the cross-reactive material is a dimer containing a subunit identical in electrophoretic mobility to subunit I of the seed lectin and another subunit of higher molecular mass than the seed lectin subunit. The subunits may represent different degrees of completion or modification of a common polypeptide chain. Leaf and seed lectins that exhibited similar properties have also been identified from *Griffonia simplicifolia* and *Phaseolus vulgaris*.

Leaves and flowers of *Sophora japonica* also contain galactose lectins.

Seeds of only a few legumes have been known to contain two distinct sugar-specific lectins. *D. lablab* seeds contain two lectins with distinct sugar specificities. The results obtained in this study clearly demonstrate that the stems and leaves of the *lablab* bean seeds contain strong agglutinating activities that can be inhibited by galactose and lactose. The general properties of the galactose-specific seed lectin and the stem lectin are similar and possibly the stem and leaf lectin represents the dimeric form of the tetrameric galactose specific seed lectin, as the native molecular mass of the stem and leaf lectin is about half that of the native molecular mass of the galactose-specific seed lectin. The fact that the antibody to the mannose-specific seed lectin cross reacts with the galactose-specific seed lectin as well as with the stem and leaf lectin suggests that the lectins have related antigenic sites. Additional evidence for this comes from the fact that the galactose-specific seed lectin antibody also cross-reacts with the mannose-specific seed lectin (data not shown) and with the galactose-specific lectin from stems and leaves. Further, while the seeds express both the mannose and galactose-specific lectins, the stems and leaves seem to contain predominantly galactose-specific lectin only. Considering the experimental data on the binding affinities as well as the physico-chemical and biological properties of the galactose-specific seed lectin and the stem and leaf lectin, it is evident that both lectins are unusual and related. Our future work aims at understanding the structural similarities of the two galactose-specific lectins as well as the mannose-specific lectin.


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**Colony morphology mutants of chemolithotrophic *Acidithiobacillus ferrooxidans* are associated with altered genomic distribution of family 1 repetitive DNA sequence**

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Two classes of colony morphology mutants, large spreading and non-spreading small colonies, were isolated from *Acidithiobacillus ferrooxidans* in thiosulphate–agarose and tetrahydrothiophene–agarose medium, respectively. The spreading colony morphology mutants exhibited an increased chemotaxis toward thiosulphate compared to the wild type or the non-spreading mutants. Inward primers designed from the ends of two distinct families of repetitive DNA elements of *A. ferrooxidans* produced PCR amplicons of 1.3 and 1.4 kb, respectively, from both the wild type as well as mutant strains. Restriction fragment length polymorphism compared

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between the genomic DNA of wild type and mutant strains showed altered distribution of family 1 repetitive DNA element in the genome of the mutants. The possibility of transposition of repetitive DNA element resulting in the mutation and genome rearrangement within a population of *A. ferrooxidans* cells is discussed.

BACTERIAL genome generally contains insertion sequences (IS) which can promote spontaneous mutations and DNA sequence rearrangements that may lead to genetic and phenotypic diversity and could play an important role in evolution of strains in a bacterial population. Another striking feature of the IS element is the frequency of transposition that varies significantly depending on the culture conditions as well as growth phase. IS elements also serve as novel biological switches capable of switching off or on the expression of nearby genes as a consequence of insertion or excision. Since these elements are inherited, strains that share a recent common ancestor would also tend to share some or all the sites at which insertion sequences are located in the genome. Therefore, the distribution of members of insertion sequence(s) in the genome is also of some interest in understanding the population dynamics of the elements and species-specific IS element may be used for interspecies strains differentiation.

*Acidithiobacillus ferrooxidans* (formerly *Thiobacillus ferrooxidans*) is a Gram-negative, chemolithoautrophic, acidophilic bacterium that obtains energy from the oxidation of ferrous iron or inorganic sulphur compounds. The bacterium oxidizes sulphur compounds to *SO\(_4^{2-}\)* and *Fe\(^{3+}\) to *Fe\(^{2+}\)* that cause metal leaching from sulphide minerals. This microbial metal-leaching process has tremendous potential in bio-hydro-metallurgy for the recovery of copper, uranium and gold. The traditional bacterial genetic techniques are hard to implement in *A. ferrooxidans* for its extreme acidophilic and obligate lithoautotrophic characteristics. Consequently, only a little progress has been achieved in the genetics or molecular biology of this bacterium and sulphur lithotrophy in particular. However, a complete genome sequence programme has been undertaken, and a gapped genome sequence which identified 2712 potential genes in 2.6 Mb genomic sequence of *A. ferrooxidans*, has recently been published. The genome of *A. ferrooxidans* contains two distinct families (family 1 and family 2) of repetitive DNA elements. ISf7e1 and ISf72, cloned from *A. ferrooxidans* ATCC19859, represent family 1 and family 2, respectively, and are shown to exhibit the characteristics of a typical prokaryotic insertion sequence. Our laboratory has cloned and sequenced a copy of family 1 repetitive DNA sequence; designated ISf7445 (ref. 14), from *A. ferrooxidans* NCIB8455, which bears extensive sequence homology with ISf7e1.

Colony formation of *A. ferrooxidans* in agar plate is very difficult because of high acidity of the medium. However, low concentrations of purified agar or agarose were recommended in a specific medium containing ferrous sulphate that could be effective to develop colonies after a long period of incubation. On the other hand, large spreading colony (LSC) morphology mutants were selected when plated on an agarose medium containing two oxidizable substrates, iron and thiosulphate. The authors claimed that these mutants had lost the iron-oxidizing activity. Subsequently, it was shown that in one of the LSC strains, a copy of family 1 element was mapped in a gene of cytochrome *c* biosynthesis that seemed to be essential in iron oxidation by *A. ferrooxidans*. In the present study, we demonstrated altered pattern in the distribution of family 1 repetitive element in the genome of colony morphology mutants compared to the wild type *A. ferrooxidans*.

* A. ferrooxidans ATCC13728, ATCC19859, NCIB8455, DSM583, and AS1.5 [a strain isolated in our laboratory from a culture (a gift from Dr G. R. Chaudhury, Regional Research Laboratory, Bhubaneswar, enriched from an acid mine water of Indian origin)] were clonally purified on ferrous-sulphate-agarose and maintained in mineral salts media containing ferrous sulphate or elemental sulphur. The colony morphology mutants of NCIB8455, ATCC19859 or AS1.5 were isolated in the following medium: 200 ml of solution A containing (NH\(_4\))\(_2\)SO\(_4\) 3.0 g, MgSO\(_4\) \(_7\)H\(_2\)O 0.5 g, CaCl\(_2\) 2H\(_2\)O 0.25 g, Na\(_2\)S\(_2\)O\(_3\) 5H\(_2\)O 5.0 g adjusted to pH 4.0 with 1(N) H\(_2\)SO\(_4\) and autoclaved at 10 lb/in\(^2\) for 10 min, was added to 800 ml of solution B containing 7.0 g agarose separately autoclaved at 15 lb/in\(^2\) for 15 min. Tetrahydrosulphide-agarose medium was prepared by mixing solution A (containing K-tetrathionate, 5.0 g instead of thiosulphate) with 780 ml solution B and lastly adding filter-sterilized 20 ml solution of sodium sulphide (0.5 g in distilled water). Oxidation of ferrous iron and sulphur compounds by cell suspensions was determined in an oxygen electrode cell (Yellow Springs Instrument Co, Inc, Ohio) of 3.0 ml working volume with constant temperature control at 30°C (refs 17, 18). Chemotaxis was assayed by a modification of Adler's method described earlier. A microfuge tube (1.5 ml) contained 100 µl of the attractant solution, ranging from 8 to 40 mM of thiosulphate. The cell suspension was taken in a capillary tube and one end of the filled capillary was introduced into the microfuge tube. After a definite period of incubation at 30°C, the cells, which had accumulated into the microfuge tube, were counted in a Neubauer improved counting chamber. Genomic DNA was prepared as described by Yates and Holmes. The DNA digested with restriction endonuclease HindIII was resolved in 0.8% agarose gel in Tris-acetate-EDTA buffer (TAE) followed by vacuum transfer onto nylon
To determine the distribution pattern of family 1 repetitive element among the host genome, endonuclease-restricted genomic DNA of wild type and mutants was probed with family 1 repetitive element. The probe (Figure 1) was prepared in a polymerase chain reaction (PCR), using the following primer pair: F1 (5'-GGCTCTCTCTGCGATAGA-3', nucleotides 1–18 of IST445) and R1 (5'-GGCTCTCTGCTATTTTCAG-3', nucleotides 1202–1219, complementary of IST445); EMBL accession no. Y1830914. The PCR reactions were performed using ELONGase (Gibco/BRL) with 10 ng genomic DNA in 50 μL containing 200 μM each of deoxynucleoside triphosphates, 500 nM each of the primer and the buffer with 1.5 mM MgSO4. The reactions were programmed as follows: 94°C for 5 min and then subjected to 30 cycles each consisting of 30 s at 94°C, 30 s at 50°C and 3 min at 68°C, and finally at 68°C for 7 min to complete the primer extension. Non-radioactive labelling, hybridization and detection were performed as recommended by the manufacturer (BioNick labelling and Blue-Gene detection system, BRL, USA) and as described earlier1,2.

Thiosulphate–agarose or tetrahionate-sulphide-agarose did not allow wild type cells to form colonies, but were selectively effective in allowing the development of two kinds of specific colony morphology mutants within A. ferrooxidans population at a frequency of 10−7 to 10−8 cell−1 after about 10 days of incubation. The colonies started appearing on thiosulphate-agarose as white dots that spread as cloudy white layers and continued to cover the entire surface (spreading phenotype; Figure 2a) of a petri dish on further incubation. The colonies on tetrahionate-sulphide-agarose were creamish, small, round-shaped with an average diameter of 1.5–2.0 mm (non-spreading phenotype; Figure 2b). The spreading or the non-spreading colony morphology mutants grew like wild type in ferrous sulphate containing broth or ferrous-iron-agarose plates. The rates of ferrous-iron oxidation by wild type and the mutants were similar (Table 1). This observation does not comply with those of Schrader and Holmes15 who claimed that the spreading phenotype was accompanied with the loss of iron oxidation. The rate of thiosulphate or sulphur oxidation by the non-spreading mutants was higher compared to the wild type or the spreading mutants, but the spreading mutants were found altered in sulphide oxidation activity (Table 1). It is possible that the mutants might be distinct in the oxidative activity towards sulphur (Table 1), but more studies would be required to link the effect of such variation in the sulphur lithotrophic properties with the colony morphology phenotype of the mutants.

Table 1. Oxygen uptake in presence of iron, elemental sulphur, thiosulphate and sulphide by sulphur-grown cells of wild type and colony morphology mutant strains of A. ferrooxidans

<table>
<thead>
<tr>
<th>Strain</th>
<th>Elemental Iron</th>
<th>Sulphur</th>
<th>Thiosulphate</th>
<th>Sulphide</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCIB 8455</td>
<td>75 (8)</td>
<td>221 (23)</td>
<td>115 (15)</td>
<td>65 (5)</td>
</tr>
<tr>
<td>RC1000</td>
<td>80 (10)</td>
<td>225 (22)</td>
<td>166 (16)</td>
<td>115 (11)</td>
</tr>
<tr>
<td>RCM1</td>
<td>85 (10)</td>
<td>396 (12)</td>
<td>200 (20)</td>
<td>65 (6)</td>
</tr>
<tr>
<td>ATCC 19859</td>
<td>ND</td>
<td>221 (13)</td>
<td>114 (5)</td>
<td>66 (8)</td>
</tr>
<tr>
<td>RC2000</td>
<td>80 (15)</td>
<td>220 (24)</td>
<td>79 (15)</td>
<td>150 (25)</td>
</tr>
<tr>
<td>RC201b</td>
<td>80 (10)</td>
<td>200 (16)</td>
<td>13 (4)</td>
<td>39 (7)</td>
</tr>
<tr>
<td>RCM2b</td>
<td>ND</td>
<td>400 (18)</td>
<td>180 (20)</td>
<td>64 (5)</td>
</tr>
</tbody>
</table>

*Oxygen uptake was estimated with cell suspension (equivalent to 300 μg protein) as described in the text. Reaction mixture contained 50 μmol ferrous sulphate or 100 μg elemental sulphur or 50 μmol thiosulphate or 10 μmol sulphide. Standard deviation for three independent experiments is given in parentheses.

*RC100 (spreading mutant) and RCM1 (non-spreading mutant) were selected from NCIB 8455; RC200 or RC201 (spreading mutant), and RCM2 (non-spreading mutant) were selected from ATCC 19859.
transferred to a thiosulphate-agarose plate from a spreading zone of a colony, the colony size reached an average diameter of 2.5 cm within only 5 days of incubation, when the rate of increase of colony diameter was 4.16 μm min⁻¹. Development of swarm colony in Proteus has intrigued many microbiologists to study morphogenetic processes in bacteria²²,²³. These studies suggest that colony growth and expansion, a multicellular and cooperative event, is a dynamic process involving movement over the solid surface of specially differentiated cells. Cellular morphology, motility, rate of colony expansion and colony morphology of the spreading mutants of A. ferrooxidans, described in the present report, resemble the swarm colony development of Proteus. In an earlier study, we reported chemotaxis toward thiosulphate by A. ferrooxidans and showed that the chemoreception of thiosulphate was constitutive because the cells grown in iron, sulphur, thiosulphate or tetrathionate exhibited similar chemotactic activity¹⁸. It is interesting to note that the sulphur or iron-grown cells of the spreading mutant, RC100, exhibited an increased chemotaxis compared to the wild type or the non-spreading mutants and responded maximally at 20 mM (Figure 3) which was equal to the concentration of thiosulphate used in a solid plate. The spreading phenotype of the mutants may result from enhanced chemotaxis that ultimately manifested as an additional selective advantage to colonize on the thiosulphate-agar plate. We showed²⁴ that adhesion to a sulphide agar plate, chalcopyrite, was significantly less with several chemodeficient mutants than the wild type A. ferrooxidans NCIB8455. Further, in a copper leaching process from sulphide ore, cells of A. ferrooxidans were found attached to thin section of ore, but in the areas containing copper sulphide only²⁵. So, the detailed characterization of the colony morphology mutants, including the colonization on sulphide ores associated with metal-leaching activity should be worth pursuing for both academic and biotechnological interest.

A. ferrooxidans genome contains 10 to 20 copies of each of the two families of repetitive DNA¹¹,¹⁴ which constitute more than 5% (ref. 11) of the total genome of A. ferrooxidans. Inward primers designed from the ends of IS1445 or IS172 were successfully used in PCR to generate amplicons of 1.3 or 1.4 kb corresponding to family 1 and family 2 repetitive DNA elements, respectively, and no detectable differences in the restriction analyses among the PCR amplicons (generated from wild type strains or colony morphology mutants) were noted (data not shown). Thus, the DNA sequence in family 1 and family 2 repetitive DNA elements in the genome of A. ferrooxidans should not be considered polymorphic in nature.

Strain-specific distinctive RFLP pattern was evident in an earlier study¹⁴, among the independent natural isolates of A. ferrooxidans, when genomic DNA was probed with IS1445 or IS172. This is further extended in the present study when RFLP pattern of AS1.5 was compared with wild type strains NCIB8455 and ATCC19859 (compare lane 1 or lane 5 of Figure 4a with lane 1 of Figure 4b). There are several examples where the genomes of independent strains of the same species exhibit significant multiple insertion/deletion differences due to integrated accessory elements (e.g. mobile IS elements), which can contribute to individual genome differences²⁶. Additionally, IS elements can make the genomic rearrangements due to their recombination potential²⁷. However, such variation in RFLP may also be contributed by random sequence polymorphisms in the genome.

Unlike extrachromosomal replicons (e.g. plasmids), IS elements do not have autonomous replication function. They encode specific transposase protein, which can mobilize and insert an element in a new site of the genome. Nevertheless, distribution patterns of the IS elements along the host genome must be necessarily nonrandom, because their marked influence on target genes imposes positional constraints since the fitness of the host must be maintained²⁸. Thus, patterns of positional IS distributions within bacterial strains could be of considerable interest to follow their drift in the course of strain geniealogy. This would not only help to

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**Figure 3.** Chemotactic responses of A. ferrooxidans [wild type NCIB8455, the spreading colony morphology mutant (RC100) and non-spreading colony mutant (RCM1)] toward thiosulphate. Chemotactic assay¹⁷ is described in the text. Cell concentration, 10⁶ ml⁻¹; capillary diameter 0.5 mm; length 6.0 cm; incubation period, 15 min. △, sulphur-grown NCIB100; □, iron-grown NCIB100; □, sulphur-grown RCM1; ●, iron-grown RCM1; ●, sulphur-grown NCIB8455; ●, iron-grown NCIB8455. Accumulation without thiosulphate was 0.02%.
Figure 4. Southern blot analysis of HindIII restricted genomic DNA probed with family 1 repetitive element (Figure 1). a, Lane 1, Wild type NCIB8455; Lane 2, Spreading colony morphology mutant, RC100 (isolated from NCIB8455); Lane 3, Non-spreading colony morphology mutant, RCM1 (isolated from NCIB8455); Lane 4, Biotin labelled x HindIII; Lane 5, Wild type ATCC19859; Lane 6, Non-spreading colony morphology mutant, RCM2 (isolated from ATCC19859); Lanes 7 and 8, Spreading colony morphology mutants RC200 and RC201, respectively (isolated from ATCC19859). b, Lane 1, Wild type AS1.5; Lanes 2–5, Spreading colony morphology mutants AS1.5.1, AS1.5.2, AS1.5.3, AS1.5.4, respectively, isolated from AS1.5.

establish strain relationship, but also contribute to model evolutionary studies in limited fast-evolving systems.1,2 The present study showed alteration in the banding pattern in RFLP of the mutants from the respective mother strains (Figure 4a and b). The Dice coefficient (Sd) values (Sd = 2x,y/(n1 + n2)), where n1 is the number of hybridized bands present in both the sample pair, n1 and n2 are the total number of hybridized bands in the two samples, respectively3,27, calculated from RFLP and scored in pair-wise combination (data not shown) between a mother strain and the respective mutants or between two mutants originated from the same mother strain were much higher than the value scored between two mutants of different origins or that scored between two wild type strains. Thus, the DNA fingerprints of spreading and non-spreading mutants of NCIB8455 (Figure 4a, lanes 2 and 3) when compared with the corresponding spreading and non-spreading mutants of ATCC19859 (Figure 4a, lanes 6 to 8) confirm the distinct origin of the mutants. Likewise, similarity in the RFLP of the four spreading mutants, selected from AS1.5, indicates common origin of the strains, yet distribution of family 1 repetitive element among these mutants or between a mutant and AS1.5 was not identical (Figure 4b).

The new pattern of hybridization in the mutants suggests altered distribution of family 1 repetitive DNA element in the genome of the mutants compared to wild type that might have resulted from transposition of family 1 element. Evidently IS elements strongly influence the functions of their immediate genetic environment. Besides disruption of gene sequences, gene functions may be affected by promoter or terminator properties of IS sequences causing adjacent genes to be over-expressed, silenced or newly expressed in the case of cryptic genes28,29. The movement of IS element, in many instances, is influenced by environmental conditions, and has raised the primary question concerning whether the activation of transposition or excision of IS elements is a programmed response29. In Mycobacterium tuberculosis, reduced oxygen atmosphere was shown to induce transposition of indigenous IS6110 resulting in selection of a colony morphology mutant phenotype30. Similar observation of stress-induced transposition was also noted in other bacterial species. The wild type A. ferrooxidans could not grow to develop colonies on sulphur compounds–agarose plate (present study; ref. 16). This starvation or stressed condition to the cells may induce transposition of indigenous IS element resulting in the distinct RFLP pattern, including additional hybridized bands (compare lanes 6 and 8 with lane 5 of Figure 4a; or compare lane 3 with lane 1 of Figure 4b) observed in the genomic DNA of the mutants. It is conceivable that the colony morphology mutants possessed certain advantages of colonization on the surface of the sulphur compounds–agarose medium which was not suitable for wild type cells. In other words, transposition or excision (compare lanes 2–5 with lane 1 of Figure 4b) event might have caused certain mutation resulting in specific colony morphology mutant phenotype. Certain mutations, e.g. mutation in rpoS allow non-growing cells to survive under conditions in stationary phase culture and consequently select rpoS mutations in such a stress condition31.
The mutation leading to spreading colony with loss of iron oxidation phenotype and reversion to wild type (with iron oxidation phenotype) was thought to be phenotypic switching in A. ferrooxidans mediated by a programmed transposition and excision of IS elements. But the spreading phenotype described in the present study was not associated with loss of iron oxidation activity of the mutants. Moreover, any specific insertion or excision of IST445 associated with the mutant phenotype is yet to be confirmed. Characterization of the unique band(s) in RFLP in the mutants, not present in the respective mother strain, would help to elucidate the role of repetitive elements in the evolution of strains and stress-induced specific transposition event in particular. Through the inverse PCR technique using IST445-specific outward primer pair, we have cloned IS-flanked genomic region (from several distinct insertion sites) of a colony morphology mutant (unpublished). These clones will be characterized and hopefully would be of great value in predicting the function of the affected genomic region. Finally, we note that the development of colony morphology mutants described here or the phenotypic switching reported by Schrader and Holmes could be of a simple but very useful model to envisage the molecular mechanism of adaptive or nonrandom mutation, a phenomenon that challenges the dogma of spontaneous mutation.


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