compared to a standard Westernised microbiome (Clemente et al., 2015). This community has the highest levels of bacterial diversity reported for humans, undoubtedly because of 11,000 years without both antibiotics and Westernisation (Clemente et al., 2015). This is a clear demonstration of the limiting effect of both modern life and liberal antibiotic use on the human microbiome.

It is obvious from studies of disease caused by antibiotic mediated dysbiosis and analysis of un-medicated isolated communities that antibiotics profoundly damage our microbiomes **(**Jernberg et al., 2010, Dethlefsen et al., 2008, Modi et al., 2014). It is important that the medical profession stops evaluating antibiotic treatment solely on the basis of an individual’s nuclear genome. This excludes the exceptional influence of the microbiome constituent and its phenotypic influence on health. The use of hologenomic theory in medicine will allow the use of a more microbial ecological approach to treatment that will hopefully lead to a future not endangered by the risk of antibiotic mediated disease or resistant bacteria.

Hologenomic theory has led to a scenario of conscious phenotypic alteration never before experienced. Unlike our nuclear genome, we have an ability to change the actual composition of our microbiota, and therefore experience any alterations to human physiology it imparts. This implies is that we can manipulate our own phenotype and drive contemporary medical evolution by employing hologenomic theory in modern treatment.

In the same ways, microbial equilibrium can be disrupted with antibiotic use, research has also provided methods to return our hologenomic to a balanced state. Probiotic pills, supplements or foods can return order to a microbiome in dysbiosis (Floch et.al. 2006). These have achieved unheard of popularity in the western world, with sales valued at over $30 000 million US dollars in 2014 (Statista, 2014). These probiotics can be applied in both prescribed and over the counter forms, and in many cases, are still considered a ‘health food’ by the FDA (Islam, 2016). In medicine, probiotics have been used with increasing success against a variety of conditions such as irritable bowel syndrome, prevention of cardiovascular disease, improvement of immune response, with the most promising results in treatment of antibiotic associated diarrhoea and pouchitis (Floch et.al. 2006, Islam, 2016). Probiotic supplements are composed of non-pathogenic live bacteria such as *Lactobacilius, Bifidobacterium*, as well as yeast strains such as *Saccharomyces boulardii* (Islam, 2016). Exact mechanisms of the benefits gained from ingestion of probiotics is still largely unknown, with it hypothesised that the live microbes refill the spaces that pathogenic bacteria could otherwise fill, add diversity to the internal ecosystem, or could be additionally involved in immunomodulation (Scarpellini et al., 2008, Eloe-Fadrosh et al., 2015).

The name, probiotics, or pro-life is in stark contrast to that of antibiotics (Islam, 2016). It is not just in a name however; antibiotics and probiotics are opposing in their microbial consequences and disease ramifications. A condition mentioned above, antibiotic associated diarrhoea (AAD) causes symptoms such as dehydration and toxic megacolon, and depending on the antibiotic used is experienced in 5-39% of administered patients (Gupta & Garg, 2009, McFarland, 1998, Hood et al., 2014). Probiotics are used in prophylactic prevention of AAD in high risk patients. They supplement the microbiome with additional helpful bacteria, promoting greater diversity. This attainment of variety is advantageous as the insurance hypothesis claims, that the higher species numbers, the larger the resilience of the microbial ecosystem against environmental fluctuations (Yachi & Loreau, 1999). A meta-analysis study conducted by McFarland showed that use of probiotics as prophylaxis could reduce the risk of AAD by 44-57% (McFarland, 2006). This preventative effect has been further proven by more recent studies such as those conducted by Avadhani & Miley in 2011 and Videlock & Cremonini in 2012. Despite these supportive findings, supplementation with preventative probiotics during antibiotic treatment is not recognised procedure in hospitals, despite a study by Wong et al. in 2015 establishing that many medical professionals agree probiotic practices could be beneficial to patient treatment.

Humans have reaped probiotic benefit before the existence of microbes was even conceived (McFarland, 2015). The benefits were so well known that Ancient Egyptian hieroglyphs depict the drinking of fermented milk (McFarland, 2015, Yang et al., 2014). Yet the medical profession remains resistant to the use of probiotics. Difficulties arise from insufficient research combined with a heterogeneity of microbe studies. The variety of conclusions reached on specific microbes make any deductions hard to reach about the effectiveness of overall probiotic treatment (Thomas, 2016). For probiotics to become more accepted as both prophylactic and therapeutic treatments, efforts such as the Human Microbiome Project need to continue to creating awareness around the importance of maintaining a healthy microbiome (Turnbaugh et al., 2007, Sperber et al., 2012).

Probiotics are a hopeful tool in the fight against antibiotic related disorders, and have been shown to have promising applications in cancer research and metabolic disorders, as well as preventative effects (Thomas, 2016). These microbe boosts in pill form might be the next step on the evolutionary journey towards optimal microbiota health in order to maximise human hologenomic fitness.

**Conclusion**

Hologenomic theory, with all its Lamarckian aspects has the potential to revive evolutionary philosophy. It does not alter the basic tenets of traditional theories of Darwinian natural selection, Mendelian inheritance or modern synthesis but it does adds on an important layer of microbial influence to consider in all three of these models (Gilbert et al., 2015). Human phenotype has morphed over time, not just due to selection, inheritance and variation from the nuclear genome, but from the influence of a huge variety of organisms found in the human microbiome. Due to this phenotypic influence, microbiota has played an instrumental role in human evolution, influencing aspects from early cellular eukaryotic organisms, to mediating changes in human diet and environment, and the early development of the adaptive immune system. Further analysis of individuals’ microbe’s role in phenotype and evolution, will allow development of treatments for diseases that have up to now proved severely detrimental to human fitness. In this way, modern medicine, by acknowledging the importance of hologenomic theory, will be able to alter the worldwide human disease profile, shaping contemporary evolution.

It is important to note that this is a very wide overview of the topic of hologenomic theory. In my research, I noticed the tendency of researchers to zero in on very specific topics, for example the advantage of one specific microbe over another (Qin et al., 2012, Dodd et al., 2015). This is incredibly helpful as knowledge of the intricate workings of a subject allow great overall mastery of the topic itself. Hologenomic theory however, is still young and many areas of its composition are left gaping with empty spaces that need to be researched. The principle of hologenomic thought rests on aspects of ecological theory, a philosophy that requires the study of the interaction of all the contributing parts. Due to holistic nature of the theory itself, therefore, I thought it would be suitable to examine in overview, and this would allow identification of those topics most in need of discussion and further scientific enquiry.

Despite the conclusions I have reached, in much of my research I found the field lacking in research. As with any new topic on the scientific horizon, many questions need to be solved to move forward in hologenomic theory. It seems the role of microbiota and the mechanism of its phenotypic influence are still relatively unexplored and in order to reach more definitive suppositions on this, continual research needs to be conducted (McFall-Ngai et al., 2013). Studies into the heritability of microbes are needed for hologenomic principles to be truly claimed as evolutionary theory, for example more research investigation of the mechanism behind foetal microbial colonisation is necessary (Perez-Muñoz et al., 2017, Bordenstein and Theis 2015). It would also be interesting to examine the possible existence of a ‘core’ human microbiome as this would prove useful, especially in the creation of probiotic treatments that could restore this ‘core’ composition to unhealthy individuals (Ley et al., 2007).

Research also needs to continue in the areas of antibiotic and probiotic use in medicine. The acceptance of microbial consideration and practice has so far been resisted by the medical industry. For the damage inflicted by antibiotics to be recognised and for realisation of the potentially prophylactic and therapeutic role probiotics, further research will need to continue. The medical utilisation of probiotics will only occur with regulation through administrations such as the FDA, and for that many more controlled, randomised and placebo based clinical trials as well as continuing meta-analysis of data on probiotic use will need to occur (Videlock and Cremonini, 2012, Islam, 2016). These metagenomics studies are instrumental in not only identifying diversity and numbers of microbes but specifying the species constituents of microbiomes across individuals (Ross et al., 2013). Mustata & Mustata in 2014 claimed that probiotics might be the upcoming ‘secret weapon’ in modern medicine, but for this to be believed, much greater volumes of more controlled research need to be conducted.

Apart from the advantages that research will provide, studies of hologenomic theory are beneficial to the scientific pursuit. As we merge symbionts and the host into one model of the holobiont, we also allow assimilation of many different scientific disciplines that assist with research, such as medicine, surgery, epidemiology, agriculture, biotechnology, and behavioural, computer and ecosystem science (The University of Chicago, 2017, Mustata and Mustata, 2014, McFall-Ngai et al., 2013). The Human Microbiome Project, first launched in 2007, is an example of a convergence of disciplines used in hologenomic theory (Turnbaugh et al., 2007).

It is interesting to consider a future where medical acceptance of hologenomic theory is widespread. While dubious in its privacy, fascinating possibilities stretch on the horizon for the personal investigation and identification of a patient’s microbiome, the information of which is then stored on a medical database to be consulted by the patient’s doctors (Ley et al., 2007). An initial ‘core’ microbiome could be documented that was then updated regularly with addition microbes and activity resulting from lab analysis of stool samples. In this way, a stool sample would become like a blood test. This would be particularly beneficial for those individuals that do experience phenotypic change and disease due to activity within their microbiome. A database would enable monitoring of changes throughout a patient’s life, examination of microbial mediated disease, as well as easy analysis of the effects of antibiotics, probiotics, or even simple lifestyle change, with before and after studies. Not only would this allow greater monitoring of symptoms and diseases, it would allow direct comparison and easy analysis of humans as hologenomic entities (Ley et al., 2007).

Hologenomic theory has the potential to not only revitalise tradition evolutionary theories but to allow significant improvement of human health. Analysis of human history and phenotypic alteration can provide insight into how we can maximise our health in the future, driving a contemporary advancement. We need to extend the view of the human as the sum of their own genetic variation to include that of the genome of their microbes, especially in consideration of phenotype and disease outcome. It seems only suiting, considering the sheer number of microbes that inhabit our body that we devote sufficient consideration to them. The helpful symbionts that provide advantages in fitness can quickly turn hostile under differing conditions. There is a delicate balance, therefore, that needs to be maintained but we still know very little about the right equilibrium that constitutes a ‘healthy microbiome’. This is an area that needs intense exploration to utilise medications such as antibiotics and probiotics to our hologenomic advantage. I have no doubt that investigation will ensue, as above all, my research has demonstrated to me that the hologenomic bug is catching.

# Bibliography

Acheson, D.W.., Luccioli, S., 2004. Mucosal immune responses. Best Practice & Research Clinical Gastroenterology 18, 387–404. doi:10.1016/j.bpg.2003.11.002

Adler, C.J., Dobney, K., Weyrich, L.S., Kaidonis, J., Walker, A.W., Haak, W., Bradshaw, C.J.A., Townsend, G., Soltysiak, A., Alt, K.W., Parkhill, J., Cooper, A., 2013. Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. Nature Genetics; New York 45, 450–5, 455e1.

Alegado, R.A., Brown, L.W., Cao, S., Dermenjian, R.K., Zuzow, R., Fairclough, S.R., Clardy, J., King, N., 2012. A bacterial sulfonolipid triggers multicellular development in the closest living relatives of animals. eLife 1, e00013. doi:10.7554/eLife.00013

Avadhani, A., Miley, H., 2011. Probiotics for prevention of antibiotic-associated diarrhea and Clostridium difficile-associated disease in hospitalized adults—A meta-analysis. Journal of the American Academy of Nurse Practitioners 23, 269–274. doi:10.1111/j.1745-7599.2011.00617.x

Bäckhed, F., Ding, H., Wang, T., Hooper, L.V., Koh, G.Y., Nagy, A., Semenkovich, C.F., Gordon, J.I., 2004. The gut microbiota as an environmental factor that regulates fat storage. Proc. Natl. Acad. Sci. U.S.A. 101, 15718–15723. doi:10.1073/pnas.0407076101

Bordenstein, S.R., Theis, K.R., 2015. Host Biology in Light of the Microbiome: Ten Principles of Holobionts and Hologenomes: e1002226. PLoS Biology; San Francisco 13. doi:http://dx.doi.org.virtual.anu.edu.au/10.1371/journal.pbio.1002226

Brucker, R.M., Bordenstein, S.R., 2013a. The capacious hologenome. Zoology 116, 260–261. doi:10.1016/j.zool.2013.08.003

Brucker, R.M., Bordenstein, S.R., 2013b. The Hologenomic Basis of Speciation: Gut Bacteria Cause Hybrid Lethality in the Genus Nasonia. Science 341, 667–669. doi:10.1126/science.1240659

Burcelin, R., 2012. Regulation of Metabolism: A Cross Talk Between Gut Microbiota and Its Human Host. Physiology 27, 300–307. doi:10.1152/physiol.00023.2012

Butzer, K.W., 1977. Environment, Culture, and Human Evolution: Hominids first evolved in mosaic environments, but stone toolmaking accelerated the emergence of Homo, and both culture and environment subsequently served as catalysts for evolution. American Scientist 65, 572–584.

Chapman, M.J., Dolan, M.F., Margulis, L., 2000. Centrioles and Kinetosomes: Form, Function, and Evolution. The Quarterly Review of Biology 75, 409–429.

Chuong, E.B., 2013. Retroviruses facilitate the rapid evolution of the mammalian placenta", BioEssays, vol. 35, no. 10, pp. 853-861.

Clemente, J.C., Pehrsson, E.C., Blaser, M.J., Sandhu, K., Gao, Z., Wang, B., Magris, M., Hidalgo, G., Contreras, M., Noya-Alarcón, Ó., Lander, O., McDonald, J., Cox, M., Walter, J., Oh, P.L., Ruiz, J.F., Rodriguez, S., Shen, N., Song, S.J., Metcalf, J., Knight, R., Dantas, G., Dominguez-Bello, M.G., 2015. The microbiome of uncontacted Amerindians. Science Advances 1, e1500183. doi:10.1126/sciadv.1500183

Coburn, B., Guttman, D.S., 2015. The human microbiome. Canadian Medical Association. Journal: CMAJ; Ottawa 187, 825.

David, L.A., Materna, A.C., Friedman, J., Campos-Baptista, M.I., Blackburn, M.C., Perrotta, A., Erdman, S.E., Alm, E.J., 2014. Host lifestyle affects human microbiota on daily timescales. Genome Biology 15, R89. doi:10.1186/gb-2014-15-7-r89

Dedeine, F., Vavre, F., Fleury, F., Loppin, B., Hochberg, M.E., Boulétreau, M., 2001. Removing symbiotic Wolbachia bacteria specifically inhibits oogenesis in a parasitic wasp. PNAS 98, 6247–6252. doi:10.1073/pnas.101304298

Dethlefsen, L., Huse, S., Sogin, M.L., Relman, D.A., 2008. The Pervasive Effects of an Antibiotic on the Human Gut Microbiota, as Revealed by Deep 16S rRNA Sequencing. PLOS Biology 6, e280. doi:10.1371/journal.pbio.0060280

Dodd, D., Tropini, C., Sonnenburg, J.L., 2015. Your gut microbiome, deconstructed. Nat Biotech 33, 1238–1240. doi:10.1038/nbt.3431

Donia, M.S., Fischbach, M.A., 2015. Small molecules from the human microbiota. Science 349, 1254766. doi:10.1126/science.1254766

Duncan, S.H., Hold, G.L., Barcenilla, A., Stewart, C.S., Flint, H.J., 2002. Roseburia intestinalis sp. nov., a novel saccharolytic, butyrate-producing bacterium from human faeces. Int. J. Syst. Evol. Microbiol. 52, 1615–1620. doi:10.1099/00207713-52-5-1615

Duncan, S.H., Louis, P., Flint, H.J., 2004. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. Appl. Environ. Microbiol. 70, 5810–5817. doi:10.1128/AEM.70.10.5810-5817.2004

Eloe-Fadrosh, E.A., Brady, A., Crabtree, J., Drabek, E.F., Ma, B., Mahurkar, A., Ravel, J., Haverkamp, M., Fiorino, A.-M., Botelho, C., Andreyeva, I., Hibberd, P.L., Fraser, C.M., 2015. Functional Dynamics of the Gut Microbiome in Elderly People during Probiotic Consumption. mBio 6, e00231–15. doi:10.1128/mBio.00231-15

Ettema, T.J.G., 2016. Evolution: Mitochondria in the second act. Nature 531, 39–40. doi:10.1038/nature16876

Flint, H.J., 2012. The impact of nutrition on the human microbiome. Nutr Rev 70, S10–S13. doi:10.1111/j.1753-4887.2012.00499.x

Francino, M.P., 2016. Antibiotics and the Human Gut Microbiome: Dysbioses and Accumulation of Resistances. Frontiers in Microbiology 6. doi:10.3389/fmicb.2015.01543

Funkhouser, L.J., Bordenstein, S.R., 2013. Mom Knows Best: The Universality of Maternal Microbial Transmission. PLOS Biology 11, e1001631. doi:10.1371/journal.pbio.1001631

Gilbert, S.F., 2014. Symbiosis as the way of eukaryotic life: The dependent co-origination of the body. Journal of Biosciences; Dordrecht 39, 201–9. doi:http://dx.doi.org.virtual.anu.edu.au/10.1007/s12038-013-9343-6

Gilbert, S.F., Bosch, T.C.G., Ledón-rettig, C., 2015. Eco-Evo-Devo: developmental symbiosis and developmental plasticity as evolutionary agents. Nature Reviews. Genetics; London 16, 611–622. doi:http://dx.doi.org.virtual.anu.edu.au/10.1038/nrg3982

Gilmore, M.S., Ferretti, J.J., 2003. The Thin Line Between Gut Commensal and Pathogen. Science 299, 1999–2002. doi:10.1126/science.1083534

Goff, L.J., 1982. Symbiosis and Parasitism: Another Viewpoint. BioScience 32, 255–256. doi:10.2307/1308531

Gupta, V., Garg, R., 2009. Probiotics. Indian Journal of Medical Microbiology; Chandigarh 27, 202–9. doi:http://dx.doi.org.virtual.anu.edu.au/10.4103/0255-0857.53201

Hehemann, J.-H., Correc, G., Barbeyron, T., Helbert, W., Czjzek, M., Michel, G., 2010. Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. Nature 464, 908–912. doi:10.1038/nature08937

Hood, K., Nuttall, J., Gillespie, D., Shepherd, V., Wood, F., Duncan, D., Stanton, H., Espinasse, A., Wootton, M., Acharjya, A., Allen, S., Bayer, A., Carter, B., Cohen, D., Francis, N., Howe, R., Mantzourani, E., Thomas-Jones, E., Toghill, A., Butler, C.C., 2014. Probiotics for Antibiotic-Associated Diarrhoea (PAAD): a prospective observational study of antibiotic-associated diarrhoea (including Clostridium difficile-associated diarrhoea) in care homes. Health Technology Assessment 18, 1–84. doi:10.3310/hta18630

Islam, S.U., 2016. Clinical Uses of Probiotics: Medicine 95, e2658. doi:10.1097/MD.0000000000002658

J. Lederberg, A.T. McCray, 2001. Ome sweet ’omics–A genealogical treasury of words Scientist, 17 (2001), p. 8.

Jayasinghe, T.N., Chiavaroli, V., Holland, D.J., Cutfield, W.S., O’Sullivan, J.M., 2016. The New Era of Treatment for Obesity and Metabolic Disorders: Evidence and Expectations for Gut Microbiome Transplantation. Frontiers in Cellular and Infection Microbiology 6. doi:10.3389/fcimb.2016.00015

Jernberg, C., Löfmark, S., Edlund, C., Jansson, J.K., 2010. Long-term impacts of antibiotic exposure on the human intestinal microbiota. Microbiology 156, 3216–3223. doi:10.1099/mic.0.040618-0

Jiménez, E., Marín, M.L., Martín, R., Odriozola, J.M., Olivares, M., Xaus, J., Fernández, L., Rodríguez, J.M., 2008. Is meconium from healthy newborns actually sterile? Research in Microbiology 159, 187–193. doi:10.1016/j.resmic.2007.12.007

Kang, D.-W., Adams, J.B., Gregory, A.C., Borody, T., Chittick, L., Fasano, A., Khoruts, A., Geis, E., Maldonado, J., McDonough-Means, S., Pollard, E.L., Roux, S., Sadowsky, M.J., Lipson, K.S., Sullivan, M.B., Caporaso, J.G., 2017. Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. Microbiome; London 5. doi:http://dx.doi.org.virtual.anu.edu.au/10.1186/s40168-016-0225-7

Kilian, M., Chapple, I.L.C., Hannig, M., Marsh, P.D., Meuric, V., Pedersen, A.M.L., Tonetti, M.S., Wade, W.G., Zaura, E., 2016. The oral microbiome – an update for oral healthcare professionals. Br Dent J 221, 657–666. doi:10.1038/sj.bdj.2016.865

Knights, D., Costello, E.K., Knight, R., 2011. Supervised classification of human microbiota. FEMS Microbiology Reviews 35, 343–359. doi:10.1111/j.1574-6976.2010.00251.x

Lee, Y.K., Mazmanian, S.K., 2010. Has the Microbiota Played a Critical Role in the Evolution of the Adaptive Immune System? Science 330, 1768–1773. doi:10.1126/science.1195568

Ley, R.E., Knight, R., Gordon, J.I., 2007. The human microbiome: eliminating the biomedical/environmental dichotomy in microbial ecology. Environmental Microbiology 9, 3–4. doi:10.1111/j.1462-2920.2006.01222\_3.x

Li, B.-L., 2000. Why is the holistic approach becoming so important in landscape ecology? Landscape and Urban Planning 50, 27–41. doi:10.1016/S0169-2046(00)00078-5

Li, J., Nasidze, I., Quinque, D., Li, M., Horz, H.-P., André, C., Garriga, R.M., Halbwax, M., Fischer, A., Stoneking, M., 2013. The saliva microbiome of Pan and Homo. BMC Microbiology 13, 204. doi:10.1186/1471-2180-13-204

Makino, H., Kushiro, A., Ishikawa, E., Kubota, H., Gawad, A., Sakai, T., Oishi, K., Martin, R., Ben-Amor, K., Knol, J., Tanaka, R., 2013. Mother-to-infant transmission of intestinal bifidobacterial strains has an impact on the early development of vaginally delivered infant’s microbiota. PLoS ONE 8, e78331. doi:10.1371/journal.pone.0078331

Margulis, L., 1981. Symbiosis in cell evolution: life and its environment on the early Earth, W H Freeman, San Francisco.

Martin H. Floch, MD,\* Karen K. Madsen, PhD,w David J. A. Jenkins, MD, PhD, DSc,z, Stefano Guandalini, MD,y Jeffery A. Katz, MD,J Andrew Onderdonk, PhD,z, W. Allan Walker, MD,\*\* Richard N. Fedorak, MD,w and Michael Camilleri, MD, 2006. Recommendations for Probiotic Use.

Mattner, J., Schmidt, F., Siegmund, B., 2016. Faecal microbiota transplantation—A clinical view. International Journal of Medical Microbiology 306, 310–315. doi:10.1016/j.ijmm.2016.02.003

McFall-Ngai, M., Hadfield, M.G., Bosch, T.C.G., Carey, H.V., Domazet-Lošo, T., Douglas, A.E., Dubilier, N., Eberl, G., Fukami, T., Gilbert, S.F., Hentschel, U., King, N., Kjelleberg, S., Knoll, A.H., Kremer, N., Mazmanian, S.K., Metcalf, J.L., Nealson, K., Pierce, N.E., Rawls, J.F., Reid, A., Ruby, E.G., Rumpho, M., Sanders, J.G., Tautz, D., Wernegreen, J.J., 2013. Animals in a bacterial world, a new imperative for the life sciences. Proceedings of the National Academy of Sciences of the United States of America 110, 3229–3236.

McFarland, L.V., 2015. From yaks to yogurt: the history, development, and current use of probiotics. Clin. Infect. Dis. 60 Suppl 2, S85–90. doi:10.1093/cid/civ054

McFarland, L.V., 2006. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. Am. J. Gastroenterol. 101, 812–822. doi:10.1111/j.1572-0241.2006.00465.x

McFarland, L.V., 1998. Epidemiology, risk factors and treatments for antibiotic-associated diarrhea. Dig Dis 16, 292–307. doi:16879

Metcalfe, N.H., 2007. In what ways can human skeletal remains be used to understand health and disease from the past? Postgrad Med J 83, 281–284. doi:10.1136/pgmj.2006.051813

Modi, S.R., Collins, J.J., Relman, D.A., 2014b. Antibiotics and the gut microbiota. Journal of Clinical Investigation; Ann Arbor 124, 4212–8.

Muscatine, L., Porter, J.W., 1977. Reef Corals: Mutualistic Symbioses Adapted to Nutrient-Poor Environments. BioScience 27, 454–460. doi:10.2307/1297526

Mustata, G., Mustata, M., 2014. The Microbiome - the Secret Weapon of Modern Medicine. International Journal of Medical Dentistry; Iasi 4, 49–63.

O’Mahony, C., Scully, P., O’Mahony, D., Murphy, S., O’Brien, F., Lyons, A., Sherlock, G., MacSharry, J., Kiely, B., Shanahan, F., O’Mahony, L., 2008. Commensal-Induced Regulatory T Cells Mediate Protection against Pathogen-Stimulated NF-κB Activation. PLoS Pathog 4. doi:10.1371/journal.ppat.1000112

Perez-Muñoz, M.E., Arrieta, M.-C., Ramer-Tait, A.E., Walter, J., 2017. A critical assessment of the “sterile womb” and “in utero colonization” hypotheses: implications for research on the pioneer infant microbiome. Microbiome 5, 48. doi:10.1186/s40168-017-0268-4

Phillips, M.L., 2009. Gut Reaction: Environmental Effects on the Human Microbiota. Environ Health Perspect 117, A198–A205.

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., Liang, S., Zhang, W., Guan, Y., Shen, D., Peng, Y., Zhang, D., Jie, Z., Wu, W., Qin, Y., Xue, W., Li, J., Han, L., Lu, D., Wu, P., Dai, Y., Sun, X., Li, Z., Tang, A., Zhong, S., Li, X., Chen, W., Xu, R., Wang, M., Feng, Q., Gong, M., Yu, J., Zhang, Y., Zhang, M., Hansen, T., Sanchez, G., Raes, J., Falony, G., Okuda, S., Almeida, M., LeChatelier, E., Renault, P., Pons, N., Batto, J.-M., Zhang, Z., Chen, H., Yang, R., Zheng, W., Li, S., Yang, H., Wang, J., Ehrlich, S.D., Nielsen, R., Pedersen, O., Kristiansen, K., Wang, J., 2012. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 490, 55–60. doi:10.1038/nature11450

Rigaud, T., Pennings, P.S., Juchault, P., 2001. Wolbachia Bacteria Effects after Experimental Interspecific Transfers in Terrestrial Isopods. Journal of Invertebrate Pathology 77, 251–257. doi:10.1006/jipa.2001.5026

Rollo, F., Luciani, S., Canapa, A., Marota, I., 2000. Analysis of bacterial DNA in skin and muscle of the Tyrolean iceman offers new insight into the mummification process. Am. J. Phys. Anthropol. 111, 211–219. doi:10.1002/(SICI)1096-8644(200002)111:2<211::AID-AJPA7>3.0.CO;2-M

Rosenberg, E., Sharon, G., Atad, I., Zilber-Rosenberg, I., 2010. The evolution of animals and plants via symbiosis with microorganisms: Evolution via symbiosis. Environmental Microbiology Reports 2, 500–506. doi:10.1111/j.1758-2229.2010.00177.x

Ross, E.M., Moate, P.J., Marett, L.C., Cocks, B.G., Hayes, B.J., 2013. Metagenomic Predictions: From Microbiome to Complex Health and Environmental Phenotypes in Humans and Cattle. PLOS ONE 8, e73056. doi:10.1371/journal.pone.0073056

Sapp, J., 1994. Evolution by association: a history of symbiosis, Oxford University Press, New York.

Scarpellini, E., Cazzato, A., Lauritano, C., Gabrielli, M., Lupascu, A., Gerardino, L., Abenavoli, L., Petruzzellis, C., Gasbarrini, G., Gasbarrini, A., 2008. Probiotics: Which and When? DDI 26, 175–182. doi:10.1159/000116776

Singh, Y., Ahmad, J., Musarrat, J., Ehtesham, N.Z., Hasnain, S.E., 2013. Emerging importance of holobionts in evolution and in probiotics. Gut Pathogens 5, 12. doi:10.1186/1757-4749-5-12

Soen, Y., Knafo, M., Elgart, M., 2015. A principle of organization which facilitates broad Lamarckian-like adaptations by improvisation. Biology Direct; London 10.

Sperber, A.D., Drossman, D.A., Quigley, E.M.M., 2012. The Global Perspective on Irritable Bowel Syndrome: A Rome Foundation-World Gastroenterology Organisation Symposium. The American Journal of Gastroenterology; Cambridge 107, 1602–1609. doi:http://dx.doi.org.virtual.anu.edu.au/10.1038/ajg.2012.106

Sperber, G.H., 2013. The role of teeth in human evolution. British Dental Journal; London 215, 295–7. doi:http://dx.doi.org.virtual.anu.edu.au/10.1038/sj.bdj.2013.878

Statista 2014, (accessed 5.4.17)

 https://www.statista.com/statistics/252941/probiotic-products-sales-worldwide-by-region/

Thomas, L.V., 2016. Probiotics- the journey continues. International Journal of Dairy Technology 69, 469–480. doi:10.1111/1471-0307.12354

Tilman, D., Reich, P.B., Knops, J.M.H., 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature 441, 629–632. doi:10.1038/nature04742

Turnbaugh, P.J., Ley, R.E., Hamady, M., Fraser-Liggett, C.M., Knight, R., Gordon, J.I., 2007. The Human Microbiome Project. Nature; London 449, 804–10. doi:http://dx.doi.org.virtual.anu.edu.au/10.1038/nature06244

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., Gordon, J.I., 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature; London 444, 1027–31. doi:http://dx.doi.org.virtual.anu.edu.au/10.1038/nature05414

Van Leuven, J.T., Meister, R.C., Simon, C., McCutcheon, J.P., 2014. Sympatric Speciation in a Bacterial Endosymbiont Results in Two Genomes with the Functionality of One. Cell 158, 1270–1280. doi:10.1016/j.cell.2014.07.047

Van Nood, E., Vrieze, A., Nieuwdorp, M., Fuentes, S., Zoetendal, E.G., de Vos, W.M., Visser, C.E., Kuijper, E.J., Bartelsman, J.F., Tijssen, J.G., Speelman, P., Dijkgraaf, M.G., Keller, J.J., 2013. Duodenal Infusion of Donor Feces for Recurrent Clostridium difficile. The New England Journal of Medicine; Boston 368, 407–15.

Videlock, E.J., Cremonini, F., 2012. Meta-analysis: probiotics in antibiotic-associated diarrhoea. Aliment Pharmacol Ther 35, 1355–1369. doi:10.1111/j.1365-2036.2012.05104.x

Waddington, C.H., 1953. Genetic Assimilation of an Acquired Character. Evolution 7, 118–126. doi:10.2307/2405747

Walter, J., Ley, R., 2011. The Human Gut Microbiome: Ecology and Recent Evolutionary Changes [WWW Document]. http://dx.doi.org.virtual.anu.edu.au/10.1146/annurev-micro-090110-102830. URL http://www.annualreviews.org.virtual.anu.edu.au/doi/10.1146/annurev-micro-090110-102830 (accessed 4.30.17).

Warinner, C., Speller, C., Collins, M.J., Lewis, C.M., 2015. Ancient human microbiomes. Journal of Human Evolution 79, 125–136. doi:10.1016/j.jhevol.2014.10.016

West-Eberhard, M.J., 2005. Phenotypic accommodation: adaptive innovation due to developmental plasticity. J. Exp. Zool. B Mol. Dev. Evol. 304, 610–618. doi:10.1002/jez.b.21071

Wilcox, M.H., 2003. Clostridium difficile infection and pseudomembranous colitis. Best Practice & Research Clinical Gastroenterology 17, 475–493. doi:10.1016/S1521-6918(03)00017-9

Woese, C.R., 2004. A New Biology for a New Century. Microbiol. Mol. Biol. Rev. 68, 173–186. doi:10.1128/MMBR.68.2.173-186.2004

Wong, S., Saif, M., O’Driscoll, J., Kumar, N., Smith, É., Roels, E., van Nes, I., Faber, W., McKeown, E., Hirani, S.P., Jamous, A., 2015. Use of Probiotics in Preventing Antibiotic Associated Diarrhoea and Clostridium Difficile Associated Diarrhoea in Spinal Injury Centres: An International Multicentre Survey. International Journal of Probiotics & Prebiotics; Coppell 10, 85–90.

Yachi, S., Loreau, M., 1999. Biodiversity and ecosystem productivity in a fluctuating environment: The insurance hypothesis. PNAS 96, 1463–1468. doi:10.1073/pnas.96.4.1463

Yang, Y., Shevchenko, A., Knaust, A., Abuduresule, I., Li, W., Hu, X., Wang, C., Shevchenko, A., 2014. Proteomics evidence for kefir dairy in Early Bronze Age China. Journal of Archaeological Science 45, 178–186. doi:10.1016/j.jas.2014.02.005

Yatsunenko, T., Rey, F.E., Manary, M.J., Trehan, I., Dominguez-Bello, M.G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R.N., Anokhin, A.P., Heath, A.C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J.G., Lozupone, C.A., Lauber, C., Clemente, J.C., Knights, D., Knight, R., Gordon, J.I., 2012. Human gut microbiome viewed across age and geography. Nature 486, 222–227. doi:10.1038/nature11053

Zaffiri, L., Gardner, J., Toledo-Pereyra, L.H., 2012. History of Antibiotics. From Salvarsan to Cephalosporins. Journal of Investigative Surgery 25, 67–77. doi:10.3109/08941939.2012.664099

Zilber-Rosenberg, I., Rosenberg, E., 2008. Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. FEMS Microbiology Reviews 32, 723–735. doi:10.1111/j.1574-6976.2008.00123.x