**Prompt:** 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?

Defining a species at the microbial level is difficult given their relatively small genome size, which can range from a mere 0.2 to 13.0 megabases, and the advent of Horizontal Gene Transfer (Koonin and Wolf, 2008). Using *E. coli* as an example, the sequence similarity required to be classified the same species has been shown to vary greatly, complicating the definition of a species (Welch et al.). Horizontal Gene Transfer can also complicate the phylogenetic relationships between organisms as the relationship can be incongruent (Philippe et al.). As prokaryotic genome sizes are small, they are under evolutionary pressure to lose genes to be more streamlined (Morris et al.). These genes that are lost from the organism are not lost from the community , since they can be transferred to other organisms via Mobile Genetic Elements (MGE) that could be incorporated into another organism’s genome (Oliveira et al.). These changes in gene composition in microbial genomes through Horizontal Gene Transfer can be accumulated over time with environmental evolutionary pressures and can change the biogeochemical landscape of ecosystems as the cycling genes are gained and lost (McDaniel et al.).

Given that Horizontal Gene Transfer can largely alter the smaller genome of the different strains of a microbial species as seen in *E. coli*, there is the challenge of having a clear definition of a microbial species where their genomes are quite distinct from each other. As mentioned in Welch *et al.*, 2002, the sequence similarity between the three *E.coli* strains was 39.2%, which is significantly lower than the 99.5% sequence similarity between humans (Levy et al. 2007). As different strains contain various pathogenicity islands that can change the disease potential of a strain (Welch). Horizontal Gene Transfer has been suspected due to changes in codon usage frequencies and drastically different genes were found in different islands that are inserted at certain regions in the genome (Welch). This can create a big change in the genome length depending on what is inserted or substituted, which can reduce the similarity between the different strains of *E. coli*. With varying genome lengths within the same species, it complicates the process of defining the criteria for a prokaryotic species.

The mechanisms that can be used by Horizontal Gene Transfer include transformation, conjugation and transduction, in which Mobile Genetic Elements (MGEs) help facilitate. MGEs can include plasmids, bacteriophages, transposons and Integrative conjugative elements that can move DNA from one spot to another on the genome or to other organisms that may or may not be closely related (Oliveira et al., Frost et al.). This creates an avenue for Horizontal Gene Transfer to occur in the community. Prokaryotes use an operon to organize the genes, which are highly conserved, but the more you move downstream from them, the more likely introduced genes are to attach (Oliveira et al.). This creates hotspots for new genes to be integrated to the genome whether it be through prophages or the integrative conjugative elements that can be several hundred bases long, and can be self managed based on genes encoded.

Phylogenetic relationships can also introduce a source of confusion, whereby a gene can jump from branch/leaf to other, which can distort the phylogenetic relationship in a small manner. This can have an effect on the inferences that come from these new branches/leaves and can cause them to become congruent since a gene may have changed due to Horizontal Gene Transfer and no longer corresponds to what worked before (Phillipe et al.). This can create a bias due to evolutionary pressure since only a few samples were taken. Although they are in a tree and are connected to each other, even if they have sequence similarity, doesn’t mean they have the same metabolic pathways due to Horizontal Gene Transfer and incongruent sequences (Philippe et al.).

Horizontal gene transfer can also occur in oceanic environments, where selective pressures force the prokaryotes to produce DNA in response to changing environmental conditions that can affect the survival of prokaryotes (McDaniel et al.). As genes are accumulated over time and/or are more expressed in the environment, Horizontal Gene Transfer can cause insertions and substitutions that could increase the function or introduce a new one. To gain a fitness advantage Horizontal Gene Transfer can insert into the genome, but there is nothing that can be done at deeper waters. However, this should come at the cost of other genes that are not really used given that acceptor organisms will have a smaller genome (Morris et al.). Also selecting for certain genes allows the genome to be more streamlined, which can conserve limiting resources in the ocean (Morris et al.).

Over time, when the new, horizontally acquired genes accumulate, new pathways emerge and only the ones that can adapt or live in these conditions will remain. These changes in the biogeochemical cycling will happen over longer time scales as these processes are often on a larger scale (Morris et al.). Also, as the genomes of the organism will change due to Horizontal Gene Transfer it would have to withstand the test of time to be conserved at the species level.

Given the possible drastic changes in genetic makeup within species, it may seem difficult and impossible to determine a single definition for a prokaryotic species. However, if a set of criteria that can reliably find the conserved genes across all strains within a species, then that could be used to clearly define a microbial species. As long as two organisms share a certain amount of sequence homology, then they can classified as the same species, like 39.2% for *E.coli* (Welch et al.).

Overall, the genetic shuffling within the genome as performed by mobile genetic elements can cause big changes that can change the genome size and the functions as well. These changes can happen at hotspots, where higher frequencies of insertions occur, but conserved sequences are still there. When more genes are inserted through Horizontal Gene Transfer, this decreases the sequence similarity threshold needed to be called the same species as long as it has the right amount of conserved sequences. This increases the complexity in identifying the species definition due to the high within-species variability when dealing with Horizontal Gene Transfer and while it is useful to have a clear definition of a prokaryotic species, it may not be feasible with many possible exceptions to the rules and all the possible changes in genomes associated.

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