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There is evidence that L. ferrooxidans is also capable of fixing atmospheric nitrogen. Genomic DNA from the L. ferrooxidans type strain was reported to give a positive hybridization signal with a nifHDK gene probe from Klebsiella pneumoniae. L. ferrooxidans was also shown to reduce acetylene to ethylene and oxidize ferrous iron to ferric iron at low oxygen concentrations. This ability was repressed by added ammonium ions, which is indicative of the ability to fix nitrogen. The ability of T. thiooxidans to fix nitrogen is uncertain. No hybridization signal was obtained when a nifHDK gene probe from Klebsiella pnuemoniae was used against chromosomal DNA from T. thiooxidans ATCC 8085, but a positive signal was obtained when a T. ferrooxidans nifHDK probe was hybridized to an unidentified T. thiooxidans isolate. The role of nitrogen fixation in bioleaching operations is difficult to predict. The dissolution of atmospheric ammonia in acid solutions could provide sufficient ammonium to suppress nitrogen fixation.Furthermore, nitrogen fixation is inhibited under fully aerobic conditions therefore might not occur in a well-aerated leaching operation. In the highly-aerated, high oxidation rate, BIOX tanks used to pretreat gold-bearing arsenopyrite ores, addition of a small amount of ammonia in the form of low-grade fertilizer is required to enhance mineral oxidation. 1.5 Energy Sources 1.5.1 Iron oxidation As stated earlier, the energy requirements for growth of both T. ferrooxidans and L. ferrooxidans are able to be met by the oxidation of ferrous to ferric iron under aerobic conditions.@ Work by Blake and colleagues on the components of iron oxidation in acidophilic bacteria has revealed that the ability to oxidize iron appears to have evolved several times. At least four unique iron-oxidation mechanisms exist.Two of these mechanisms are found in the mesophilic acidophiles. The pathway for iron oxidation in T.ferrooxtdans is characterized by the presence of large amounts of the small copper protein, rusticyanin and c-type cytochromes. Rusticyanin is not detectable in L. ferrooxidans or in any of the moderately or extremely thermophilic iron-oxidizers. A novel red cytochrome (cytochrome 579) which is clearly different from cytochrome a-, b-or c-type hemes and not found in the other iron-oxidizers, dominates the electron transport chain of L. ferrooxidans. This unique cytochrome was redox active with ferrous sulfate. The components of the iron-oxidation pathway in T. ferrooxidans have been relatively well studied.These are a 92 kDa membrane porin, an Fe ( II ) oxidase, cytochrome C55v rusticyanin and a cytochrome c oxidase of the aa3-type. All the above components have been isolated and characterized, the amino acid sequence for rusticyanin has been determined and gene for the Fe () oxidase have been cloned and sequenced. The exact order of the components and particularly, the position of rusticyanin in the passage of the electrons is uncertain. In a recent review it has been postulated that the role of rusticyanin is to broaden the electron pathway from cytochrome C552 to the cytochrome oxidase as illustrated below.

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