Chapter 13
Bacillus spp.: A Prolific Siderophore Producer

Arif Khan, Hiren V. Doshi, and Mukund Chandra Thakur

Abstract *Bacillus* species comprises of several hundred species and is characterized as non-spore- or endospore-forming, straight or slightly curved Gram-positive rods, which may turn Gram-negative with age, and single or multi-flagellate and grows in aerobic or facultative anaerobic conditions. *Bacillus* spp. include xenobiotic biodegraders, plant growth promoters, siderophore producers and human & plant pathogens.

Iron is a micronutrient and the fourth most abundant element in the earth's crust. Bacteria need iron for a range of metabolic and signaling functions including electron transport, peroxide reduction, amino acid & nucleoside synthesis, DNA synthesis, photosynthesis and most importantly – some virulence traits. *Bacillus* spp. have developed a mechanism for acquiring iron by the use of siderophores. Siderophores are small iron-chelating molecules that have high affinity for iron. Siderophores show a wide range of variety in their structure. Some siderophores are comprised of a peptide backbone with various coordinating iron-ligating groups. *Bacillus* spp. produce a wide variety of siderophores such as bacillibactin, pyoverdine, pyochelin, schizokinen, petrobactin, etc. which play a crucial role in its existence.

Keywords *Bacillus subtilis* • Siderophore • Iron chelator • Bacillibactin • Iron-chelating receptor

A. Khan • M.C. Thakur (✉)
Department of Biotechnology, Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences,
ADIT Campus, New Vidyangar 388121, Anand, Gujarat, India
e-mail: mukundthakur@aribas.edu.in

H.V. Doshi
Department of Pharmaceutical Chemistry, Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences,
ADIT Campus, New Vidyangar 388121, Anand, Gujarat, India

M.T. Islam et al. (eds.), *Bacilli and Agrobiotechnology*,
DOI 10.1007/978-3-319-44409-3_13

13.1 Introduction

Bacillus is a genus of Gram-positive, rod-shaped (bacillus) bacteria and a member of the phylum *Firmicutes*. *Bacillus* species can be obligate aerobes (oxygen reliant), or facultative anaerobes (having the ability to be aerobic or anaerobic), and are ubiquitous in nature. They give positive test for the enzyme catalase when oxygen is used or present. *Bacillus* include both free-living (nonparasitic) and parasitic pathogenic species. Under stressful environmental conditions, bacteria can produce oval endospores that are not true *spores* but which the bacteria can reduce themselves and remain in a dormant state for very long periods. These characteristics originally defined the genus, but not all such species are closely related, and many have been moved to other genera of *Firmicutes*.

Many species of *Bacillus* can produce copious amounts of enzymes which are made use of in different industries. Some *Bacillus* species can form intracellular inclusions of polyhydroxyalkanoates under certain adverse environmental conditions, as in a lack of elements such as phosphorus, nitrogen or oxygen combined with an excessive supply of carbon sources.

B. subtilis has proved to be an invaluable model for research. Other species of *Bacillus* are important pathogens causing anthrax and food poisoning. Many *Bacillus* species are able to secrete large quantities of enzymes. *B. amyloliquefaciens* is the source of a natural antibiotic protein barnase (a ribonuclease), alpha amylase used in starch hydrolysis, the protease subtilisin used with detergents, and the *Bam*HI restriction enzyme used in DNA research. A portion of the *Bacillus thuringiensis* genome was incorporated into corn (brinjal and cotton) crops. The resulting GMOs were then found to be resistant to some insect pests.

B. subtilis is one of the best understood prokaryotes, in terms of molecular biology and cell biology. Its superb genetic amenability and relatively large size have proved to be powerful tools required to investigate a bacterium from all possible aspects. Recent improvements in fluorescence microscopy techniques have provided novel and amazing insight into the dynamic structure of a single-cell organism. Research on *B. subtilis* has been at the forefront of bacterial molecular biology and cytology, and the organism is a model for differentiation, gene/protein regulation, and cell cycle events in bacteria.

Two *Bacillus* species are considered medically significant: *B. anthracis*, which causes anthrax and *B. cereus*, which causes food poisoning similar to that caused by *Staphylococcus*. A third species, *B. thuringiensis*, is an important insect pathogen and is sometimes used to control insect pests. The type species *B. subtilis* is an important model organism. It is also a notable food spoiler, causing ropiness in bread and related food. Some environmental and commercial strains such as *B. coagulans* may play a role in food spoilage of highly acidic tomato-based products.

An easy way to isolate *Bacillus* is by placing non-sterile soil in a test tube with water, then shaking it, ultimately plating it in melted mannitol salt agar and incubating at room temperature for at least a day. Colonies are usually large, spreading and

irregularly shaped. Under the microscope, *Bacillus* cells appear as rods and a substantial portion usually contain an oval endospore at one end, making it bulge.

The cell wall of *Bacillus* is a structure on the outside of the cell that forms the second barrier between the bacterium and the environment and at the same time maintains the rod shape and withstands the pressure generated by the cell's turgor. The cell wall is composed of teichoic and teichuronic acids. *B. subtilis* was the first bacterium for which the role of an actin-like cytoskeleton in cell shape determination and peptidoglycan synthesis was identified. It was also the first bacterium for which the entire set of peptidoglycan synthesizing enzymes was localized, thus, paving the way for understanding the role of cytoskeleton in shape generation and maintenance.

The genus *Bacillus* was named in 1835 by Christian Gottfried Ehrenberg to contain rod-shaped (bacillus) bacteria. He had 7 years earlier named the genus *Bacterium. Bacillus* was later amended by Ferdinand Cohn to further describe them as spore-forming, Gram-positive, aerobic or facultatively anaerobic bacteria (Xu and Côté 2003). Like other genera associated with the early history of microbiology, such as *Pseudomonas* and *Vibrio*, the 266 species of *Bacillus* are ubiquitous. The genus has very large ribosomal 16S diversity and is environmentally diverse.

One of the studies reconciles the exception that *Lactobacillus plantarum* does not entail iron. It might apparently manage to stimulate all its enzymatic functions with metals other than iron. After complexing iron, the ferric-siderophore complexes are taken up into the cell. The specific siderophore and its chirality are recognized by highly specific receptors in the outer membrane of bacteria. In an active and energy-dependent way, they transport the ferric complexes into the periplasm. Once the complexes are collected there, they are then handed over to the intracellular transport and storage components and finally integrated into proteins to accomplish their enzymatic functions (Archibald 1983).

Several studies have tried to reconstruct the phylogeny of the genus as mentioned in Fig. 13.1. The *Bacillus*-specific study with the most diversity covered is by Xu and Côté (2003) using 16S and the ITS region, where they divide the genus into ten groups, which includes the nested genera *Paenibacillus*, *Brevibacillus*, *Geobacillus*, *Marinibacillus* and *Virgibacillus*. However, the tree constructed by the living tree project, a collaboration between ARB-Silva and LPSN where a 16S (and 23S if available) tree of all validated species was constructed, the genus *Bacillus* contains a very large number of nested taxa and majorly in both 16S and 23S is paraphyletic to *Lactobacillales* (*Lactobacillus*, *Streptococcus*, *Staphylococcus*, *Listeria*, etc.), due to *Bacillus coahuilensis* and others. A gene concatenation study found similar results to Xu and Côté (2003), but with a much more limited number of species in terms of groups, but used *Listeria* as an outgroup. So in light of the ARB tree, it may be "inside-out."

One clade, formed by *B. anthracis*, *B. cereus*, *B. mycoides*, *B. pseudomycoides*, *B. thuringiensis* and *B. weihenstephanensis* under current classification standards, should be a single species (within 97 % 16S identity), but due to medical reasons,

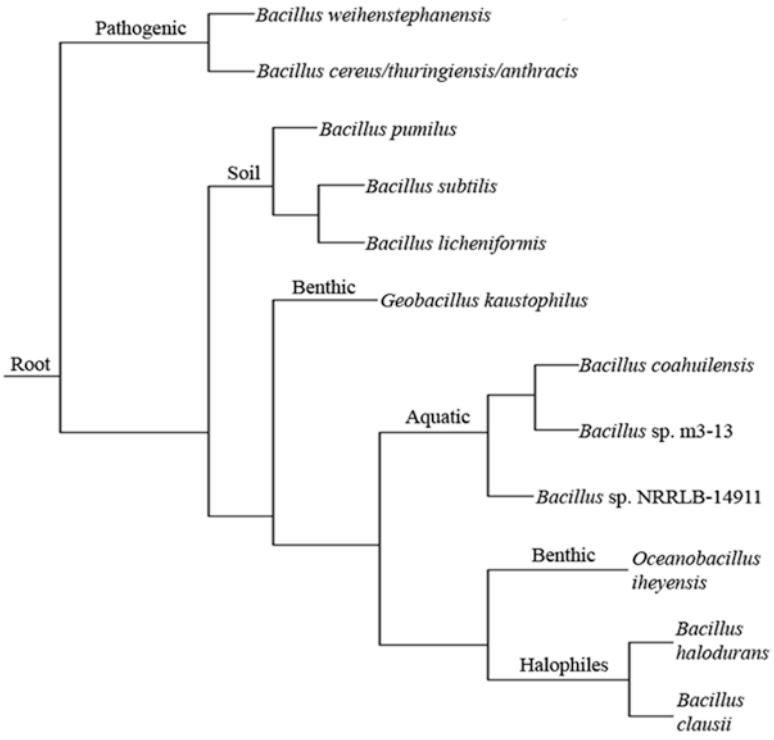

Fig. 13.1 Phylogenetic tree of genus *Bacillus*

they are considered separate species, an issue also present for four species of *Shigella* and *Escherichia coli*.

Much corroboration confirms that corynebactin was isolated from Gram-positive *Corynebacterium glutamicum* and *B. subtilis* incorporates a threonine trilactone and glycine spacers, which elongate the three chelating arms as compared with enterobactin. In *B. subtilis* (DNA with low G + C content), three Fur-like proteins have been characterized (Bsat et al. 1998). One, called Fur, regulates mainly iron uptake and siderophore biosynthesis. A second one, called PerR, regulates peroxide stress response genes and acts with manganese as corepressor. A third one, Zur, regulates genes for zinc uptake. The Zur protein found in *E. coli* shows only 25 % identity to the *B. subtilis* Zur, while the two Fur proteins have 32 % identical amino acids.

Apart from producing siderophores, some *Bacillus* spp. even help in the release of iron from siderophores by using ferri-reductase. Ferri-siderophore reductase of *B. megaterium* has been partially purified and it shows wide range of substrate specificity. The putative Mn oxidase CumA (Okazaki et al. 1997; Brouwers et al. 1999; Francis and Tebo 2001) of *P. putida*GB-1 and MnB1 is a multicopper-type oxidase enzyme that utilizes oxygen as an electron acceptor. Same is also true in other well-studied systems (Brouwers et al. 2000; Tebo et al. 2004) including *Bacillus* sp. spores (Van Waasbergen et al. 1996; Dick et al. 2006).

Webb et al. (2005) have elegantly demonstrated that the oxidation of Mn(II) to Mn(IV) by *Bacillus* spores is a two-step process involving a transient Mn(III) intermediate. Further, an enzymatically produced Mn(III) intermediate of Mn(II) oxidation by *Bacillus* sp. strain SG-1 spores has been trapped by pyrophosphate under conditions that minimized abiotic processes (Webb et al. 2005). The above observation suggests that ligands can trap Mn(III) when interacting with living systems. The differing origin of the Mn(III) in the *Bacillus* and *Pseudomonas* cases could involve the greater stability constant of PVD Mn(III) (Parker et al. 2004) than of pyrophosphate-Mn(III) (Webb et al. 2005). Beside chelation of ferric ions (iron chelator), degradation of textile dyes (Thakur et al. 2012; Joshi et al. 2013) and several other heavy metal ions such as nickel and chromium chelation have also been reported for *B. megaterium* in various studies.

Among pathogenic bacteria, characteristic features of the tubercle bacillus include its slow growth, dormancy, complex cell envelope, intracellular pathogenesis and genetic homogeneity. Generally bacteria are denoted as pathogenic because they have found out an easy way in animal system to survive where suitable temperature/environment (warmth) is always available for them. Plentiful of nutrition is also available for their survival and growth. Here the pathogenic organisms can acquire all the required minerals from the host body tissues apart from one, i.e., iron which is generally present in oxidized form Fe (III) at pH 7 and is difficult to utilize directly. The uptake mechanism of iron in *Bacillus* spp. is mentioned in Fig. 13.2.

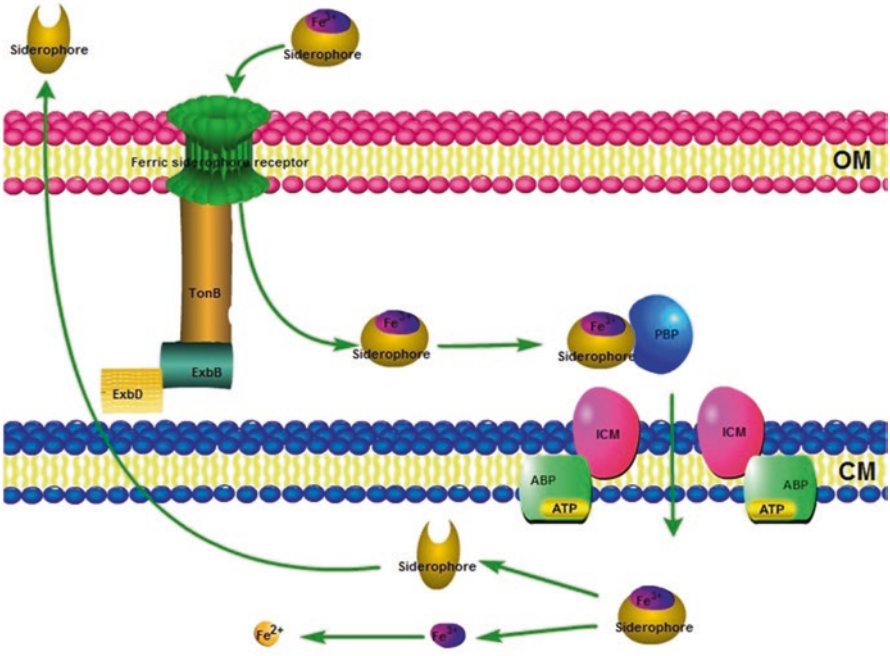

Fig. 13.2 Uptake mechanism of iron in *Bacillus* spp.

13.2 Transport of Substrates into *Bacillus* spp.

The uptake of substrates into the best-studied organism, *Bacillus subtilis*, has been outlined in general terms. The uptake of substrates take place in three steps: (1) uptake across outer membrane, (2) transport across the cytoplasmic membrane and (3) periplasmic binding protein dependent transport.

13.3 Uptake of Ferri-siderophore Complex Across Outer Membrane

Gram-negative *Bacillus* sp. is surrounded by two membranes, the outer membrane and the cytoplasmic membrane (Braun and Hantke 1981; Braun et al. 1985; Lugtenberg and Van Alphen 1983). Hydrophilic substrate not larger than 600–700 Da diffuses through water-filled pores of the outer membrane found by the most abundant proteins in this membrane (Nikaido and Vaara 1987). However, for some substrates, stereochemical recognition takes place between the substrate and an outer membrane protein (Ferenci 1989). This has been demonstrated for maltodextrins, which are recognized by the LamB protein, and for nucleosides which interact with Tsx protein (Hantke 1976; Krieger-Brauer and Braun 1980; Maier et al. 1988; Benz et al. 1988). The PhoE protein forms more efficient pore than the porin (Lugtenberg and Van Alphen 1983) both for inorganic and organic phosphate but does not seem to specifically recognize phosphate, but rather it displays a broad specificity for anions (Benz and Bauer 1988). Before uptake studies had been performed, genetic evidence pointed to the role of these proteins in transport of certain substrates. Synthesis of these proteins was regulated at the transcriptional level by maltodextrins, nucleosides, and phosphates. Maltodextrins (the intracellular regulatory compound is maltotriose) convert a protein (MalT) to an activator, nucleosides inactivates two repressor proteins (DeoR, CytR), and phosphate starvation induces a complex regulatory network resulting in PhoE synthesis. These proteins facilitate the diffusion of the substrate across the outer membrane. The smaller homologues of maltodextrins, phosphate, and nucleosides can also pass through the porins so that the specific porins are not absolutely required. They increase the rate of diffusion and are essential for the uptake of larger homologues across the outer membrane into the periplasmic space.

13.4 Transport Across the Cytoplasmic Membrane

Stereochemical recognition between the substrate and the transport proteins and actual transport against a concentration gradient occurs in the cytoplasmic membrane. Energy required for the active transport is provided in the form of an electrochemical potential across the cytoplasmic membrane by electron transport chain

located therein or by the ATP hydrolysis through the membrane-bound ATPase. Certain substrates such as lactose are transported across the cytoplasmic membrane of *B. subtilis* by FhuBC protein (Ollinger et al. 2006), by a process driven by the electrochemical potential (Hengge and Boss 1983).

13.5 Periplasmic Binding Protein-Dependent Transport (PBT)

Transport of many amino acids, peptides, certain sugars and anions follows a so-called binding protein-dependent mechanism in which protein in the periplasmic space (located between the outer and the cytoplasmic membrane) is involved (Hengge and Boss 1983; Ames 1986). The binding proteins recognize the certain substrates and delivered them to the integral membrane proteins of the cytoplasmic membrane. They are essential constituents of the transport system. The periplasmic binding proteins can be released by an osmotic shock treatment involving plasmolysis of cells in 15 % sucrose (which counterbalances the internal osmotic pressure) in the presence of EDTA (releasing Mg(II) ions supposed to stabilize the outer membrane). Upon rapid dilution of the plasmolyzed cells in to a low-salt Tris/Mg(II) buffer, periplasmic proteins are released from the cells. Alternatively, cells are converted to spheroplasts by a similar procedure but with the inclusion of lysozyme to degrade the murein (peptidoglycan) layer. Such treated cells show greatly reduced transport rates which can be restored by adding back the binding protein in the presence of Ca(II) (increases the permeability of the outer membrane) (Hengge and Boss 1983). Substrates bound to the binding protein rather than the free substrate are accepted by the cytoplasmic transmembrane protein. Usually the periplasmic proteins are synthesized in a large excess with respect to the membrane proteins and are the best characterized transport proteins. The structure of several such proteins (for arabinose, ribose, galactose, sulfate) has been resolved to the atomic scale by X-ray analysis. They exhibit similar conformations composed of two globular domains forming a cleft at the substrate binding sites, linked by a flexible hinge (Quiochio 1988). The very hydrophobic integral cytoplasmic membrane proteins accept substrates from the binding proteins. Usually two such proteins are found in a single system which exhibit the sequence similarity, for example, HisQ and HisM of histidine, MalF and MalG of maltose, PstA and PstC of phosphate, and OppB and OppC of the peptide transport system. But there are also exceptions to this rule. The high-affinity arabinose system contains only one hydrophobic protein of the usual size (34 kD) (Scripture et al. 1987), and variations also occur in the iron transport system.

Characteristic of the PBT system is the involvement of a polar but nevertheless membrane-bound protein which displays sequences also found in nucleotide-binding proteins. In fact, it has long been known that ATP either directly or indirectly

serves as an energy source for PBT. ATP binding, but not ATP hydrolysis, has been demonstrated for the MalK, HisP, and OppD proteins (Higgins et al. 1988). There is evidence that these proteins are bound to the inside of the cytoplasmic membrane (Gallagher et al. 1989).

The PBT system is a high-affinity transport system (K_m 1 μM and below) which concentrates substrates inside the cell against a very large gradient (in the order of 10^5).

13.6 Iron(III) Transport in *B. subtilis*

Iron in the ferric form in aerobic conditions and physiological pH (around 7.00) is extremely insoluble and only scarcely available for bacteria under all natural conditions. As a response to iron starvation, bacteria synthesize elaborate iron supply systems which are composed of low-molecular-weight ferric complexing compounds, termed siderophores and ferric-siderophore transport system. A number of iron(III) transport systems have been characterized in *B. subtilis* (Braun and Winkelmann 1987). Iron uptake in *B. subtilis* using various different siderophores such as bacillibactin, enantio-enterobactin, itoic acid, etc. has been reported by several researchers (May et al. 2001). Further this organism can recognize a variety of catecholate siderophores, but does so through the expression of several and sometimes overlapping membrane transport proteins.

Bacillus subtilis is prototypical for studying iron uptake in Gram-positive organisms. Recent studies of its iron metabolism have elucidated multiple aspects of siderophore synthesis, transport and regulation (Dertz et al. 2006; Miethke et al. 2006; Ollinger et al. 2006; Gaballa and Helmann 2007). *Bacillus subtilis* is a model system which produces a number of hydroxamate- and catecholate-type siderophores such as shizokinen, itoic acid, petrobactin, corynebactin, bacillibactin, etc. Corynebactin siderophore was produced by *Corynebacterium glutamicum* (Abergel et al. 2008), and later genomic data and biochemical analysis revealed that this organism is producing bacillibactin type of compound (Barbeau et al. 2002). Thus, the bacillibactin was the preferred siderophore name for the *B. subtilis* siderophore (Heinrichs et al. 2004; Koppisch et al. 2005).

Bacillibactin is a catecholate siderophore produced by many *Bacilli* (May et al. 2001). It is a cyclic trimeric ester made of three units of 2,3-dihydroxybenzoate-glycine-threonine (Fig. 13.3), joined by lactone linkages in a cyclic manner, similar to the enterobacterial siderophore enterobactin (May et al. 2001). The linear component 2,3-dihydroxybenzoylglycine, also known as itoic acid, also has iron-chelating capabilities (Ito 1993).

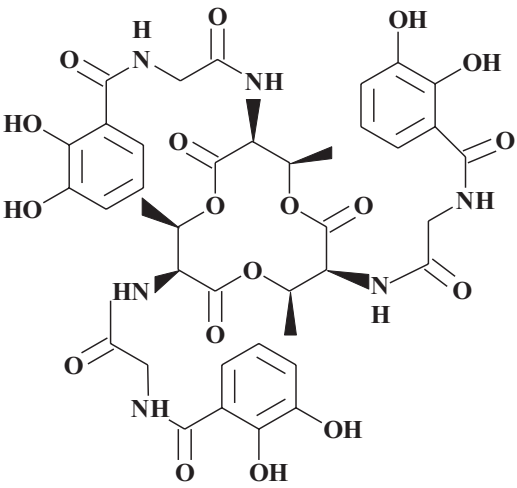

Fig. 13.3 Chemical structure of bacillibactin

13.7 Biosynthetic Pathway of Bacillibactin

Bacillibactin is synthesized by *B. subtilis* under iron-deficient conditions and secreted into the external environment where it binds with Fe(III) with high affinity and specificity. This Fe(III)-bound siderophore is called ferri-siderophore complex which is taken up into the cell by specific transport components.

Bacillibactin synthesis (Fig. 13.4) (http://www.metacyc.org/META/NEW-IMAGE?type=PATHWAY&object=PWY-5903&detail-level=3) can be divided into two parts:

1. Conversion of chorismate to 2,3-dihydroxybenzoate: 2,3-dihydroxybenzoate is synthesized from chorismate through isochorismate and 2,3-dihydroxy-2,3-dihydrobenzoate. Chorismate plays a major role as a key intermediate and branch point in the biosynthesis of many aromatic compounds.
2. Synthesis of bacillibactin from 2,3-dihydroxybenzoate, glycine and L-threonine: Synthesis of bacillibactin is a complex process catalyzed by the bacillibactin synthetase multienzyme complex and is presented by a single pathway reaction. The synthesis starts with the activation of 2,3-dihydroxybenzoate, catalyzed by 2,3-dihydroxybenzoate-AMP ligase, encoded by dhbE (May et al. 2001) in the following reaction:

$$2,3\ \text{dihydroxybenzoate} + \text{ATP} + \text{H}^{+} \rightarrow\ 2,3\ \text{dihydroxybenzoyladenylate} + \text{PPi}$$

The product (2,3-dihydroxybenzoyl adenylate) is transferred onto the aryl carrier protein (ArCP) domain of a bifunctional protein dhbB, whose other function is isochorismatase (May et al. 2001), where it is attached to the free thiol group of its cofactor, 4′-phosphopantetheine. The 4′-phosphopantetheinyl transferase protein (sfp gene product) catalyzes the cofactor attachment (Grossman et al. 1993).

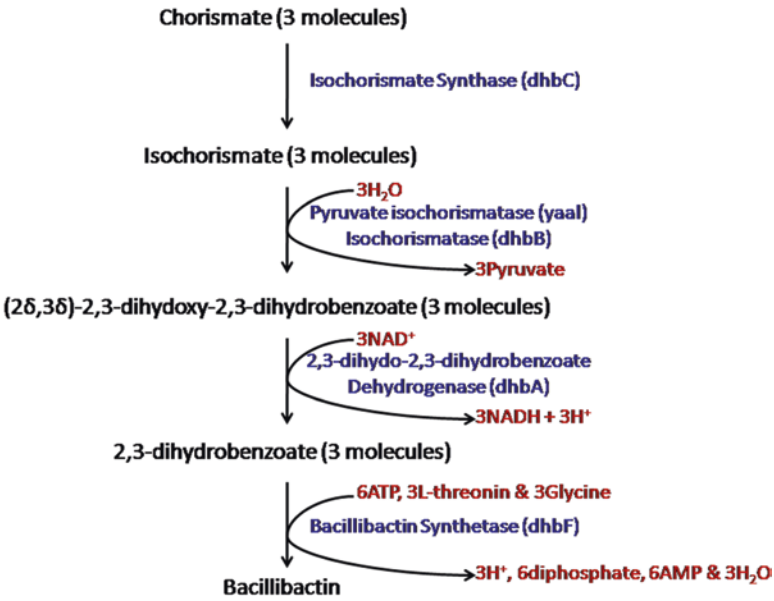

Fig. 13.4 Pathway for bacillibactin biosynthesis

The glycine and L-threonine amino acids are initially bound to 4′-phosphopantetheine cofactors via thiol groups. These 4′-phosphopantetheine cofactors carrying glycine and L-threonine then bind to the seven-domain holo-DhbF protein, thereby activating the holo-DhbF protein. The 4′-phosphopantetheinyl transferase then catalyzes the transfer of glycine and L-threonine amino acids to two peptidyl-carrier-protein domains of the seven-domain holo-DhbF protein and subsequently to the activated 2,3-dihydroxybenzoate. The reaction for binding of glycine and L-threonine amino acids to the thiol groups of the 4′-phosphopantetheine cofactors are as follows:

$$\text{glycine} + \text{ATP} = \text{glycyl-AMP} + \text{PPi}$$

$$\text{L-threonine} + \text{ATP} = \text{L-threonyl-AMP} + \text{PPi}$$

Two additional domains of 4′-phosphopantetheinyl transferase enzyme catalyze the condensation of the activated amino acids to the activated 2,3-dihydroxybenzoate which ultimately form Dhb-glycine-threonine product (May et al. 2001). The product is transferred to the last domain of holo-DhbF protein, named as Te domain. This protein then trims off the three such moieties and releases the trilactone bacillibactin.

13.8 Mechanism of Ferri-bacillibactin Uptake

The bacillibactin synthesizing operon consists of five gene sets (*dhbACEBF*) whose function is given in Table 13.1 and Fig. 13.5.

The detailed molecular analysis of iron uptake pathways in *B. subtilis* was first described by Schneider and Hantke (1993). They suggested that ABC transporter subunits are needed for the uptake of iron siderophore compounds. This hypothesis was generated independently by an analysis of the protein similarities for all of the ABC transport systems (Fig. 13.6), and corresponding surface-binding proteins, in *B. subtilis* (Quentin et al. 1999). The uptake of iron(III) by *B. subtilis* using bacillibactin siderophore requires involvement of a number of membrane-bound proteins such as substrate-binding proteins, ATPase, permeases and transporters. FeuABC is one such membrane-bound bacillibactin transporter of *B. subtilis*. FepDG is another membrane-bound heterodimeric inner membrane permease involved in transport of

Table 13.1 Functions of genes involved in biosynthesis of bacillibactin

Genes	Functions
dhbA	–
dhbC	Isochorismate synthetase
dhbE	DHB-AMP ligase
dhbB	Isochorismate lyases/ArCP
dhbF	Functionally similar to dimodular actinomycin synthetase-II of *Streptomyces chrysomallus*

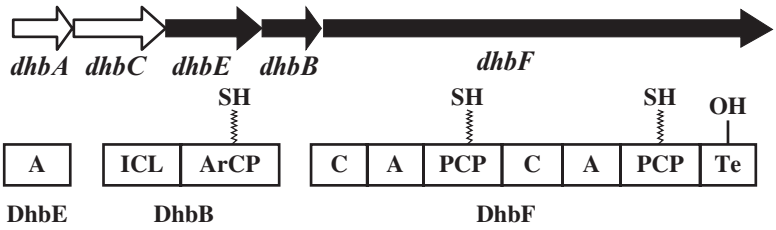

Fig. 13.5 Operon for biosynthesis of bacillibactin

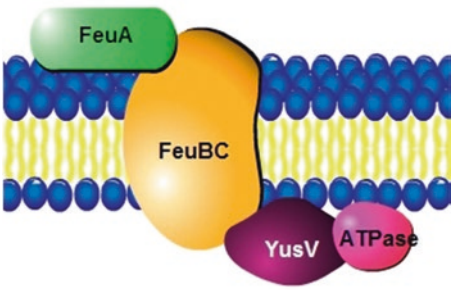

Fig. 13.6 Pathway for iron uptake in *B. subtilis*

bacillibactin. YusV acts as ATP-binding protein and provides energy for transport of ferri-bacillibactin. It is hypothesized that YuiI finally hydrolyzes bacillibactin and ferri-bacillibactin so that iron becomes available for cellular processes and bacillibactin may be recycled for further use. The whole process of iron uptake by bacillibactin in *B. subtilis* is regulated by a Fur homologue which binds directly to a Fur box. Another bacillibactin pathway regulator Mta has been recently discovered which is a MerR-type transcriptional regulator. It activates bacillibactin secretion (Miethke et al. 2008). The exact mechanism of Fur homologue and Mta binding with Fur box and metal ions is poorly understood. Hence, further studies are required in this direction to fully comprehend the regulation of metal uptake in *B. subtilis* or Gram-positive organisms in general.

13.9 Role of Siderophore in Plant Growth Promotion

Siderophores are synthesized by microorganisms and released into the environment which then can bind to iron more specifically and lead the iron unavailable for other microorganisms in the vicinity and thereby limiting their growth. This approach may be used in the biological control of plant diseases (Raymond et al. 2003).

Microorganisms that prosper in the rhizosphere employ a number of different mechanisms to kill or evade pathogens. These microorganisms and their mechanisms can be used as first line of defense and hence become biocontrol agents for plants (Walsh et al. 1971). Plant roots normally exude a variety of different secondary metabolites which chemotactically attract bacteria and fungi toward itself. These bacteria and fungi produce secondary metabolites such as siderophores like bacillibactin which play an important role in competition between microorganisms in soil. These secondary metabolites may act as plant growth promoters (PGP) (Schneider and Hantke 1993). Fusarium wilt is widely reported to cause extensive damage to pepper crop. It was recently reported by Yu et al. (2011) that *B. subtilis* CAS15 significantly suppresses the spore germination of Fusarium wilt in pepper by 8–64 %. *B. subtilis*, *B. amyloliquefaciens*, *B. cereus*, and *B. anthracis* have been reported to produce siderophores such as bacillibactin, schizokinen, petrobactin, etc. and have also been used for biocontrol against soilborne pathogens, postharvest fungal pathogens, and even foliar pathogens (Ito and Neilands 1958; Xiong et al. 2000; Barbeau et al. 2002; Moore and Helmann 2005).

References

Abergel, R. J., Zawadzka, A. M., & Raymond, K. N. (2008). Petrobactin-mediated iron transport in pathogenic bacteria: Coordination chemistry of an unusual 3,4-catecholate/citrate siderophore. *Journal of the American Chemical Society, 130*, 2124–2125.

Ames, G. F.–. L. (1986). Bacterial periplasmic transport systems: structure, mechanism and evolution. *The Annual Review of Biochemistry, 55*, 397.

Archibald, F. S. (1983). *Lactobacillus plantarum*, an organism not requiring iron. *FEMS Microbiology Letters, 19*, 29–32.

Barbeau, K., Zhang, G., Live, D. H., & Butler, A. (2002). Petrobactin, a photoreactive siderophore produced by the oil-degrading marine bacterium *Marinobacter hydrocarbonoclasticus. Journal of the American Chemical Society, 124*, 378–379.

Benz, R., & Bauer, K. (1988). Permeation of hydrophilic molecules through the outer membrane of gram-negative bacteria. *European Journal of Biochemistry, 176*, 1.

Benz, R., Schmid, A., Maier, C., & Bremer, E. (1988). Characterization of the nucleoside-binding site inside the Tsx channel of *Escherichia coli* outer membrane. *European Journal of Biochemistry, 176*, 699.

Braun, V., & Hantke, K. (1981). Bacterial cell surface receptors. In B. K. Ghosh (Ed.), *Organization of prokaryotic cell membranes* (Vol. 11, p. 1). Boca Raton: CRC Press.

Braun, V., & Winkelmann, G. (1987). Microbial iron transport. Structure and function of siderophores. In *Progress in clinical biochemistry and medicine* (vol. 5, p. 69). Heidelberg: Springer-Verlag.

Braun, V., Fischer, E., Hantke, K., Heller, K., & Rotering, H. (1985). Functional aspects of gram-negative surfaces. In D. B. Roodyn (Ed.), *Subcellular biochemistry* (Vol. 11, p. 103). New York: Plenum Press.

Brouwers, G.-J., de Vrind, J. P. M., Corstjens, P. L. A. M., Baysse, C., & de Vrind-de, J. E. W. V. (1999). *CumA*, a gene encoding a multicopper oxidase, is involved in Mn^{2+}-oxidation in *Pseudomonas putida* GB-1. *Applied and Environmental Microbiology, 65*, 1762–1768.

Brouwers, G.-J., Vijgenboom, E., Corstjens, P. L. A. M., de Vrind, J. P. M., & de Vrind-De Jong, E. W. (2000). Bacterial Mn^{2+} oxidizing systems and multicopper oxidases: An overview of mechanisms and functions. *Geomicrobiology Journal, 17*, 1–24.

Bsat, N., Herbig, A., Casillas-Martinez, L., Setlow, P., & Helmann, J. D. (1998). *Bacillus subtilis* contains multiple Fur homologues: Identification of the iron uptake (Fur) and peroxide regulon (PerR) repressors. *Molecular Microbiology, 29*(1), 189–198.

Dertz, E. A., Stintzi, A., & Raymond, K. N. (2006). Siderophore-mediated iron transport in *Bacillus subtilis* and *Corynebacterium glutamicum. Journal of Biological Inorganic Chemistry, 11*(8), 1087–1097.

Dick, G. J., Lee, Y. E., & Tebo, B. M. (2006). Maganese(II)-oxidizing Bacillus spores in Guaymas basin hydrothermal sediments and plumes. *Applied and Environmental Microbiology, 72*(5), 3184–3190.

Ferenci, T. (1989). Selectivity in solute transport: Binding sites and channel structure in maltoporin and other bacterial sugar transport proteins. *BioEssays, 10*, 3.

Francis, C. A., & Tebo, B. M. (2001). *CumA* multi-copper oxidase genes from diverse Mn(II)-oxidizing and non-Mn(II)-oxidizing *Pseudomonas* strains. *Applied and Environmental Microbiology, 67*, 4272–4278.

Gaballa, A., & Helmann, J. D. (2007). Substrate induction of siderophore transport in *Bacillus subtilis* mediated by a novel one-component regulator. *Molecular Microbiology, 66*, 164–173.

Gallagher, M. P., Pearce, S. R., & Higgins, C. F. (1989). Identification and localization of the membrane associated, ATP binding subunits of the oligopeptide permease of *Salmonella typhimurium. European Journal of Biochemistry, 180*, 133.

Grossman, T. H., Tuckman, M., Ellestad, S., & Osburne, M. S. (1993). Isolation and characterization of *Bacillus subtilis* genes involved in siderophore biosynthesis: Relationship between *B. subtilis* sfpo and *Escherichia coli* entD genes. *Journal of Bacteriology, 175*(19), 6203–6211.

Hantke, K. (1976). Phage T6-colicin K receptor and nucleoside transport in *Escherichia coli. FEBS Letters, 70*, 109.

Heinrichs, D. E., Rahn, A., Dale, S. E., & Sebulsky, M. T. (2004). In J. H. Crosa, A. R. Mey, & S. M. Payne (Eds.), *Iron transport in bacteria, iron transport systems in pathogenic bacteria: Staphylococcus, Streptococcus and Bacillus* (pp. 387–401). Washington, DC: ASM Press.

Hengge, R., & Boss, W. (1983). Maltose and lactose transport in *Escherichia coli*: Examples of two different types of concentrative transport systems. *Biochimica et Biophysica Acta, 737*, 443.

Higgins, C. F., Gallagher, M. P., Mimmack, M. L., & Pearce, S. R. (1988) A family of closely related ATP binding subunits from prokaryotic and eukaryotic cells. *BioEssays* 8:111. http://www.metacyc.org/META/NEW-IMAGE?type=PATHWAY&object=PWY-5903&detail-level=3

Ito, T. (1993). Enzymatic determination of itoic acid, a *Bacillus subtilis* siderophore, and 2,3-dihydroxybenzoic acid. *Applied and Environmental Microbiology, 59*(7), 2343–2345.

Ito, T., & Neilands, J. B. (1958). Products of "Low-iron Fermentation" with Bacillus subtilis: Isolation, characterization and synthesis of 2,3-dihydroxybenzoylglycine1,2. *Journal of the American Chemical Society, 80*, 4645.

Joshi, B., Kabariya, K., Nakrani, S., Khan, A., Parabia, F. M., Doshi, H. V., & Thakur, M. C. (2013). Biodegradation of turquoise blue dye by Bacillus megaterium isolated from industrial effluent. American Journal of Environmental Protection, 1(2), 41–46.

Koppisch, A. T., Browder, C. C., Moe, A. L., Shelley, J. T., Kinkel, B. A., Hersman, L. E., Iyer, S., & Ruggiero, C. E. (2005). Petrobactin is a primary siderophore synthesized by *Bacillus anthracis* str. *Sterne* under conditions of iron starvation. *Biometals, 18*, 577–585.

Krieger-Brauer, J., & Braun, V. (1980). Function as related to the receptor protein specified by the *tsx* gene of *Escherichia coli. Archives of Microbiology, 124*, 233.

Lugtenberg, B., & Van Alphen, L. (1983). Molecular architecture and function of the outer membrane of *Escherichia coli* and other gram-negative bacteria. *Biochimica et Biophysica Acta, 737*, 51.

Maier, C., Bremer, E., Schmid, A., & Benz, R. (1988). Pore-forming activity of the Tsx protein from the outer membrane of *Escherichia coli*: Demonstration of a nucleoside-specific binding site. *Journal of Biological Chemistry, 263*, 2493.

May, J. J., Wendrich, T. M., & Marahiel, M. A. (2001). The *dhb* operon of *Bacillus subtilis* encodes the biosynthetic template for the catecholic siderophore 2,3-dihydroxybenzoate-glycine-threonine trimeric ester bacillibactin. *Journal of Biological Chemistry, 276*, 7209–7217.

Miethke, M., Klotz, O., Linne, U., May, J. J., Beckering, C. L., & Marahiel, M. A. (2006). Ferribacillibactin uptake and hydrolysis in *Bacillus subtilis. Molecular Microbiology, 61*, 1413–1427.

Miethke, M., Schmidt, S. & Marahiel, M. A. (2008). The major facilitator superfamily-type transporter YmfE and the multidrugefflux activator Mta mediate bacillibactin secretion in Bacillus subtilis. Journal of Bacteriology, 190, 5143–5152.

Moore, C. M., & Helmann, J. D. (2005). Metal ion homeostasis in *Bacillus subtilis. Current Opinion in Microbiology, 8*(2), 188–195.

Nikaido, H., & Vaara, M. (1987). Outer membrane. In F. C. Neidhardt (Ed.), *Eschericia coli and Salmonella typhimuriu, Cellular and molecular biology* (Vol. 1, p. 7). Washington, DC: American Society for Microbiology.

Okazaki, M., Sugita, T., Shimizu, M., Ohode, Y., Iwamoto, K., DeVrind-DeJong, E. W., et al. (1997). Partial purification and characterization of manganese- oxidizing factors of *Pseudomonas fluorescens* GB-1. *Applied and Environmental Microbiology, 63*, 4793–4799.

Ollinger, J., Song, K. B., Antelmann, H., Hecker, M., & Helmann, J. D. (2006). Role of the Fur Regulon in Iron Transport in *Bacillus subtilis. Journal of Bacteriology, 188*(10), 3664–3673.

Parker, D. L., Sposito, G., & Tebo, B. M. (2004). Manganese(III) binding to a pyoverdine siderophore produced by a manganese(II)-oxidizing bacterium. *Geochimica et Cosmochimica Acta, 68*, 4809–4820.

Quentin, Y., Fichant, G., & Denizot, F. (1999). Inventory, assembly and analysis of *Bacillus subtilis* ABC transport systems. *Journal of Molecular Biology, 287*, 467–484.

Quiochio, F. A. (1988). Molecular features and basic understanding of protein-carbohydrate interaction. The arabinose binding protein sugar complex. *Current Topics in Microbiology, 139*, 135.

Raymond, K. N., Dertz, E. A., & Kim, S. S. (2003). Enterobactin: An archetype for microbial iron transport. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 3584.

Schneider, R., & Hantke, K. (1993). Iron-hydroxamate uptake systems in *Bacillus subtilis*: Identification of a lipoprotein as a part of a binding protein-dependent transport system. *Molecular Microbiology, 8*, 111–121.

Scripture, J. B., Voelker, C., Miller, S., O'Donneil, R. T., Polgar, L., Rade, J., Horazdovsky, B. F., & Hogg, R. W. (1987). High affinity L-arabinose transport operon. *Journal of Molecular Biology, 197*, 37.

Tebo, B. M., Bargar, J. R., Clement, B. G., Dick, G. J., Murray, K. J., Parker, D., et al. (2004). Biogenic manganese oxides : Properties and mechanisms of formation. *Annual Review of Earth and Planetary Sciences, 32*, 287–328.

Thakur, M. C., Khan, A., & Doshi, H. (2012). Isolation and screening of dye degrading micro-organisms from the effluents of dye and textile industries at Surat. American Journal of Environmental Engineering, 2(6), 152–159.

Van Waasbergen, L. G., Hildebrand, M., & Tebo, B. M. (1996). Identification and characterization of a gene cluster involved in manganese oxidation by spores of the marine Bacillus sp. strain SG-1. *Journal of Bacteriology, 178*(12), 3517–3530.

Walsh, B. L., Peters, W. J., & Warren, R. A. J. (1971). The regulation of phenolic acid synthesis in *Bacillus subtilis. Canadian Journal of Microbiology, 17*, 53–59.

Webb, S. M., Dick, G. J., Bargar, J. R., & Tebo, B. M. (2005). Evidence for the presence of Mn(III) intermediates in the bacterial oxidation of Mn(II) Proc. *National Academy of Sciences of the United States of America, 102*(15), 5558–5563.

Xiong, A., Singh, V. K., Cabrera, G., & Jayaswal, R. K. (2000). Molecular characterization of the ferric-uptake regulator, fur, from *Staphylococcus aureus. Microbiology, 146*(3), 659–668.

Xu, D., & Côté, J. C. (2003). Phylogenetic relationships between Bacillus species and related genera inferred from comparison of 3′ end 16S rDNA and 5′ end 16S-23S ITS nucleotide sequences. *International Journal of Systematic and Evolutionary Microbiology, 53*, 695–704.

Yu, X., Ai, C., Xin, L., & Zhou, G. (2011). The siderophore-producing bacterium, Bacillus subtilis CAS15, has a biocontrol effect on Fusarium wilt and promotes the growth of pepper. *European Journal of Soil Biology, 47*, 138–145.