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**REVIEW**

# SIDEROPHORE: STRUCTURAL AND FUNCTIONAL CHARACTERISATION – A COMPREHENSIVE REVIEW

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Plants and microbes have enormous importance in our daily life. Iron is said to be the fourth most abundant element in the earth's crust from soil, still many plants face problem in uptaking iron because it is found in insoluble form, which severely restricts the bioavailability of this metal. In response to this, microorganisms present in soil such as *Pseudomonas* sp., *Enterobacter genera*, *Bacillus* and *Rhodococcus* produce special iron carriers or iron-binding compounds called as 'siderphores' or 'siderochromes'. This paper is an attempt to review the importance of siderphores in enhancing plants' iron utilisation strategies, the mode of transport of siderophores along with iron across the memberane and depending on the difference in their chemical structure, functional moiety and their source of isolation of four different types of siderophore (hydroxamates, catecholates, carboxylates and siderophore with mixed ligand). Siderophore and their derivative have large application in agriculture as to increase soil fertility and as biocontrol for fungal pathogen. This review unlike other reviews includes (1) types of siderophore, (2) the structural difference amongst them, (3) siderophore biosynthesis, (4) transport mechanism, (5) the genetics of siderophore and (6) their efficacy in human life.

Key words: siderophore, phytosiderophore, catecholate siderophore, hydroxamate siderophores, carboxylate siderophore, transport of iron, rhizosphere bacteria

Siderophores are compounds from ancient Greek words, *sidero* 'iron' and *phore* 'carriers' meaning 'iron carriers'. These are low-molecular-weight (<10 kDa) iron-chelating compounds, produced by 'rhizospheric bacteria' under iron-limited conditions in order to enhance the plant growth by scavenging iron from the environment and making the mineral available to the cell near the root (Neilands 1952; Lankford 1973; Alexander & Zubererm 1991; Hider & Kong 2010; Maheshwari 2011; Ahmed & Holmstrom 2014). Iron is the major component for various vital functions (photosynthesis, enzyme cofactor, redox reagent, respiration, synthesis of nucleosides and amino acids) of the plant. Owing to deficiency of iron, various plants seem to rely on excretion of phytosiderophore (i.e. chelate compounds, common in grasses that sequester iron) by the roots and secretion of siderophore by group of microbes to facilitate the Fe complex uptake under iron deficiency conditions, which binds with high affinity for iron (Takagi 1976; Mino *et al.* 1983; Marschner *et al.* 1986; Neilands 1995; Kannahi & Senbagam 2014). It has been assumed that competition for iron in the rhizosphere is controlled by the empathy of the siderophores for iron (Loper & Henkels 1999; Bernd & Rehm 2008; Munees & Mulugeta 2014). Siderophores function as plant growth promoters (Yadav *et al.* 2011; Verma *et al.* 2011), biocontrol agents (Verma *et al.* 2011) and bioremediation agents (Wang *et al.* 2011; Ishimaru *et al.*

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2012), in addition to their valuable role in soil mineral weathering (Reichard *et al.* 2005; Buss *et al.* 2007; Shirvani & Nourbakhsh 2010). Alkaline soils are considered to be the potential inducers of iron deficiency in plants in spite of the presence of iron in high concentration in the soil, when the soil pH exceeds 6.5–7.0, the availability of iron in the soil is significantly reduced, whilst calcareous soils, which have high pH, decrease the affinity of plants for Fe and thus impede Fe uptake mechanism.

*Classification of Siderphores*

A great variation is seen in siderophore structure produced by many bacteria. Siderophores are generally classified on the basis of co-ordinating groups that chelate the Fe (III) ion. The most common co-ordinating groups are catecholates, hydroxamates and carboxylates (Ali & Vidhale 2013). A minority of siderophores have chemically distinct Fe (III) ion binding group, including salicylic acid, oxazoline or thizoline nitrogen. Some siderophores including pyoverdines are classified as 'mixed ligands' having co-ordinating groups that fall into chemically different classes (Table 1). Different types of siderophore were identified by electrophoretic mobility, spectrophotometric titration, proton nuclear magnetic resonance spectroscopy, mass spectrometry, acid hydrolysis and biological activity.

**1. Catecholate – siderophore**

Siderophore exhibiting phenolate or 2,3-dihydroxy benzoate (DHB) binding groups belong to the catecholate type of siderophore. Catechol, also known as pyrocatechol or 1,2-dihydroxybenzene, is an organic compound with the molecular formula $C_6H_4(OH)_2$. It is the orthoisomer of the three isomeric benzenediols. This colourless compound occurs naturally in trace amounts. In iron-limited medium, *Azotobacter vinelandii* produces three catecholate siderophores, namely, tricatecholate protochelin, the dicatecholate azotochelin and the monocatecholate aminochelin (Corbin & Bulen 1969; Page & Tigerstrom 1988; Cornish & Page 1995; Wittmann *et al.* 2001).

**1.1 Enterobactin**

This catecholate-type siderophore is a cyclic trimer composed of 2,3-dihydroxy-*N*-benzoylserine. Three molecules of the DHB-Ser formed undergo intermolecular cyclisation, yielding enterobactin. Enterobactin was the first tricatechol siderophore, isolated from *Escherichia coli*, *Aerobacter aerogenes* and *Salmonella typhimurium* (Ward *et al*. 1999). Enterobactin (Enterochelin) are produced by bacteria of the family Enterobacteriaceae, includes all strains of *E. coli* having high affinity for iron. *S. typhimurium*, *Klebsiella pneumoniae* and *Erwinia herbicola* are also known to produce enterobactin. Enterobactin is the strongest siderophore having the capacity to chelate iron even from the environment where concentration of iron is very low (Raymond *et al.* 2003). *E. coli* enterobactin has a molecular weight of 669 kDa, which exceeds the size limit for diffusion across the outer membrane. Therefore, enterobactin are synthesised with the help of active transport mechanism for transport and capturing iron (Hans *et al.* 2001; Gregory *et al.* 2012). In the year 1998, Cornish and Page observed that the catecholate siderophores were hyper-produced to offer chemical protection from oxidative damage catalysed by $O_2$ and Fe.

**2. Hydroxamate-type siderophores**

Ferrichrome-type hydroxamate siderophores are of special ecological interest because of their production by many soil fungi (Zahneret *et al.* 1963; O'Sulivan & O'Gara 1992; Schalk *et al.* 2011), including symbiotic ectomycorrhizal fungi, and their ability to mobilise iron in neutral and alkaline soils in which other naturally occurring compounds are ineffective as iron chelators because of competition from other metal ions (Cline *et al.* 1982). Experimental evidence indicates that hydroxamate siderophores can supply iron to some plant species. Hydroxamate siderophores are generally produced by fungi and belong to Zygomycotina (Mucorales), Ascomycotina (Aspergilli, Penicillia, *Neurospora-crassa*) and Deuteromycotina (*Fusarium dimerum*). Hydroxamate siderophores were mostly trihydroxamates, followed by dihydroxamates. Monohydroxamate nature was not shown by any of the reported fungal siderophores. Hydroxamate siderophores formed hexadentate, tetradentates and bidentate ligands. A good correlation was observed between hydroxamate groups and ligand property. Ferrichrome belonging to hydroxamate are either linear or crystal in structure. Certain ferrichrome deriva-

T a b l e 1

Structural, chemical and functional moiety of major bacterial siderophore

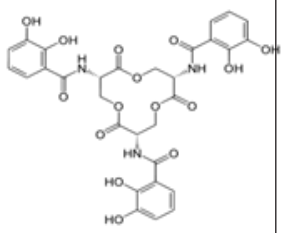
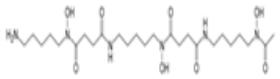
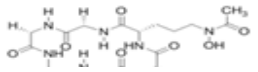
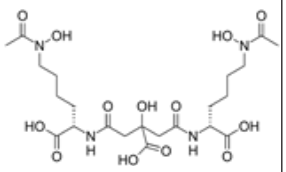
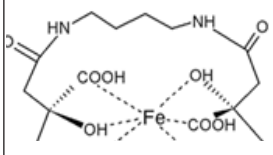
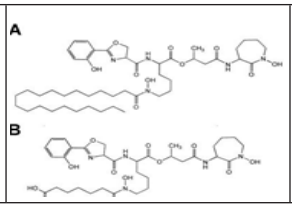
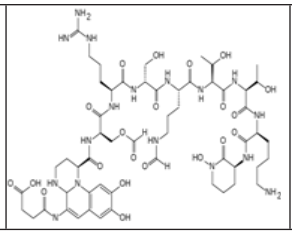
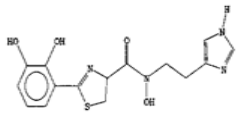
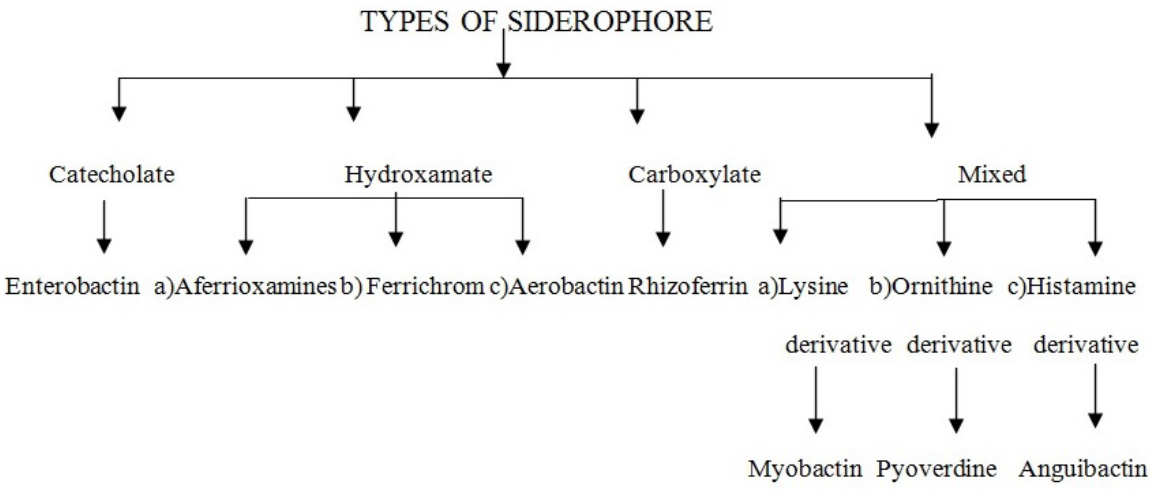
| | Type of Side-rophore | Structure | Chemical moiety | Function | Bacterial source | Reference |
|---|---|---|---|---|---|---|
| 1 | Catecholate: phenolate or 2,3-dihydroxy benzoate (DHB) binding groups | | | | | |
| | (a) Enterobactin |  | 2,3-dihydroxy -*N*-benzoylse-rine a cyclic trimer | Iron-chelating compound and used in agriculture | Family Ente-robacteriaceae, e.g. *E. coli* | Pollack & Neilands 1970; Walsh *et al*. 1990; O'Brien *et al*. 1970; Gregory *et al*. 2012; IUPAC, Commis-sion on Nomen-clature of Organic Chemistry, 1993 |
| 2 | Hydroxamate: esters or acid chlorides or carboxylic acids | | | | | |
| | (a) Aferrioxa-mines |  | Linear trihyd-roxamates | Used medically for the binding of excess blood iron in the treatment of thalsaemia | *Streptomyces* and *Nocardia* | O'Brien *et al*. 1970; Gregory *et al*. 2012; IUPAC, Commis-sion on Nomen-clature of Organic Chemistry, 1993 |
| | (b) Ferrichrome |  | Cyclic trihyd-roxamate | Growth factor for other mic-robes | Basidomyce-tes producing fungal species, e.g. *Ustilagos-phaerogena* | Jalal & van der Helm 1991; O'Brien *et al*. 1970; Gregory *et al*. 2012; IUPAC, Commission on Nomenclature of Organic Chemistry, 1993 |
| | (c) Aerobactin |  | A trihydroxy-tetraoxo tetra azatricosane-tri-carboxylic acid | Sequester iron in iron-poor environments such as the urinary tract | *Pseudomonas* of marine ori-gin, *Klebisella pneumonia*, *Aerobacterae-rogenes* | Buyer *et al*. 1991; Meyrier 1999; O'Brien *et al*. 1970; Gregory *et al*. 2012; IUPAC, Commis-sion on Nomen-clature of Organic Chemistry, 1993 |
| 3 | Carboxylate: hydroxyl carboxylate and carboxylates | | | | | |
| | Rhizoferrin |  | Diaminopropa-neacylated with citric acid via amine bonds to the terminal carboxylate of citric acid | Application in biotechnology: metal-binding properties and the ability to be easily degra-ded by various microorganism | Fungi, specifi-cally mem-bers of the zygomycetes | Winkelmann 1991; Stephan *et al*. 1996; O'Brien *et al*. 1970; Gregory *et al*. 2012; IUPAC, Commis-sion on Nomen-clature of Organic Chemistry, 1993 |

Table 1 continued

| | Type of Side-rophore | Structure | Chemical moiety | Function | Bacterial source | Reference |
|---|---|---|---|---|---|---|
| | Siderophore with mixed ligand | | | | | |
| 1 | Lysine derivative | | | | | |
| | Myobactin |  | Two structural classes based on the presence or absence of a 2-hydroxyphe-nyloxazoline ring system | Chemotaxano-mic markers for identification of mycobacteria upto species level | *M. tuber-culosis*, *M. smegmatis* | Stephen *et al*. 1998; De Voss *et al*. 1999; Varma & Podila 2005 |
| 2 | Ornithine derivative | | | | | |
| | Pyoverdine |  | Chromophore, (1S)-5-ami-no-2,3-dihyd-ro- 8,9-dihyd-roxy-1H-py-rimido[1,2-a] quinoline-1-car-boxylic acid | Prevention of iron overload toxicity Inhibition of pathogenic bacterial growth | *Pseudomonas aeruginosa* | Wendenbaum *et al*. 1983; Lamont & Martin 2003; Meyer 2000 |
| 3 | Histamine derivative | | | | | |
| | Anguibactin |  | ω-N-hyd-roxy-ω-[[2´ -(2",3!-dihyd-roxyphenyl) thiazolin-4´ -yl]-carboxy] histamine | Inhibits iron uptake by living cells, removes iron from other siderophores and ferric hydroxide, aqueous soluti-ons, including cell-culture media | *Vibrio angu-illarum* | Jalal *et al*. 1989 |



tives (ferrioxamines) display antibiotic activity and have been designated as ferrimycines (Bickel 1960). Hydroxamate siderophore family include rhodotorulic acid, dimerium acid, alcaligens and putribactin.

**2.1. Aferrioxamines**

These are linear trihydroxamates siderophores with a molecular formula of $C_{25}H_{48}N_6O$, produced by *Streptomyces* and *Nocardia*. These are used therapeutically for the binding of excess blood iron in the treatment of thalsaemia. It is on the World Health Organization's List of Essential Medicines, a list of the most important medication needed in a basic health system WHO Model List of Essential Medicines (World Health Organization 2013).

**2.2. Ferrichrome**

The ferrichromes comprise one large family of hydroxamate siderophores. These are cyclic trihydroxamate siderophore. Ferrichromes are cyclic hexapeptide siderophores composed of three *N*-acyl-*N*-hydroxyl-L-ornithine, two variable amino acids (alanine, serine, or glycine), and a glycine linked by way of peptide bonds. Five acyl groups at may be acetyl, malonyl, trans-β-methylglutaconyl, trans-anhydromevalonyl and cis-anhydromevalonyl. The acyl groups are homogeneous in each ferrichrome produced by Basidomycetes, except for the asperchromes, which are synthesized by *Aspergillus ochraceous* (Jalal & Van der Helm 1991; Renshaw *et al.* 2002; Ali *et al.* 2011). Ferrichrome produced by the fungus *Ustilago sphaerogena* was the first siderophore to be isolated and shown to be a growth factor for other microbes (Neilands 1981).

**2.3. Aerobactin**

Aerobactin is a bacterial iron-chelating agent (siderophore) found in *E. coli* with a molecular formula of $C_{22}H_{36}N_4O_{13}$ (Neilands 1995; Johnson *et al.* 1988). This hydroxamate siderophore is an exogeneous siderophore of *Pseudomonas* (marine origin), *K. pneumonia, A. aerogenes*, *E. coli* and other bacteria (Buyer *et al.* 1991). It is a virulence factor enabling *E. coli* to sequester ironin iron-poor environments. Under iron-limiting conditions, the *Pseudomonas sp*. produced aerobactin, a dihydroxamate siderophore previously found only in the family Enterobacteriaceae.

**3**. **Carboxylate-type siderophore – Rhizoferrin**

This is a novel class of siderophore whose members neither possess hydroxamate nor phenolate ligands rather iron binding is achieved by hydroxyl carboxylate and carboxylates (Schwyn & Neiland 1987). Rhizoferrin is composed of diaminopropane symmetrically acylated with citric acid via amine bonds to the terminal carboxylate of citric acid (Drechsel *et al.* 1992). These siderophore are found in the kingdom of bacteria as well in the realm of fungi. Rhizoferrin is the only known carboxylate siderophore produced by fungi, specifically synthesised by members of the zygomycetes. Carboxylate siderophore include *Rhizobacteria*, *Staphyloferrin* and *Rhizopherrin* (Stephan *et al.* 1996). Interestingly, both fungi and bacteria produce rhizoferrin, fungi produce only *R*, *R*-rhizoferrin, whilst a few bacteria produce enantio-rhizoferrin *S*, *S*-rhizoferrin (Munzinger *et al.* 1999).

**4**. **Siderophore with mixed ligand**

Apart from above siderophores, certain siderophores have the mixed ligands of lysine, ornithine and histamine derivatives.

**4.1. Lysine Derivative**

**4.1.1. Myobactin**

Mycobactins are 2-hydroxy phenyl oxazoline containing siderophore molecules for the acquisition of iron. Two chemical structures of siderophores produced by *Mycobacterium tuberculosis* are reported, one of the structure being more lipophilic in nature, whereas the other structure is more hydrophilic. The only chemical difference between the two is the nature of the *N*-acyl chain on the hydroxylated lysine in the middle of the molecule. Myobactin siderophore isolated from *Myobacteria* revealed that in mycobactin, two hydroxamate, a phenolate and a oxazoline nitrogen, were present. *M. tuberculosis* produces only the mycobactin class of siderophore molecule, which contains this salicylic-acid-derived moiety. Saprophytic mycobacteria such as *Mycobacterium smegmatis* produce both this and a peptidic siderophore, called as exochelin (De Voss *et al.* 1999; Varma & Podila 2005).

### 4.2. Ornithine derivative

#### 4.2.1. Pyoverdines

Pyoverdine/pseudobactins contain a dihydroxyquinoline derivative. The structure of the peptide differs between pseudomonads and more than 40 structures have been described, whilst the chromophore, (1S)-5-amino-2,3-dihydro-8,9-dihydroxy-1H-pyrimido [1,2-a]quinoline-1 carboxylic acid, is the same with the exception of azobactin from *A. vinelandii*, which possesses an extra urea. The chromophore derived from 2,3-diamino-6,7-dihydroxyquinoline are linked to a peptide chain exhibiting two hydroxamate groups or one hydroxamate and one hydroxycarboxylate group. A variety of fluorescent chromopeptide siderophore termed pseudobactin and pyoverdines are synthesised by *Pseudomonas* species. *Pseudomonas* spp. are of considerable importance both in agriculture and as human pathogens (Kloepper *et al.* 1980). Two important siderophore-mediated iron uptake systems have been found in these bacteria: one involving the fluorescent siderophore pseudobactin (also known as pyoverdin) and the other involving the siderophore pyochelin (Abddalah 1991; Meyer 2000; Meneely & Lamb 2007).

### 4.3. Histamine derivative

#### 4.3.1. Anguibactin

The chemical structure of anguibactin has been identified as ω-*N*-hydroxy-ω-[[2′-(2″,3″-dihydroxyphenyl)thiazolin-4′-yl]-carboxy]histamine, isolated from marine pathogen *Vibrio anguillarum* (Jalal *et al.* 1989). Anguibactin acts as an inhibitor for uptake of iron by living cells, wrests iron from vertebrate tissues, removes iron from other siderophores and ferric hydroxide and removes ferric ion from aqueous solutions, including cell culture media.

*Pathways for siderophore biosynthesis*

There are two major pathways for siderophore biosynthesis:

A. Non-ribosomal peptide synthetases (NRPSs) multienzymes dependant.

B. NRPS independent.

NRPSs are large, multimodular enzymes that perform non-ribosomal peptide (NRP) synthesis in which each module is responsible for the incorporation of one amino acid into the peptide chain. The number and order of the modules usually dictate the number and order of amino acids in the peptide product (Crosa & Walsh 2002). The activation (A)-domain of each module of the NRPS recognises and activates a specific amino acid as its acyl adenylate by reaction with ATP. This activated ester is then covalently linked as its thio-ester on the thiolation (T) domain. The condensation (C) domain catalyses the direct transfer to another acyl amino acid intermediate on the adjacent downstream module to form a peptide bond (Ravell & Cornelis 2003). NRPSs synthesises both the chromophores (Mossialos *et al.* 2002) and the peptide chains of pyoverdine (Crosa & Walsh 2002).

(A) NRPS-dependent biosynthesis

The enzyme involved in the synthesis is ATP pyrophosphate, which is widely used in exchange assay and to investigate the substrate specificity of adenylation domains within the synthetase multienzymes. Formation of hydroxmic acid by trapping of activated carboxyl group with hydroxylamine is an alternative method of assay for enzymes involved in the formation of acyladenylate. Hydroxamic acid can be converted to its ferric complex and detected spectrophotometrically.

NRPS is an assemblage of peptides of wide structural diversity and biological activity. For recognition, activation and modification of each amino incorporated, they have a different unit structure, and each unit is responsible for its specific function to finally form a peptide product (Lautru & Challis 2004). The number and order of unit in the NRPSs is responsible for the determination of size and sequence of NRP.

NRPS chain extension unit contains three domains:

1. Adenylation domain (A): Adenylation domain specifically recognises the substrate and catalyses the adenylation of its carboxyl group.

2. Thiolation domain (T): Thiolation domain is also known as peptidyl carrier protein (PCP) domain. It uses terminal thiol of a post-translationally installed phosphopantetheine arm to capture the activated carboxyl group of the adenylate.

3. Condensation domain (C): Catalyses acylation of the resulting thioester with activated acyl group

attached to the T domains in upstream unit. In some NRPS unit, C domain is replaced by a heterocyclisation (CY) domain that catalyses hetrocycle formation by a reaction of β-amino thiol group in the substrate attached to the T domain of the upstream unit.

Chain initiation unit contains only A and T domains. The growing chain is covalently bound to the T domain in successive unit throughout the assembly process.

Thioesterase domain (TE): This domain is usually present in the final unit. Hydrolysis or cyclisation results in the release of assembled chain from the NRPS. Some TE domains catalyse NADH-dependent cleavage of the acyl thioester attached to the T domain (Nadia & Challis 2009).

(B) NRPS-Independent biosynthesis

In comparison to the NRPS-dependent pathway, the enzymology of non-ribosomal-peptide-synthetases-independent siderophore (NIS) biosynthesis was overlooked for almost three decades. The first genetic characterisation of the NIS biosynthetic pathway to aerobactin was reported by Neilands and co-workers in 1980 (DeLorenzo & Neilands 1986; Challis 2005). The siderophore aerobactin is a common metabolic product of different bacteria including *Vibrio, Yersinia, Salmonella* and *E. coli* (Challis 2005). The enzymes involved in aerobactin biosynthesis is LucD, an $FADH_2$-dependent monooxygenase that convert L-lysine to L-N6-huydroyllysine using molecular oxygen as a co-substrate (Challis 2005). The enzymes envolved possess substrate-specific properties. According to the biochemical characterisation, different NIS synthetase were identified, for example, DesD, a cluster of gene des ABCD, pubC and put AB (Ledyand & Buttler 1997; Challis 2005; Barona *et al.* 2006; Kadi *et al.* 2008).

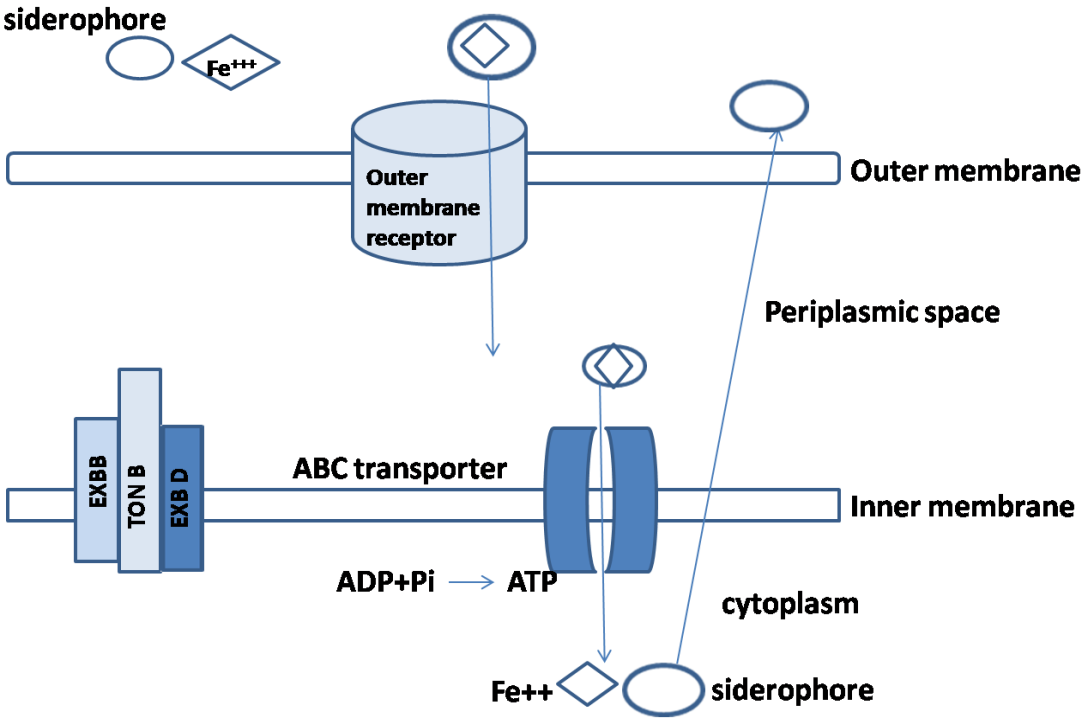



Figure 1. Transport of iron across the outer and inner membrane:

- Outer membrane contains receptor for binding and transporting the siderophore–iron complex to the periplasmic space.
- The siderophore–iron complex in the periplasmic space binds to periplasmic binding protein and is transported via ABC transporters composed of a protein channel in the cytoplasmic membrane to the cytosol.
- TonB, ExbB and ExbD are inner membrane protein complex that helps for the transportation of siderophore–iron complex across the outer memebrane.

*Transport of Fe by Siderophores*

Siderophore trap traces of iron under the form of very stable complexes (Winkelmann 1991) as they are excreted by iron-starved microorganisms, and after the complexes have formed, they are internalised into the cell by specific cell receptors (Neilands 1982). Microorganisms including bacteria and fungi use siderophores to fulfill their iron requirements. To transport iron in the cytoplasm, bacteria capture iron-loaded siderophores at the cell surface and transport them into the cytosol. The binding constants of siderophores for Fe (III) are extremely high, implying that these compounds can effectively scavenge Fe (III) from a variety of complexes found in natural environment (Stintzi *et al.* 2000; Bernd & Rehm 2008).

In gram-negative bacteria, this typically requires a combination of protein pattern:

- An outer membrane receptor that specifically binds the ferric–siderophore complex on the outer membrane and transfer it into the periplasm.
- A protein complex containing TonB transduces energy from the proton motive force into transport-proficient structural changes of the receptor.
- A binding protein located in the periplasm transfers the siderophore bound iron to cytoplasmic-membrane-associated trasporter.

Once released into the periplasm, siderophore are rapidly bound by the specific periplasmic binding protein FhvA (hydroxamate siderophores) (Coulton *et al.* 1986), FepB (Enterobactin) and FecB (ferric dicitrate) (Pressler *et al.* 1988).

The membrane-associated transporter or ABC transporter composed of a protein channel in the cytoplasmic membrane coupled with a cytoplasmic ATPase that envolves ferric siderophore internalisation at the expense of ATP hydrolysis in the cytoplasm (Figure 1).

The ABC transporter system is assembled of two proteins, one to separate the membrane acting as a permease and a second on which it can hydrolyse to provide the energy for transport. Transmembrane permease Fhu B for hydroximate, FepDG for enterobactins and Fec CA for ferric dicitrate.

Ferric siderophores are released from the transport system at the cytoplasmic side of the cytoplasmic membrane. Eventually, iron is rapidly released from the siderophore complex via reduction. Different iron transport mechanism has been suggested for an outer membrane transport of pyoverdine

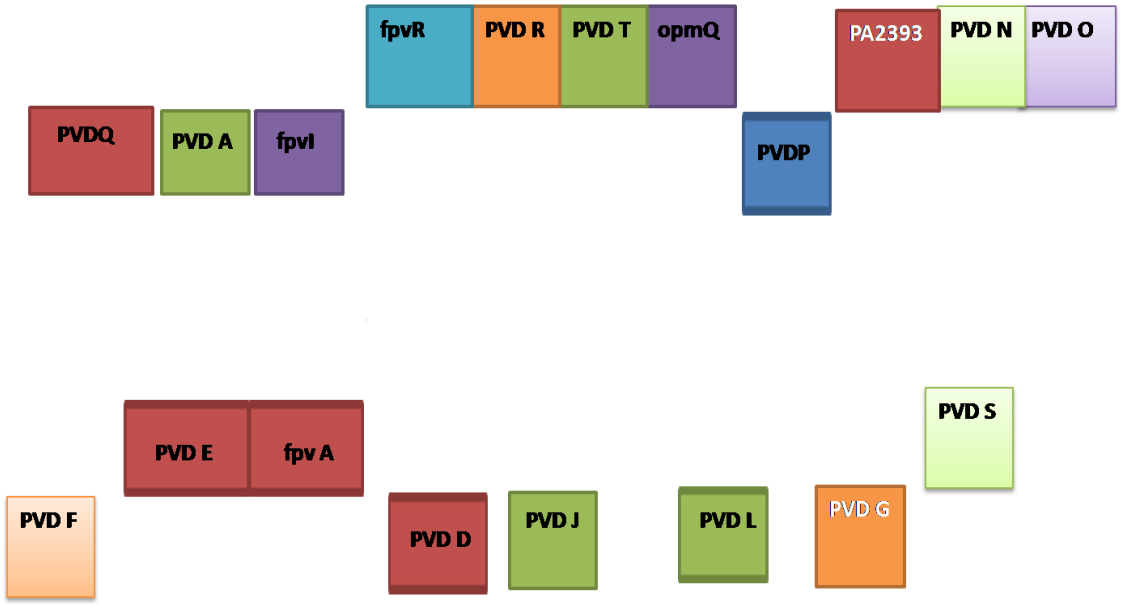



Figure 2. Arrangement of genes involved in siderophore biosynthesis in *Pseudomonas aeruginosa* as per the ORF region

via the FpvA receptor in *Pseudomonas aeruginosa*. The ligand exchange step occurs at the cell surface and involves the exchange of iron from a ferric pyoverdine to an iron-free pyoverdine strongly bound to the receptor FpvA. This mechanism suggest an increase in concentration of iron-free siderophore (Schalk *et al.* 2001) .

*Genes involved in siderophore biosynthesis*

Many species of *Pseudomonas* produce fluorescent siderophore. There is extensive homology between the DNA from different species, consistent with the suggestion that the different siderophore synthesis genes have evolved from the same ancestor set of gene (Hohnadel & Meyer 1988; Rombel & Lamont 1992), suggested the characteristics of pseudobactins and pyoverdin involved in the synthesis of pigments consist of achromophore joined to a short peptide; the chromophore is conserved between species, whereas the exact nature of the peptide is species specific, with differences reflecting specificities of uptake (Hohnadel & Meyer 1988) (Figure 2, Table 2).

*Siderophore uptake regulation*

Protein named Fur (ferric uptake regulator) has been identified, which is responsible for transcriptional regulation of these genes. Fur gene product acts as a classical repressor requiring $Fe^{2+}$ as an activator. When the iron concentration is high, fur forms a complex with $Fe^{2+}$; in the promoter region, Fur binding prevents transcription of these genes. Under the conditions of iron deficiency, $Fe^{2+}$ is removed from Fur, and fur-dependant genes are transcribed. Thus Fur regulates the oxidation state of the main components of iron metabolism (Hall & Foster 1996; Bsat *et al.*1998; Hantke 2001).

Fur is related to the repression of genes involved in biosynthesis, export and import of siderophore (Figure 3).

*Application and importance of siderophore*

The importance of microbial siderophores extends beyond their immediate role in microbial physiology and their role in biotechnology. Applications of microbial siderophores for sustainability is enormous. These are produced by various bacteria

T a b l e 2

Genes involved in siderophore production

| Gene | ORF | Function |
|---|---|---|
| pvdA | PA2386 | Ornithine hydroxylase (Visca *et al*. 1994) |
| fpvI | PA2387. | ECF sigma factor required for the expression of fpvA (Beare *et al*. 2003) |
| fpvR | PA2388 | Anti-sigma factor for PvdS and FpvI (Lamont & Martin 2003, Beare *et al*. 2003) |
| pvdF | PA2396. | N5-Hydroxyornithine transformylase (McMorran *et al*. 2001) |
| pvdE | PA2397 | ABC transporter (secretion) (McMorran *et al*. 1996) |
| fpvA . | PA2398 | Ferripyoverdine receptor protein (Poole *et al*. 1993) |
| pvdD | PA2399 | Pyoverdine peptide synthetase (Merriman *et al*. 1995) |
| pvdJ | PA2400/1 | Pyoverdine peptide synthetase (Lehoux 2000) |
| pvdI | PA2402 | Pyoverdine peptide synthetase (Lehoux 2000) |
| pvdS ECF iron sigma factor | PA2426 | ECF iron sigma factor (Cunliffe *et al.*1995; Miyazaki *et al*. 1995) |
| pvcABCD | PA2254–PA2257 1996 | Synthesis of the pyoverdinechromophore (Stintzi *et al*. 1999) |

having wide application in various field such as agriculture to improve soil fertility and biocontrol, environmental application and medicinal application.

*Agricultural application*

Most soil microorganisms can promote mineral weathering by the production of siderophores. Siderophores provide an efficient Fe-acquisition system because of its high affinity for Fe (III) complexation by means of mineral dissolution (McGrath *et al.* 1995; Kraemer 2004). Siderophores plays a significant role in iron dissolution, making it available for microorganisms and plants. Inoculation of soil with pseudobactin produced by *Pseudomonas putida* increases growth and yield of various plants (Kloepper *et al.* 1980).

Although there is sufficient iron in most soils for plant growth, plant iron deficiency is a problem in calcareous soil, because of the low solubility of iron (III) hydroxide. Calcareous soil accounts for 30% of the world's farmland. Under such conditions, graminaceous plants (grasses, cereals and rice) secrete phytosiderophores into the soil. Poaceae (grasses) including agriculturally important species such as barley and wheat are able to efficiently sequester iron by releasing phytosiderophores via their root into the surrounding soil rhizosphere (Hershko *et al.* 2002). Evidences suggest that plants have an ability to incorporate and use the $Fe^{3+}$ of siderophores into their biomass and this was confirmed at molecular level also. The plant's enzyme NADH was able to function as ferric siderophore reductase indicating that plants accept iron through available iron siderophore chelates (Becker *et al.* 1985; Castignetti & Smarrelli 1986) increasing the availability of iron. Plants such as oats are able to assimilate iron via these microbial siderophores. Application of microbial siderophorogenic bioinoculants have been extensively studied; it was found that this approach has protected groundnut crop from iron chlorosis. An improvement in overall growth and health of plants has been observed after treatment of seeds with siderophorogenic bioinoculants. Increased per-

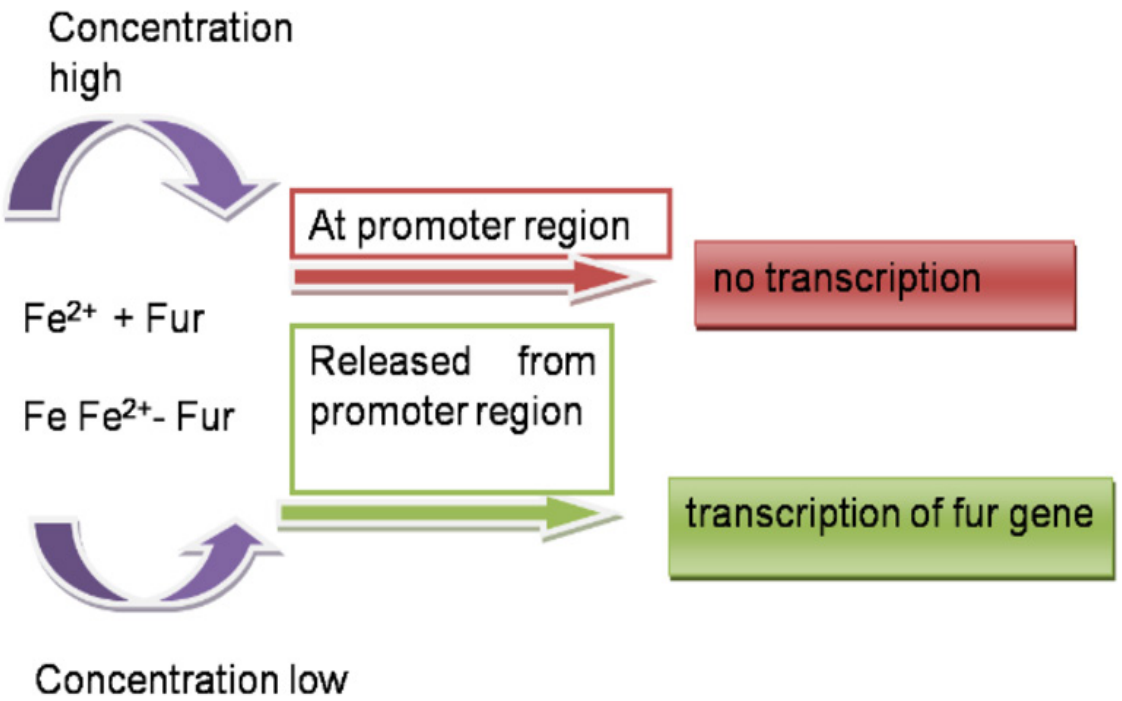



Figure 3. Regulation model of siderophore profuction because of Fur repression: Fur protein (ferric uptake regulator) is responsible for the transcriptional regulation of iron uptake. High iron concentration leads to the formation of Fur and $Fe^{2+}$ complex in the promoter region, preventing the transcription of genes involved in transport, whilst at low concentration, $Fe^{2+}$ is released from Fur proteins, leading to the transcription of iron transporting genes

centage of germination, root ramification, nodulation, height, foliage and chlorophyll content were achieved only because of seed bacterisation with siderophoregenic *Pseudomonas* (Manwar *et al*. 2001). The direct benefits of bacterial siderophores on the growth of plants have been demonstrated in several different types of experiments. For example, (i) several studies using radiolabelled ferric siderophores as a sole source of iron showed that plants are able to take up the labelled iron, (ii) mung bean plants inoculated with the siderophore-producing *Pseudomonas* strain GRP3 and grown under iron-limiting conditions showed reduced chlorotic symptoms and an enhanced chlorophyll level compared to uninoculated plants (Sharma *et al*. 2003), (iii) an increase in iron inside plant tissues of *Arabidopsis thaliana* plants because of uptake of Fe–pyoverdine complex synthesised by *Pseudomonas fluorescens* C7 to improved plant growth (Vansuyt *et al*. 2007). The provision of iron to plants by soil bacteria is even more important when the plants are exposed to an environmental stress such as heavy metal pollution. In these cases, siderophores help to alleviate the stresses imposed on plants by high soil levels of heavy metals.

The repeated and continuous use of fungicides has led to the development of fungal-resistant strains, transforming fungicides ineffective. Microbial metabolites may help improve the control of plant pathogens either by enhancing the action of antagonistic microorganisms or by providing tools to develop healthier alternatives than synthetic chemical fungicides. Some bacterial strains that do not use any other means of biocontrol can act as biocontrol agents using the siderophores that they produce. Some studies have included the use of mutants that were defective in siderophore production and found that these strains were less effective than the wild-type strains at protecting plants against fungal pathogens (Buysens *et al*. 1996). On the other hand, one study observed that siderophore overproducing mutants were more effective at protecting plants against fungal pathogens (Vandenbergh & Gonzalez 1984). *Fluorescent Pseudomonads* form a line of siderophores associated with improved plant growth through a direct effect on the plant through control of noxious organisms in the soil. Many bacteria suppress the growth of deleterious microorganism by the production of siderophore, antibiotics and cyanide (Husen 2003). Siderophores are themselves growth inhibitors of various phytopathogenic fungi, such as *Phytophthora parasitica* (Seuk *et al.* 1988), *Phythium ultimum* (Hamdan *et al*. 1991), *Fusarium oxysporum* veri *dianthi* (Buysens *et al.* 1996) and *Sclerotinia sclerotiorum* (Kraemer *et al.* 2006).

*Clinical applications*

In the treatment of thalassemia and certain other anemias, periodic whole blood transfusions are required (Pietrangelo *et al.* 2002). A siderophore from *Streptomyces pilosus*, desfarrioxamine B, is marketed as mesylate salt under the trade name Desferol and is advocated for the removal of excess iron resulting from the suppurative therapy for Thalassemia. As there is no specific physiological mechanism for the excretion of iron in man, continued transfusion therapy leads to a steady buildup of iron overload diseases such as hemochromatosis and hemosiderosis, and accidental iron poisoning, require the removal of iron from the body, especially from the liver. Such disease can be efficiently treated with siderophore-based drug and siderophore act as principal model (Chua *et al.* 2003).

The potency of common antibiotics has been elevated by binding into the iron-binding functional groups of siderophores. Siderophore used for the clearance of non-transferrin bound iron in serum that occurs in cancer therapy is a result of some chemotherapies (Chua *et al.* 2003). Antimalarial siderophore have been found to be useful in the treatment of malaria caused by *Plasmodium falciparum* (Gysin *et al.* 1991). Siderophore produced by *K. pneumoniae* act as antimalarial agent (Simon *et al.* 1993). Malaria parasites is highly dependent on $Fe^{3+}$ for its growth. Its growth is arrested in the presence of $Fe^{3+}$ chelators. Iron sources affected by desfemoxamine (DFO) and related hydroxamates reside inside the parasite and not at the level of host red blood cell (RBC) or serum source. Possible mechanisms involved in the antimalarial action of these iron chelators are sequestration of $Fe^{3+}$ from vital sources, such as storage proteins; low-molecular-weight siderophores; iron centers of key parasite enzymes, such as ribonucleotide reductase; or the scavenging of iron from degraded hemoglobin. DFO is an iron chelator with remarkable therapeutic performance,

including antimalarial activity both *in vitro* and *in vivo*. However, because of its relatively slow and apparently selective permeation into the advanced growth stages of cells infected by *P. falciparum*, its biological activity is slow to develop, that is, it demands relatively long exposures of cells at mature stages of parasite growth. The major prototype of a novel family of antimalarial agents that is termed as reversed siderophore (RSF, siderophores with relatively high lipophilic properties) subserve their permeation into cells. Because $Fe^{3+}$ binding to RSFs completely abolished their antimalarial effect, it was implied that the arrest of parasite growth involved sequestration of critical iron and not the formation of intracellular toxic $Fe^{3+}$ ligand complexes, as proposed for other classes of iron $Fe^{3+}$ chelators. The studies indicate that permeation plays a dominant role in the antimalarial activity of iron chelators and is a major determinant in the irreversible inhibition of parasite growth (Garbisu & Alkorta 1997).

Sideromycin an iron-chelating antibiotic produced by *Streptomyces* species showed good antimicrobial activity.

*Environmental applications*

Although heavy metals (Cd, Cr, Cu, Hg, Pb and Ni) are naturally present in the soil, geologic and anthropogenic activities increase the concentration of these elements to amounts that are harmful to both plants and animals. Excessive accumulation of heavy metals is toxic to most plants, water and soil possing a major environmental and human health problem. Some of the activities are mining and smelting of metals, burning of fossil fuels, use of fertilisers and pesticides in agriculture, production of batteries and other metal products in industries, sewage sludge and municipal waste disposal. Heavy metals cannot be degraded during bioremediation but can only be transformed from one organic complex or oxidation state to another. Owing to a change in their oxidation state, heavy metals can be transformed to less toxic, easily volatilised, more water soluble (and thus can be removed through leaching), less water soluble (which allows them to precipitate and become easily removed from the environment) or less bioavailable (Wang *et al.* 1989). Several microorganisms especially bacteria (*Bacillus subtilis, P. putida* and *Enterobacter cloacae*) have been successfully used for the reduction of Cr (VI) to the less toxic Cr (III) (Lelie *et al.* 1999; Haja *et al.* 2010). *B. subtilis* has also been reported to reduce nonmetallic elements. For instance, Garbisu *et al.* (1995) recorded that *B. subtilis* reduced the selenite to the less toxic element Se. *B. cereus* and *B. thuringiensis* increase the extraction of Cd and Zn from Cd-rich soil and soil polluted with effluent from metal industry (Ruggiero *et al*. 2000). It is assumed that the production of siderophore by bacteria have facilitated the extraction of these metals from the soil; this is because heavy metals have been reported to simulate the production of siderophore and this consequently affects their bioavailability (Von Gunten & Benes 1995). For instance, siderophore production by *A. vinelandii* was increased in the presence of Zn (II) Siderophore-producing rhizobacteria (SPR) are a group of plant-growth-promoting rhizobacteria, being able to play an important role in assisting the phytoremediation of heavy-metals-contaminated soils. Hence, heavy metals influence the activities of siderophore-producing bacteria which in turn increases the mobility and extraction of these metals in soil. Siderophores have the potential to resolve these environmental problems such as heavy metal accumulation, rust removal, biofouling, dye degradation, sewage treatment and bioleaching. Hydroxamate-type siderophore present in soil plays important role to immobilise these heavy metals.

Siderophores may be used to treat radioactive waste before storage (Von Gunten & Benes 1995; Bouby *et al.* 1998). Some species of fungi such as *Fusarium* sp. and bacteria such as *P. aeruginosa* were able to produce element-specific ligands (siderophores) that are able to change pH and enhance the chelation of some elements such as Uranium ($U^{6+}$) and Thorium ($Th^{4+}$) (Joshi *et al*. 2014).

*P. putida* increases its siderophore secretion in response to the availability of benzyl alcohol, a model aromatic substrate. Accelerated siderophore secretion in response to aromatic substrates provides an iron 'boost' which is required for the effective functioning of the iron-dependent oxygenases responsible for ring opening. Stimulated siderophore secretion might be a key factor in successful integration and proliferation of this organism as a bioaugmentation agent for aromatic degradation. It not only facilitates efficient aro-

matic utilisation but also provides better opportunities for iron assimilation amongst diverse microbial communities, thereby ensuring better survival and proliferation (Joshi *et al.* 2014).

Enormous volumes of effluent are generated at different stages of textile manufacturing loading waste residues such as toxic reactive dyes, chlorolignin residues and dark colouration to the water bodies resulting the change in the ecological balance. The biological breakdown of the chlorolignin residues and the chromophoric groups responsible for the dark coloration of the textile effluent can be accomplished by the use of enzymes and siderophores from the white rot fungus, *Phanerochaete chrysosporium* (Asamudo *et al.* 2005).

## CONCLUSIONS

Microorganisms and plants sustain themselves under iron-deprived conditions by releasing siderophores. Bacteria produce four types of siderophores: hydroxamate, catecholate, salicylate and carboxylate. These siderophores play an important role in the extra cellular solubilisation of iron from minerals or organic substances. Some important siderophore-producing bacteria includes *E. coli, Salmonella, K. pneumoniae, Vibrio cholerae, V. anguillarum, Aeromonas, Aerobacter raerogenes, Enterobacter, Yersinia and Mycobacterium* species. Present review highlights recent updates in siderophore production, importance, structural/functional moiety and their source of isolation. A brief description about different Fe–siderophore complexes, their transport and synthesis pathway (NRPS-dependent and NRPS-independent pathway) has been included. It also explains the genes involved in the biosynthesis of sideophore along with its regulation. Iron uptake system synthesises siderophore and represents central organic compound providing better health of plants. Understanding the chemical structures of different siderophores and the membrane receptors involved in Fe uptake has opened new areas for research.

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