MICB425 – Microbial Ecological Genomics
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"Discuss the challenges involved in defining a microbial species and how HGT complicates matters, especially in the context of the evolution and phylogenetic distribution of microbial metabolic pathways. Can you comment on how HGT influences the maintenance of global biogeochemical cycles through time? Finally, do you think it is necessary to have a clear definition of a microbial species? Why or why not?"

The number of bacterial species in the world was previously estimated to be in the range of 10^7 to 10^9, meaning that accurately define new microbial species while maintaining an exponentially growing reference database can be a daunting task for researchers (Schloss and Handelsman 2004). Horizontal gene transfer (HGT) – a mechanism that enables the sharing of individual genetic materials or entire metabolic pathways between organisms – adds an additional challenge when defining microbial species (Schloss and Handelsman 2004, Souey *et al*. 2015). However, HGT played a significant role in preserving major metabolic pathways of global biogeochemical cycles through time by distributing them among the diverse species of microbes living in different environments. Even though there are many difficult challenges in defining a microbial species, it is necessary to have a clear definition of a microbial species as it plays vital roles in saving countless patients and in maintaining a functional reference database of Earth's microbial species. To be more specific, this paper will expand on the challenges involved in defining a microbial species and how HGT complicates matters (in the context of the evolution and phylogenetic distribution of microbial metabolic pathways), the influence of HGT in the preservation of global biogeochemical cycles through time, and the necessity of having a clear definition of a microbial species.

Challenges Involved in Defining Microbial Species

There are numerous challenges involved in defining a microbial species. To list a few:

- Many of the world's microbial species remain unclassified and the lack of knowledge limits the success of growing uncultured clades on standard media (Stewart 2012).
- Motivations of finding ways to classify microbial species – such as the drive to devise and deploy categories versus the desire to recognize and understand evolutionary groups – often create different views, which in turn leads to different definitions for microbial species (Doolittle and Papke 2006).
- Frequent events of HGT such as transformation, conjugation, and transduction in bacteria combined with genetic mutations result in various microbial species sharing similar genes (Trevors 1999).

The combination of HGT and genetic mutations alone poses an especially challenging problem when defining microbial species. The paper described how transformation evolved as a mechanism in DNA repair and genome preservation in bacteria and how conjugation and transduction evolved as different mechanisms to aid in the spread of genetic materials via plasmids and phages, respectively (Trevors 1999). Using transformation as a mechanism in DNA repair is not completely foolproof since genetic information cannot be transmitted over extended

periods of time without some changes to their contents via mutations – such as point mutations, insertions, and deletions – and/or recombination events (Trevors 1999). Combined with the rapid cell division of microbial species, HGT enables the rapid distribution of individual genes, or even entire metabolic pathways, among its descendants and other bacterial population and communities via conjugation and transduction (Trevors 1999).

Influence of HGT on the Global Biogeochemical Cycles

According to various sources, HGT played a vital role in the maintenance of entire metabolic pathways through time (Falkowski *et al*. 2009, Riley and Lizotte-Waniewski 2009, Pál *et al.* 2005, Trevors 1999). The evidence examined by these sources – such as gene order, distribution of metabolic processes, and detailed phylogenetic analyses – all demonstrated that individual genes and entire metabolic pathways were being horizontally transferred in response to changing environments (Falkowski *et al*. 2009, Pál *et al.* 2005). In eukaryotes, gene duplicates are still considered the main source of evolution (Pál *et al.* 2005). Thus, the theoretical models and systematic analyses from other studies (mentioned in Pál *et al.*) are mainly focused on the effects of gene duplication in relation to the evolution of metabolic networks in bacteria (Pál *et al.* 2005). However, Pál *et al.* and other papers argued that gene duplication may have played a significant role in the early evolution of metabolic pathways only and that HGT became the more dominant genetic mechanism that contributed to the recent expansion of the microbial metabolic pathways (Falkowski *et al*. 2009, Pál *et al.* 2005, Kechris *et al*. 2006).

The evidence presented above can be connected to Falkowski *et al.*'s observation of HGT's influence on the global biogeochemical cycles through time. It was noted that many of the major metabolic pathways were distributed among the individual Bacterial and Archaeal lineages (Falkowski *et al*. 2009). For instance, the dissimilatory sulfite reductases were found in modern sulfate-reducing d-proteobacteria, Gram-positive bacteria, and Archaea (Falkowski *et al*. 2009). In addition to that, the nitrogen genes such as nitrogenases, ammonia monooxygenases, and *nif* genes were also noted to be widely distributed among Bacteria and Archaea (Falkowski *et al*. 2009, Kechris *et al*. 2006). For larger pathways transferred by HGT, there was also evidence for transfers of large "superoperons" that encoded entire anoxygenic photosynthetic apparatus (Falkowski *et al*. 2009). The evidence provided in these papers demonstrates the influence of HGT on the maintenance of global biogeochemical cycles through time: By distributing the diverse biogeochemical reactions among different organisms living in different environmental conditions, HGT enables the survival of major biogeochemical pathways throughout the extreme environments of Earth's systems billions of years ago.

Necessity of a Clear Definition for Microbial Species

The necessity of having a clear definition of microbial species is vital in both the clinical and reference laboratory settings. Having a clear definition of microbial species in the clinical laboratory setting can mean the difference between life and death of a patient. In order to assist physicians in finding the appropriate treatments for patients, clinical laboratory scientists must be able to isolate, identify, and determine the antimicrobial susceptibility patterns of various human disease agents (Baron *et al*. 1996). Clinical laboratory scientists must also keep up with microbial name changes or identification of new microbial species and inform the clinical staff

via background information and a reference (Baron *et al*. 1996). In addition, having a clear definition of microbial species is also vital in maintaining a reference database in a cost-effective and efficient manner. A clear definition of microbial species can minimize the misidentification of atypical strains or rare or newly described species that are not present in the database (Baron *et al*. 1996). This may also help in the categorization of microbes by optimizing the use of specialized tests. Using the correct specialized tests – such as genetic probes, plasmid profiles, or DNA – may save time and resources in the maintenance of reference databases (Baron *et al*. 1996). Therefore, having a clear definition of microbial species will not only save countless patient lives, it will also be vital in the maintenance of reference databases as new microbial species are discovered.

There are a number of approaches that can be used to define microbial species. Using the phylogenetic approach, one can attempt to define species via comparisons of gene sequences in a given strain with gene sequences for all known species in the databases, but this requires a significant amount of computation (Baron *et al*. 1996). Instead, the total DNA of one organism can be used to define species with the basis of DNA relatedness using the following five factors: Genome size, G+C content, DNA relatedness under conditions optimal for DNA re-association, thermal stability of related DNA sequences, and DNA relatedness under conditions supra-optimal for DNA re-association (Baron *et al*. 1996). The advantages of using this approach are that this can be applied equally to all organisms and that it cannot be affected by phenotypic variation, mutations, or presence/absence of metabolic or other plasmids (Baron *et al*. 1996). Alternatively, the standard approach would be the polyphasic approach, which involves the phenotypic grouping of strains by their morphological, physiological, and biochemical features (Baron *et al*. 1996, Prakash *et al*. 2007). However, the integration of additional parts is required – such as the use of complete 16S rRNA gene sequencing and DNA-DNA hybridization studies with related organisms – since these parts are not foolproof nor applicable in some situations (Prakash *et al*. 2007, Janda and Abbott 2007).

The challenges involved in defining microbial species are often due to our lack of knowledge on the unclassified microbial species and our different views in classification of microbial species. However, HGT complicates this significantly by enabling microbial species to distribute individual genomes and/or entire metabolic pathways among different Bacteria and Archaea lineages on a global scale through time. Even though HGT complicates the process of defining a microbial species, they also played a vital role in the maintenance of global biogeochemical cycles through time. The different types of HGT played significant roles in maintaining the major genes or metabolic pathways from mutations accumulated through time (Transformation) and acted as mechanisms of transfer of genetic materials between other microbial species (Conjugation and Transduction) during environmental changes. Finally, it is necessary to clearly define microbial species in a clinical and reference laboratory settings to find optimal treatments for patients and to maintain references in databases for research consistency and efficiency, respectively. An optimal definition would involve a mix of the phylogenetic approach with the current polyphasic approach for improved accuracy in defining novel microbial species. Without a clear definition of microbial species, research into novel microbial species may be delayed indefinitely and countless patient lives may be lost from ambiguous microbial species definitions as more unclassified microbial species are identified.

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