ABSTRACT

Bioleaching can be defined as an hydrometallurgical dissolution process assisted by microorganisms for the recovery of metals from their ores or concentrates usually from their insoluble form. Bioleaching places the metal of interest in the solution phase during oxidation. Microorganisms are also used as successful agents in cleaning up metal polluted environments from various sources. These concepts of the usefulness of microbes has been exploited in the present study using acidophilic iron oxidizing bacteria *Thiobacillus ferrooxidans* and *Serratia marsescens*. Though the nutritional requirement of these two organisms are different, they definitely do share some common properties at the molecular level in terms of their metal resistance proteins and blue copper proteins. Some of the properties are that *Serratia* sp are also found in acidophilic habitats and tolerate low levels of pH in growth medium. The mercuric reductase gene of *Thiobacillus ferrooxidans* and *Serratia marsescens* share similarity. The chromate resistance protein of the bacteria *Thermoplasma* share similarity with *Pseudomonas* sp. The rusticyanin protein of *Thiobacillus ferrooxidans* and two proteins copA and copC in *Serratia* are blue copper proteins. With the genomic sequencing being partially complete for both *Thiobacillus ferrooxidans* and *Serratia marsescens*, from microbiological point of view the metal resistance and metal leaching abilities of these two organisms were chosen as the topic of the work.

The isolation and culturing of microorganisms from soils or any other source is definitely not difficult. In conditions where microoganisms like *Thiobacillus ferrooxidans* grow only under highly acidophilic conditions, traditional plating methods poses a problem as agar does not solidify at low pH. A direct and simple plating technique was designed which is less cumbersome than the existing plate preparations for acidophilic bacteria. In addition, a colored plate assay was designed which would support only the growth of iron oxidizing acidophiles and detect them based the ferro-ferric ion complex formation. The time course of growth of microorganisms can be monitored from the optical density measurement in most of the

cases, but in the case of acidophiles due to their extremely low biomass, an indirect measurement of growth has to monitored. Since the iron oxidizing acidophiles oxidize ferrous to ferric during the growth of the organism a technique called the ferron assay was designed successfully and it has proven to be less cumbersome and more stable for the detection of the ferric formed.

Rusticyanin in *Thiobacillus ferrooxidans* is suppose to be the first electron acceptor in the electron transport chain. Blue copper proteins are present in both prokaryotic and eukaryotic sources. Owing to vast amount of sequences deposited in the databases, data mining for a particular kind of protein becomes more tedious. The broad range signature already present in the databases for blue copper protein does not mine out any one kind of blue copper protein. Keeping the concept of utilizing specific primers rather than random primers to amplify a particular sequence from the DNA of any organism, functional protein signatures for each kind of a blue copper protein from different species using online databases was designed. We have designed sixteen specific functional protein signatures and it has proved to be better in mining out specific blue copper proteins sequences.

Serratia sp has also been reported to be one of the organisms found in acidophilic habitats such as in acid mine drainages. The *Serratia marsescens* isolate used for this work was isolated from the soil. There are few references to identify the metal resistance characteristics of *Serratia marsescens*. Before going in to study the effect of metals, *Serratia marsescens* used in this study being an isolate from soil, the concept of developing a cheap and novel medium, for the dual purpose of the enhanced growth of the organism and the enhanced production of the pigment was met with success. The pigment prodigiosin is medicinally known for its antiproliferative and antibacterial activity. The peanut medium in water showed enhanced pigment production of ~ thirty nine mg/ml over the nutrient broth which was less than two mg/ml. The pigment production was uniform irrespective of a range of pH from four to ten. The temperature did show a regulation in pigment production as already reported, but the concept that the temperature regulation varies with medium and the

nutritional substrates is shown for the first time. On comparing the different substrates used it was concluded that the fatty acid did play a major role as a substrate for the organism.

Peanut on its own contains few of the metals as reported earlier. The metals chosen for the study were the commonly used alkaline earth metals, alkali metals and transition metals in the microbiological mediums. At a concentration of 0.2 mg/ml only four of the metals namely nickel, copper, cobalt and chromium showed a complete block on the pigment production but not on the growth of the organism the rest of the metals did not show any block on the pigment nor on the growth of the organism. The minimum metal concentration to block the pigment production and the maximum tolerance limit was studied. The effect on protein biosynthesis in the presence and absence of the metal was studied.

In all the three cases of study of temperature, nutrition and metals regulating the pigment production, a ~94 kDa protein was found to play a major in the regulation of the prodigiosin pigment production which has not been reported so far in *Serratia marsescens*. A part of the high molecular weight protein of *Serratia marsescens* was amplified using the primers designed for the first 900 base pairs of *Streptomyces coelicolor*. With *Serratia marsescens* plasmid sequence having been completely sequenced, copper and chromium resistance proteins from other sources showed similarity with certain sequences in the plasmid sequence of *Serratia marsescens*. The blue copper protein rusticyanin from *Thiobacillus ferrooxidans* and copA and copC protein from *Serratia marsescens* was found to have high homology when aligned using the Clustal W software.

Owing to the multifaceted property of prodigiosin, the pigment was used for the first time to dye plastic, textile and leather. Plastic was found to be dyed uniformly and amongst the cloth bits used the socks material was found to dye more efficiently. Both sides of chamois leather was dyed pink in color.