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Phylogenetic Studies of Newly Isolated Freshwater Magnetospirilla Using \textit{cbb} and \textit{mam} Genes

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Abstract

The phylogeny and relatedness of prototrophs is determined by comparison of \textit{cbb} and \textit{mam} gene sequences. Comparisons between other gene sequences have been used for this purpose and some have been used for \textit{cbb} and \textit{mam} gene sequences (Jolley, 2010). However, \textit{cbb} and \textit{mam} gene sequences have not been used in this way. The purpose of this study was to determine phylogenetic relatedness of a large number of newly isolated freshwater magnetospirilla using sequences from \textit{cbb} and \textit{mam} genes and \textit{cbb} and \textit{mam} gene sequences to compare these results to those from sequence analysis of the \textit{cbb}, \textit{cbb}, and \textit{mam} genes.

Methods

Isolation of new strains of magnetospirilla. Magnetically purified MTB from various water samples were inoculated into a semi-solid D-glucose enrichment medium similar to that described by Bazylnski et al. (2004) except that the medium was designed for non-aerobic \textit{Gammaproteobacteria}. Differentiation of the resulting bacterial colonies was used to identify 13 \textit{Magnetospirillum} and \textit{Magnetotacticum} strains. Phylogenetic relatedness of the \textit{cbb} and \textit{mam} gene sequences was determined using the BioEdit sequence alignment editor. The phylogeny of 

Results (cont.)

Table 2. Presence of \textit{cbb}, \textit{cbb}, \textit{mam}, and \textit{mam} genes in strains of magnetospirilla as determined by PCR. A negative result does not mean the gene is necessarily absent; it could mean that the sequence of the gene is different enough that the degenerate primers used are not effective. ISS-2 and BW-2 are \textit{Gambacottiacoccus} MTB used as positive PCR control for \textit{cbb} and \textit{mam} genes.

Table 1. Degenerate PCR primers used for the amplification of 16S \textit{RNA}, \textit{cbb}, \textit{cbb}, \textit{mam}, and \textit{mam} genes.

Table 3. Phylogenetic tree of magnetospirilla based on \textit{cbb} and \textit{mam} gene sequences. Red = new \textit{cbb} and \textit{mam} genes; black = \textit{Magnetotacticum} species; orange = \textit{MTB} and blue = magnetospirilla. Bootstrap values at nodes are percentages of 1,000 replicates. Bar represents 5% sequence divergence.

Discussion

Phylogeny and relatedness of magnetospirilla based on \textit{16S} rRNA \textit{gene} sequences. The current standard method for determining phylogenetic relatedness of prototrophs is by comparison of \textit{16S} rRNA \textit{gene} sequences. This analysis was performed on a number of newly isolated MTB with the \textit{ ENTEROBACTERIACEAE} and \textit{Gammaproteobacteria}. The results of the analysis show that most of the strains belong in the \textit{Magnetospiron} family, either as new strains of existing species (sequence identity >97%) or a new species (97% to 96%). When the data is taken in its entirety, magnetospirilla appear to be phylogenetically more related to each other than either to an \textit{MTB} or \textit{MTB}. This may be due to the presence of an \textit{MTB} in each strain. The data also show that the \textit{MTB} should be reclassified and included in \textit{MTB}

Future Directions for Research

In this study, we have shown some interesting genetic trends. However, before major conclusions can be drawn from the data, some additional \textit{PCR} and sequencing reactions must be completed. Because it is unlikely that magnetospirilla lack \textit{16S} rRNA \textit{gene} sequences, this may be due to the presence of \textit{MTB} in each strain. The data also show that the \textit{MTB} should be reclassified and included in \textit{MTB}.

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References


