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Genetic Regulations of the Biosynthesis of Microbial Surfactants: An Overview

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Abstract

Microbial biosurfactants are surface active metabolites synthesized by microbes growing on a variety of substrates. In spite of having great potential for commercial, therapeutic and environmental applications, industrial level production has not been realized for their low yields and productivities. One vital factor determining their biosynthesis is the genetic makeup of the producer organisms. Studies on molecular genetics and biochemistry of the synthesis of several biosurfactants have revealed the operons, the enzymes and the metabolic pathways required for their extracellular production. Surfactin, a cyclic lipopeptide biosurfactant is a potent antimicrobial agent and is produced as a result of non-ribosomal biosynthesis catalyzed by a large multienzyme peptide synthetase complex called the surfactin synthetase. Pathways for the synthesis of other lipopeptides such as iturin, lichenysin and arthrofactin are also mediated by similar enzyme complexes. These non-ribosomal peptide synthetases (NRPSs) responsible for lipopeptide biosynthesis display a high degree of structural similarity among themselves even from distant microbial species. Plasmid-encoded- *rhl*A, B, R and I genes of *rhl* quorum sensing system are required for production of glycolipid biosurfactants by *Pseudomonas* species. Molecular genetics of biosynthesis of alasan and emulsan by *Acinetobacter* species and of the fungal biosurfactants such as mannosylerythritol lipids (MEL) and hydrophobins have been deciphered. However, limited genetic information is available about biosynthesis of other biosurfactants such as viscosin, amphisin and

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Abbreviations: ATCC: American Type Culture Collection; ATP: Adenosine triphosphate; BHL (*Pseudomonas*): n-butanoyl-L-homoserine lactone; BHL (*Serratia*): n-butyryl-L-homoserine lactone; DNA: Deoxyribonucleic acid; HHL: N-hexanoyl-L-homoserine lactone; Kb: Kilobases; KDa: Kilodaltons; MEL: Mannosylerythritol lipids; NRPS: Non-ribosomal peptide synthetases; ORF: Open reading frame; SDS-PAGE: Sodium dodecyl sulphate polyacrylamide gel electrophoresis

putisolvin produced by some strains of *Pseudomonas* species. Understanding of the genetic regulatory mechanisms would help to develop metabolically engineered hyper-producing strains with better product characteristics and acquired capability of utilizing cheap agro-industrial wastes as substrates. This article thus provides an overview of the role and importance of molecular genetics and gene regulation mechanisms behind the biosynthesis of various microbial surfactants of commercial importance.

Introduction

Microbial surfactants or biosurfactants are the surface-active molecules derived from a large number of microorganisms. These microbially produced surface-active compounds possess the ability to reduce the surface and interfacial tension between two immiscible fluid phases. They are found in the nature in a wide variety of chemical structures including glycolipids, lipopeptides and lipoproteins, fatty acids, neutral lipids, phospholipids, polymeric and particulate lipids. Biosurfactants are different from synthetic surfactants in being non-toxic, more effective and environment-friendly. Contrary to the chemical surfactants that are generally produced from petroleum feedstock, the microbial surfactants can be produced by using a wide variety of cheap agro-based raw materials. The features that make them commercially superior to their chemically synthesized counterparts are their stability at extremes of temperatures, pH and salinity. These properties are desirable in various industrial processes such as in food processing, pharmaceutical formulations, and enhanced oil recovery and in environmental bioremediation. Apart from the classical applications (Desai and Banat, 1997), biosurfactants have also been reported to possess antibacterial**,** antifungal**,** antitumor**,** antimycoplasmic and antiviral properties (Cameotra and Makkar, 2004; Singh and Cameotra, 2004). Due to an increasing concern over the emergence of various multi-drug resistant pathogens, these molecules have emerged as potential drug molecules (Das *et al*. 2008). In spite of having such clear cut advantages, these molecules have not been commercialized extensively due to lower yields at the cellular level. This low level of production roots back to the genetics of these producers strains and thus, to increase the productivity it is essential to use recombinant and mutant hyper-producing varieties of microorganisms. Although significant increase in the production was obtained by optimization of growth medium and environmental conditions (Sen, 1997; Sen and Swaminathan, 1997; Sen and Swaminathan, 2004), the real breakthrough in their production enhancement can be obtained only by using hyper-producing recombinant and mutant varieties, as these have been reported to increase the yield manifolds. The development and use of these hyper-producers however demands a deep insight into the genetics of these producers (Mukherjee *et al*. 2006).

A fairly good number of reviews on microbial surfactants mainly focused on their types and commercial potential (Banat, 2000), their natural roles (Ron and Rosenberg, 2001) their use in environmental bioremediation (Mulligan, 2005), their production on cheap substrates (Haba *et al*. 2000; Nitschke *et al*. 2005; Dubey and Juwarkar, 2001) and their biomedical and therapeutic properties (Singh and Cameotra, 2004; Rodrigues *et al*. 2006) are available. However the genetics of microbial surfactant synthesis, which is a primary factor determining their productivity, has not been properly reviewed. The present review serves this purpose by focusing on molecular genetic regulation for the biosynthesis of a wide variety of microbial surfactants.

Genetic regulation of biosurfactant synthesis

Biosurfactants having a variety of chemical structures (*Table 1*) such as lipopeptides (Arima *et al*. 1968; Thaniyavarn *et al*. 2003; Morikawa *et al*. 1993; Tran *et al*. 2007; Rahman *et al*. 2006; Yakimov *et al*. 1995; He *et al*. 2001; Lee *et al*. 2007; Trischmann *et al*. 1994; Hasumi *et al*. 1995; Gurjar *et al*. 1995), glycolipids (Kitamoto *et al*. 1990a; Kobayashi *et al*. 1987; Morita *et al*. 2007; Hisatsuka *et al*. 1971; Guerra-Santos *et al*. 1994; Guerra-Santos *et al.* 1996; Wu *et al.* 2007; Patel and Desai, 1997; Robert *et al.* 1989; Raza *et al.* 2007; Mercade *et al.* 1993; Benincasa *et al.* 2002), flavolipids (Bodour *et al.* 2004), polymeric and particulate types (Shabtai, 1990; Panilaitis *et al.* 2006; Cirigliano and Carman, 1985; Ito *et al.* 1980; Itoh and Suzuki, 1974; Deshpande and Daniels, 1995; Franzetti *et al*. 2008; Persson *et al*. 1988) constitute the major examples of biosurfactants of commercial importance. These molecules are produced by both bacteria and fungi (Vance-Harrop *et al*. 2003).

Among all the biosurfactants reported till date, the molecular biosynthetic regulation of rhamnolipid, a glycolipid type biosurfactant produced by *Pseudomonas aeruginosa* and a lipopeptide biosurfactant called surfactin produced by *Bacillus subtilis* were the first to be deciphered. Other biosurfactants whose molecular genetics have been delineated in the recent years include arthrofactin from *Pseudomonas* species, iturin and lichenysin from *Bacillus* species, mannosylerythritol lipids (MEL) from *Candida* and emulsan from *Acinetobacter* species. The biosynthetic regulation of some other less known biosurfactants such as alasan, serrawettin, viscosin, amphisin, putisolvin, hydrophobin, lokisin and tensin are mostly unknown leaving a few isolated reports. Quorum sensing, a cell density dependent gene regulation process allowing bacterial cells to express certain specific genes on attaining high cell density, regulates the production of some biosurfactants**.** It had been reported that low-molecular-mass signal molecules (such as the furanosyl borate diester AI-2) are involved in biosurfactant production from different bacteria (Daniels *et al*. 2004). However, whether quorum sensing is the environmental cue to biosurfactant production in general is not known.

Biosurfactants from *Bacillus* species

The *Bacillus* species are the most well known as the producers of microbial surfactants. Lipopeptides, a group of biosurfactants whose structure consists of a fatty acid and peptide group is produced by this group of microorganisms. Surfactin, the first and the most well known of the microbial surfactants is a member of this group. The molecular genetics governing biosurfactant production by *Bacillus* sp. have been investigated worldwide in the recent years. In the light of this research a brief description of the various lipopeptide biosurfactants produced by *Bacillus* species and the underlying genetic regulation of their biosynthesis follows:

SURFACTIN

Surfactin, a potent biosurfactant consists of a heptapeptide moiety attached to a fatty acid chain. Surfactin biosynthesis is catalyzed non-ribosomally by a large multienzyme peptide synthetase complex called the surfactin synthetase consisting of three protein

Table 1. Biosurfactants with their microbial sources

Biosurfactants	Microbial origin	
	Bacteria	Fungi
Surfactin	*Bacillus subtilis* (Arima *et al*. 1968) *Bacillus licheniformis* F2.2 (Thaniyavarn *et al*. 2003) *Bacillus subtilis* ATCC 21332 (Nitschke and Pastore, 2003) *Bacillus subtilis* LB5a (Nitschke and Pastore, 2006) *Bacillus subtilis* MTCC 1427 and MTCC 2423 (Makkar and Cameotra, 1999)	-
Surfactant BL86	*Bacillus licheniformis* 86 (Horowitz and Currie, 1990)	-
Arthrofactin	*Arthrobacter* sp.MIS38 (Morikawa *et al*. 1993)	-
Viscosin	*Pseudomonas fluorescens* (Neu and Poralla, 1990)	-
Plipastatin	*Bacillus licheniformis* F2.2 (Thaniyavarn *et al*. 2003)	-
Massetolides	*Pseudomonas fluorescens* SS101 (Tran *et al*. 2007)	-
Iturin	*B. amyloliquefaciens* B94 (Yu *et al*. 2002) *Bacillus subtilis* RB14 (Rahman *et al*. 2006)	-
Lichenysin A	*Bacillus licheniformis* BAS50 (Yakimov *et al*. 1995)	-
Lichenysin B, C	*Bacillus* sp. (Yakimov *et al*. 1995, Yakimov *et al*. 1998, Yakimov *et al*. 1999)	-
Bamylomycin	*B. amyloliquefaciens* (Lee *et al*. 2007)	-
Halobacillin	Marine *Bacillus sp.* (Trischmann *et al*. 1994)	-
Isohalobacillin	*Bacillus sp.* A1238 (Hasumi *et al*. 1995)	-
Bioemulsifier	*Bacillus stearothermophilus* VR-8 (Gurjar *et al*. 1995)	*Candida lipolytica* IA 1055 (Vance- Harrop *et al*. 2003)
Flavolipid	*Flavobacterium* sp. MTN11 (Bodour *et al*. 2004)	-
Mannosylerthritol	-	*Candida antarctica* lipid (MEL) (Kitamoto *et al*. 1990a) *Candida* sp. KSM-1529 (Kobayashi *et al*. 1987) *Pseudozyma antarctica* JCM 10317^{T} (Morita *et* *al*. 2007)
Rhamnolipids Rl and R2	*Pseudomonas aeruginosa* (Hisatsuka *et al*. 1971, Guerra-Santos *et al*. 1984, Guerra-Santos *et al*. 1986)	-

Table 1. Contd.

Biosurfactants	Microbial origin	
	Bacteria	Fungi
Rhamnolipid	*P. aeruginosa* EM1 (Wu *et al*. 2007) *Pseudomonas aeruginosa* GS3 (Patel and Desai 1997) *Pseudomonas aeruginosa* BS2 (Dubey and Juwarkar 2001) *Pseudomonas aeruginosa* 44T1 [40, 46] *P. putida* 300-B mutant *(*obtained from *Pseudomonas putida* 33 wild strain by gamma ray mutagenesis) (Robert *et al*. 1989)	-
Rhamnolipid RL1 and RL2	*Pseudomonas* sp. 47T2 NCIB 400044 (Mercade *et al*. 1993)	-
Rhamnolipids (RL_{LBI})	*Pseudomonas aeruginosa* strain LBI (Benincasa *et al*. 2002)	-
Emulsan	*Acinetobacter calcoaceticus* ATCC 31012 (RAG-1) (Shabtai 1990) *Acinetobacter venetianus* RAG-1 (Panilaitis *et al*. 2006)	-
Liposan	-	*C. lipolytica* (Cirigliano and Carman 1985)
Biodispersan	*A. calcoaceticus* A2 (Shabtai 1990)	-
Lactonic sophorose lipid	-	T. *bombicola* KSM-36 (Ito *et al*. 1980)
Fructose-lipids	*Arthrobacter* sp*., Corynebacterium* sp*., Nocardia* sp*., Mycobacterium* sp. (Itoh and Suzuki, 1974)	-
Sophorolipids	-	*Candida bombicola* (Deshpande and Daniels 1995)
Bioemulsan	*Gordonia* sp. BS29 (Franzetti *et al*. 2008)	-
Circulocin	*Bacillus circulans*, J2154 (He *et al*. 2001)	-
AP-6	*Pseudomonas fluorescens* 378 (Persson *et al*. 1988)	-

subunits-SrfA, ComA (earlier known as SrfB) and SrfC. The peptide synthetase required for amino acid moiety of surfactin is encoded by four ORFs in the srfA operon namely SrfAA, SrfAB, SrfAC and SrfAD or SrfA-TE. This operon also contains comS gene lying within and out-of-frame with the *srf*B. While SrfAD is not essential for surfactin biosynthesis, the other three ORFs are absolutely essential for this process as had been indicated by deletion analysis. However, currently this region is thought to be involved in the lactonization process (Venkataramana and Karanth, 1989). *sfp* is another gene encoding phosphopantetheinyl transferase required for activation of surfactin synthetase by posttranslational modification. It is absolutely essential for surfactin production because few mutants had been found which have all the genes required for surfactin biosynthesis with the exception of *sfp*. Another gene is that of acyl transferase which is responsible for the transfer of hydroxy fatty acid moiety to

SrfAA but it is yet to be characterized (Peypoux *et al*. 1999). Recently *B. subtilis* has been found to regulate surfactin production by a cell density-responsive mechanism not based on homoserine lactone but utilizing a peptide pheromone, ComX (Menkhaus *et al.* 1993).

When the cell density is high, ComX, a signal peptide, accumulates in the growth medium. ComX becomes a signal peptide after being modified by the gene product of *com*Q. Quorum sensing controls *srf*A expression by ComX which when interacts with ComP and ComA activates the signal transduction system. The histidine protein kinase ComP donates a phosphate to the response regulator ComA, which gets activated and stimulates the transcription of the *srf* operon. *srf* transcription is also activated by the pheromone CSF by inhibiting the ComA-phosphate phosphatase RapC. Since CSF is an extracellular peptide factor it has to be imported inside the cell and is done so by the oligopeptide permease Spo0K. ComR and SinR also influence *srf*A expression – ComR post-transcriptionally enhances *srf*A expression and SinR negatively controls srfA possibly by regulating *com*R (Cosby *et al.* 1998; Luttinger *et al.* 1996; Liu *et al.* 1996).

LICHENYSIN

Lichenysin is formed during growth of *Bacillus licheniformis* JF2 under both aerobic and anaerobic conditions (Yakimov *et al.* 1995). It lowers the surface tension of water from 72 mN m^{-1} to 28 mN m^{-1}. Structural genes required for lichenysin synthesis have been isolated and they show high sequence homology with those of surfactin. Therefore, it can be expected that the biosyntheses of both of these substances follow similar pathways. Lichenysin like surfactin is synthesized non-ribosomally by a multienzyme peptide synthetase complex. Identification of the cloned putative lichenysin A synthetase operon revealed that it contains seven amino acid activation-thiolation, two epimerization and one thioesterase domain similar to that of surfactin (Yakimov *et al.* 1998). The lichenysin biosynthesis operon from *B. licheniformis* ATCC 10716 had been cloned and sequenced. The lichenysin operon consists of three peptide synthetase genes *lic*A, *lic*B and *lic*C and they are transcribed in the same direction (Marahiel *et al.* 1999). The *lic* operon of *B. licheniformis* is 26.6 kb long and consists of genes *lic*A (three modules), *lic*B (three modules) and *lic*C (one module). The domain structures of these seven modules resemble that of surfactin synthetases SrfA-C. The modular organization of lichenysin synthetases LicA to LicC was also found to be exactly identical with that of surfactin synthetases. There is another gene called *lic*TE which codes for a thioesterase like protein (Yakimov *et al.* 1998).

ITURIN

Iturin A is an antifungal lipopeptide biosurfactant produced by certain *Bacillus subtilis* strains such as *B. subtilis* RB14. Iturin A operon is composed of four open reading frames, *itu*D, *itu*A, *itu*B, and *itu*C. The *itu*D gene encodes a putative malonyl coenzyme A transacylase, whose disruption results in a specific deficiency in iturin A production. The striking feature of ItuA is that the three functional domains homologous to β-ketoacyl synthetase, amino transferase, and amino acid adenylation are combined.

The *itu*B gene encodes a peptide synthetase consisting of four amino acid adenylation domains, two of which are flanked by an epimerization domain. The *itu*C gene encodes another peptide synthetase that has two adenylation domains, one epimerization domain, and a thioesterase domain which probably helps in peptide cyclization. When the promoter of the iturin operon was replaced by the *repU* promoter of the plasmid pUB110 replication protein, threefold increase in the production of iturin A was observed (Tsuge *et al.* 2001).

Structural similarity of lipopeptide synthetase genes

The genes responsible for lipopeptide biosurfactant biosynthesis code for non-ribosomal peptide synthetases (NRPSs) which are multimodular enzyme complexes. They display a high degree of structural similarity among themselves e. g. the lipopeptide production genes from *Bacillus* and *Pseudomonas* species show high degree of similarity in structural organization. A comparative illustration of this organization of the lipopeptide biosynthesis genes are given in *Figure 1*.

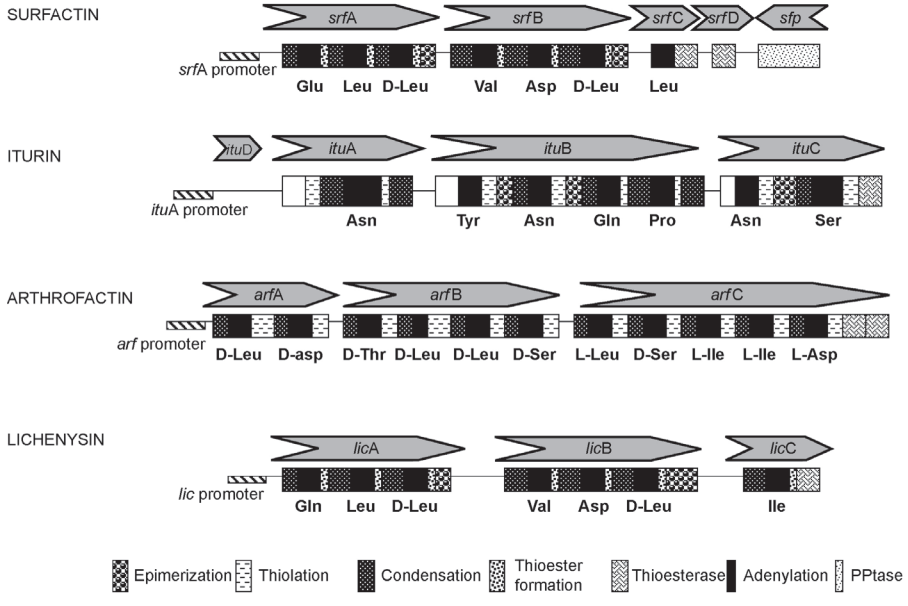

Figure 1. Structural organization of the genes encoding various lipopeptide biosurfactant synthetases. These genes show a high degree of structural similarity. *srf* operon of *Bacillus subtilis* which codes for surfactin is more than 15 kb long. It has four ORFs namely *srf*A, *srf*B, *srf*C and *srf*D, which codes for the surfactin synthetase enzyme. It also contains *sfp* gene encoding phosphopantetheinyl transferase enzyme, required for posttranscriptional modification of surfactin. Iturin synthetase encoded by *itu* operon is 38 kb long and is composed of four ORFs *itu*D, *itu*A, *itu*B and *itu*C transcribing in the same direction. The operon coding for arthrofactin synthetase consists of three ORFs *arf*A, *arf*B and *arf*C which are situated on 38.7 kb long stretch of DNA. Similarly the lichenysin operon that is about 26.6 kb long consists of three ORFs namely *lic*A, *lic*B and *lic*C. The ORFs in these genes consist of multiple regions coding for functional domains namely, epimerization, adenylation, condensation, thioester formation etc in the corresponding lipopeptide biosurfactant synthetase enzymes.

The *srf* operon of *Bacillus subtilis* which is more than 15 kb long encodes surfactin synthetase, the three subunits of which employ the thiotemplate mechanism of nonribosomal peptide synthetases (NRPSs) for incorporation of amino acids into the lipopeptide biosurfactant. There is an ATP dependant adenylation domain which activates amino acids, a condensation domain catalyzing peptide bond formation, epimerization domain epimerizing amino acids before their addition to the growing peptide chain and a thioesterase domain which cleaves the growing peptide chain. The folding of the peptide chain is then stabilized by a subsequent intramolecular lactonization possibly involving a second thioesterase, named srfA-D (Peypoux *et al.* 1999).

The iturin operon of *Bacillus subtilis* RB14 encompassing more than 38kb of DNA is composed of four open reading frames- *itu*D, *itu*A, *itu*B and *itu*C. The *itu*D gene encodes malonyl CoA transacylase, *itu*A has three functional modules homologous to fatty acid synthetase, amino acid transferase and peptide synthetase, *itu*B and *itu*C has four and two amino acid modules respectively (Tsuge *et al.* 2001).

The three genes of arthrofactin operon of *Pseudomonas*: *arf*A, *arf*B and *arf*C encode ArfA, ArfB and ArfC containing two, four and five functional modules. Each module has condensation, adenylation and thiolation domains but there is no epimerization domain (Roongsawang *et al.* 2003).

The lichenysin operon of *B. licheniformis* is 26.6 kb long and consists of genes *lic*A (three modules), *lic*B (three modules) and *lic*C (one module). The domain structures of these seven modules resemble that of surfactin synthetases SrfA-C. There is another gene called *lic*TE which codes for a thioesterase like protein (Yakimov *et al.* 1998).

Biosurfactants from *Pseudomonas* species

Pseudomonas species form the second largest group of bacteria producing biosurfactants. Many strains of *Pseudomonas* have been reported to produce glycolipids, especially rhamnolipids. Besides rhamnolipids *Pseudomonas* strains such as *Pseudomonas* sp. MIS38 have also been reported to produce arthrofactin, a lipopeptide type of biosurfactant. Other biosurfactants produced by *Pseudomonas* include viscosin produced by *Pseudomonas fluorescens*, putisolvin produced by *Pseudomonas putida* and amphisin produced by *Pseudomonas* sp. DSS73. A brief description of the known genetics and biosynthetic regulation of their production follows.

RHAMNOLIPID: A GLYCOLIPID BIOSURFACTANT

Rhamnolipid is a glycolipid biosurfactant produced by many strains of *Pseudomonas*. The structural and regulatory genes encoding the rhamnolipid synthesis pathway had been isolated and characterized in order to reveal the details of molecular biology of rhamnolipid production. The knowledge of the complex mechanisms involved in rhamnolipid synthesis thus facilitates the overproduction of these extra cellular compounds. Furthermore, the transfer of the relevant genes into other species allows the production of rhamnolipids in heterologous hosts under controlled conditions. High yield had been obtained on a an industrial scale by continuous cultivation under

optimized media and growth conditions and using refined methods of cell recycling, gas exchange and downstream processing (Daniels *et al.* 2004). The *rhl* quorum sensing system in *P. aeruginosa* regulates the production of rhamnolipid type of biosurfactants. Rhamnolipid 1 was obtained from *P. aeruginosa* KY 4025 culture grown on 10% alkane (Ochsner *et al.* 1996). *P. aeruginosa* S_7B_1 formed rhamnolipid 2 while growing on n-hexadecane and n-paraffin and it was also the first rhamnolipid to be identified (Itoh, 1971). Rhamnolipids 3 and 4 were synthesized by resting cells only (Hisatsuka *et al.* 1971; Syldatk *et al.* 1985a, Syldatk *et al.* 1985b).

Genetic details of rhamnolipid biosynthesis were obtained from genetic complementation of mutant strain of *P. aeruginosa* PG 201 with the wild type. Genes involved in rhamnolipid biosynthesis are plasmid-encoded. *rhl*A, B, R and I genes are required for production of rhamnolipids in heterologous host (Ochsner *et al.* 1995) and they are transcribed in 5′-*rhl*ABRI-3′ direction. According to a proposed biosynthetic pathway, rhamnolipid synthesis proceeds by two sequential glycosyl transfer reactions, each catalysed by a different rhamnosyltransferase (Burger *et al.* 1963). Rhamnolipid 1 synthesis is catalyzed by the enzyme rhamnosyltransferase 1, an *rhl*AB gene product, organized in one operon. Both genes are co-expressed from the same promoter and are essential for rhamnolipid synthesis. RhlA is presumably involved in the synthesis or transport of rhamnosyltransferase precursor substrates or in the stabilization of the RhlB protein (Ochsner *et al.* 1994). The second rhamnosyltransferase, encoded by *rhl*C, had been characterized and its expression had been shown to be co-ordinately regulated with rhlAB by the same quorum sensing system (Rahim *et al.* 2001). The *rhl*R and *rhl*I act as regulators of the rhlAB gene expression. RhlI protein forms N-acylhomoserine lactones, which act as autoinducers and influence RhlR regulator protein. A mutant of this bacterium with a defect in *rhl*I gene didn't produce rhamnolipids but production occurred on addition of synthetic N-acylhomoserine lactone. Induction of *rhl*AB depends on quorum-sensing transcription activator RhlR complexed with the autoinducer N-butyryl-homoserine lactone (C4-HSL). However the induction doesn't occur in the logarithmic phase of growth even in presence of RhlR and C4-HSL.

Pseudomonas aeruginosa produces wetting agents since these are required for colonizing surfaces by swarming motility. *rhl*A and *rhl*B mutants showed that swarming requires the expression of the *rhl*A gene but does not necessitate rhamnolipid production. It was also shown that if ammonium is used instead of nitrate as a nitrogen source along with an excess of available iron, it decreases *rhl*A expression and swarming motility (Deziel *et al.* 2003). Another quorum sensing system encoded by *las*R and *las*I has an influence on rhamnolipid biosynthesis. The *las* system is both a positive and a negative regulator of the *rhl* system (Pesci *et al.* 1997). The *lasI* and *rhlI* products are *N*-oxododecanoyl homoserine lactone (OdDHL, $3OC_{12}$HSL or PAI-1) (Pearson *et al.* 1994) and *N*-butyryl homoserine lactone (BHL, C4-HSL or PAI-2) respectively (Winson *et al.* 1995). The *las* system regulates the *rhl* system which in turn regulates rhamnolipid synthesis.

Rhamnolipid production is promoted by enhanced C/N ratio (Winson *et al.* 1995) and inhibited by higher iron concentration (Guerra-Santos *et al.* 1984; Guerra-Santos *et al.* 1986). It had been found that transcription of rhlAB genes involves σ54 and this is over-expressed under nitrogen limiting conditions.

ARTHROFACTIN

Arthrofactin produced by *Pseudomonas* sp. MIS38, is the most potent cyclic lipopeptide-type biosurfactant ever reported. Three genes termed *arf*A, *arf*B, and *arf*C form the arthrofactin synthetase gene cluster and encode ArfA, ArfB & ArfC which assemble to form a unique structure. ArfA, ArfB, and ArfC contain two, four, and five functional modules, respectively. (A module is defined as the unit that catalyzes the incorporation of a specific amino acid into the peptide product. The arrangement of the modules of a peptide synthetase is usually colinear with the amino acid sequence of the peptide. The modules can be further subdivided into different domains that are characterized by a set of short conserved sequence motifs.) Each module bears a condensation domain [C] (responsible for formation of peptide bond between two consecutively bound amino acids), adenylation domain [A] (responsible for amino acid recognition and adenylation at the expense of ATP) and thiolation domain [T] (serves as an attachment site of 4-phosphopantetheine cofactor and a carrier of thioesterified amino acid intermediates). However, none of the 11 modules possess the epimerization domain [E] responsible for the conversion of amino acid residues from L to D form. Moreover, two thioesterase domains are tandemly located at the C-terminal end of ArfC. *arf*B is the gene absolutely essential for arthrofactin production as its disruption impaired this act (Roongsawang *et al.* 2003).

VISCOSIN

Viscosin is produced by *Pseudomonas fluorescens* PfA7B. It acts as a wetting agent and thus the bacterium becomes able to adhere to the broccoli heads and cause decay of the wounded as well as unwounded florets of broccoli. Viscosin deficient mutants obtained by transposon mutagenesis were able to affect wounded broccoli florets but they are devoid of the ability to decay unwounded ones unlike the wild type bacterium. Triparental matings of these mutants with their corresponding wild-type clones and the helper *E.coli* HB101 (with the mobilizable plasmid pPK2013) yielded transconjugants. Their linkage maps indicated that a 25kb chromosomal DNA after transcription & translation forms three proteins which forms a synthetase complex and is required for viscosin production. A probe made from this DNA region hybridized with DNA fragments of other phytopathogenic pseudomonads to varying degrees (Braun *et al.* 2001).

AMPHISIN

Amphisin is produced by *Pseudomonas* sp. DSS73. It has both biosurfactant & antifungal properties and brings about the inhibition of plant pathogenic fungi. The two-component regulatory system GacA/GacS (GacA is a response regulator and GacS is a sensor kinase) controls the amphisin synthetase gene (*ams*Y) (Koch *et al.* 2002). The surface motility of this bacterium requires the production of this biosurfactant as is indicated by the mutants defective in the genes *gac*S and *ams*Y. Amphisin synthesis is regulated by *gac*S gene as the *gac*S mutant regains the property of surface motility upon the introduction of a plasmid encoding the heterologous wild-type *gac*S gene from *Pseudomonas syringae* (Andersen *et al.* 2003).

PUTISOLVIN

Pseudomonas putida PCL1445 produces two surface-active cyclic lipopeptides designated as putisolvins I and II. The ORF (open reading frame) encoding the synthesis of the putisolvins bears amino acid homology to various lipopeptide synthetases (Kuiper *et al.* 2004). Putisolvins are produced by a putisolvin synthetase designated as *pso*A. Three heat shock genes *dnaK, dnaJ* and *grpE* positively regulates the biosynthesis of putisolvin (Dubern *et al.* 2005). The ppuI-rsaL-ppuR quorum sensing system controls putisolvin biosynthesis. ppuI and ppuR mutants exhibit decreased putisolvin production whereas rsaL mutants show enhanced putisolvin production (Dubern *et al.* 2006).

Biosurfactants from *Acinetobacter* species

Acinetobacter species are known to produce high molecular weight biosurfactants - Emulsan and Alasan. The RAG-1 emulsan of *Acinetobacter* is a noncovalently linked complex of a lipoheteropolysaccharide and a protein. The polysaccharide part called as apoemulsan consists of various sugar components such as D-galactosamine, D-galactosaminuronic acid and diamino-dideoxy glucosamine. The fatty acids make 12% of this biopolymer and make it amphipathic in nature. The BD4 emulsan of *Acinetobacter calcoaceticus* BD4 consists of a repeating heptasaccharide unit comprising L-rhamnose, D-glucose, D-glucuronic acid and D-mannose in molar ratios of 4:1:1:1. On the other hand Alasan produced by *Acinetobacter radioresistens* is an anionic, high molecular weight, alanine containing heteropolysaccharide and protein. A brief description of the biosynthetic regulation of their production is presented in the following section.

ALASAN BIOSYNTHESIS

Acinetobacter radioresistens KA53 produces alasan, a complex of an anionic polysaccharide containing covalently bound alanine (apoalasan) and three proteins. It is released into the extracellular fluid during the stationary phase of the life cycle of this bacterium. Preparative SDS-PAGE of the alasan complex yielded three proteins (AlnA, AlnB and AlnC) of which one i.e. AlnA is of 45kDa in which all the emulsification activity was concentrated. *aln*A gene encodes this protein. Recombinant protein AlnA had an amino acid sequence homologous to that of *E. coli* OmpA. However, *E. coli* OmpA has no significant emulsifying activity, whereas AlnA has a specific emulsifying activity higher than that of alasan (Toren *et al.* 2002). The gene encoding AlnB was cloned, sequenced and overexpressed in *E. coli*. Recombinant AlnB had no emulsifying activity but stabilized oil-in-water emulsion generated by AlnA. AlnB amino acid sequence has strong homology to the family of antioxidant enzymes known as peroxiredoxins thus expression of AlnB protects *E. coli* from toxic concentrations of organic peroxide. It has been suggested that the bacterium releases AlnA, AlnB and AlnC together as a complex under stressed conditions. The genetic detail of AlnC is awaited to provide further information about the mode of action of alasan (Rosenberg *et al.* 2005).

GENETICS OF EMULSAN BIOSYNTHESIS

Acinetobacter lwoffii RAG-1 produces a potent bioemulsifier, emulsan. The logarithmic phase cells of this bacterium secrete this compound as a minicapsule on the cell surface which is however released into the medium as a protein-polysaccharide complex when the cells reach the stationary state. This release is caused by an esterase which if removed, a polymer called apoemulsan is formed which can't bring about the emulsification of non-polar, hydrophobic, aliphatic materials (Zosim *et al.* 1986).

A 27kb gene cluster termed *wee* encodes the genes (*wza, wzb, wzc, wzx, wzy*) required for emulsan biosynthesis (Nakar and Gutnick, 2001). It was later demonstrated that Wzc and Wzb are a protein tyrosine kinase and protein tyrosine phosphatase, respectively and deletion in either of the two genes gave rise to an emulsan-defective phenotype (Nakar and Gutnick, 2003).

Acinetobacter venetianus RAG1 also forms emulsan. Removal of the protein fraction yields apoemulsan, which exhibits much lower emulsifying activity on hydrophobic substrates such as n-hexadecane. The genes encoding the biosynthetic enzymes required for the synthesis of apoemulsan had been cloned and sequenced. One key protein associated with the emulsan complex is a cell surface esterase. The esterase was cloned and overexpressed in *Escherichia coli* BL21 (DE3) behind the phage T7 promoter with the His tag system. After overexpression, most of the protein was found in inclusion bodies. Both the mixture of apoemulsan with the catalytically active soluble form of the recombinant esterase isolated from cell extracts or the solubilized inactive form of the enzyme recovered from the inclusion bodies, formed stable oil-water emulsions with very hydrophobic substrates such as hexadecane under conditions in which emulsan itself was ineffective. A series of esterase-defective mutants was also generated by site-directed mutagenesis, cloned and overexpressed in *E. coli*. Mutant proteins defective in catalytic activity as well as others apparently affected in protein conformation were also active in enhancing the apoemulsan-mediated emulsifying activity (Bach *et al.* 2003).

Biosurfactants from *Serratia* species

Serratia, a group of gram negative bacteria produces surface active cyclodepsipeptides known as serrawettin W1, W2 and W3 (Matsuyama *et al.* 1986; Matsuyama *et al.* 1989). Different strains of *Serratia marcescens* produces these different serrawettins e. g. Serrawettin W1 is produced by strains 274 and ATCC 13880 or NS 38, W2 is produced by strain NS 25 and W3 is produced by strain NS 45. Besides this *Serratia liquefaciens* produces serrawettin W2. Temperature dependant synthesis of two novel lipids – rubiwettin R1 and RG1 is observed in *Serratia rubidaea* (Matsuyama *et al.* 1990).

SERRAWETTIN BIOSYNTHESIS

Serratia marcescens forms a biosurfactant serrawettin W1. A single gene *psw*P is responsible for the production of this biosurfactant. This gene has a high homology with genes of the NRPSs (non-ribosomal peptide synthetases) family. A single muta-

tion in this gene results in the failure to produce the biosurfactant (Sunaga *et al.* 2004). Another serrawettin W1 synthetase putative gene *swrW* was identified through genetic analysis of serrawettin-less mutants of *Serratia marcescens* 274. Homology analysis of this gene demonstrated the presence of condensation, adenylation, thiolation and thioesterase domains characteristic of non-ribosomal peptide synthetases (NRPS). This putative serrawettin synthetase gene was uni-modular in contrast to multi-modular nature of NRPS. This presumed that SwrW may be the simplest enzyme in the NRPS family (Li *et al.* 2005).

Serratia liquefaciens MG1 forms a biosurfactant, serrawettin W2. Its synthesis is catalyzed by a peptide synthetase which is encoded by *swr*A gene. The population density is sensed by a homoserine lactone-dependent quorum-sensing system consisting of *swr*I and *swr*R genes. The *swr*I gene product catalyzes the formation of N-butanoyl-L-homoserine lactone (BHL) and N-hexanoyl-L-homoserine lactone (HHL). The *swr*I mutant MG44, which is defective in BHL and HHL synthesis, is impaired in surfactant production. However, addition of exogenous BHL to the growth medium restores surfactant production. This strongly suggests that the quorum-sensing mechanism controls the production of a biosurfactant (Lindum *et al.* 1998).

Fungal biosurfactants

Various fungi secrete glycolipid type of surface active agents but the genetic basis of their production is largely unknown. Mannosylerythritol lipids (MEL) were first isolated from the dimorphic fungus *Ustilago maydis* and were also detected later in *Candida antarctica, Schizonella melanogramma, and Geotrichum candidum.* Sophorose lipids are secreted by *Candida bombicola.*

Ustilago maydis produces two kinds of glycolipid biosurfactants, mannosylerythritol lipid (MEL) referred to as ustilipids (Uchida *et al.* 1989) and ustilagic acid that are cellobiose lipids. These compounds are secondary metabolites as is indicated by the fact that the mutants generated by the deletion of the genes involved in their production are not lethal. *emt*1 and *cyp*1 are the two genes involved in the synthesis of these glycolipids. *emt*1 is for MEL synthesis and *cyp*1 is for ustilagic acid production. It is assumed that Cyp1 is involved in terminal and/or sub-terminal hydroxylation of an unusual fatty acid 15,16-dihydroxyhexadecanoic acid which is present in ustilagic acid.

Trichoderma reesei forms hydrophobins, which are low molecular weight proteins having high cysteine content and high surface and amphiphilic properties, which is in the level of commercial synthetic surfactants and other biosurfactants. *hfb*1 and *hfb*2 are the genes regulating the synthesis of hydrophobins. HFBI was shown to be rather unstable to N-terminal asparagine deamidation and also in some extent to non-specific proteases while its thermostability was excellent (Askolin *et al.* 2001).

Concluding remarks

The commercialization of microbial surfactants like any other biotechnological product is dependant on its production economics which in turn depends on the final yield. At present, the prices of these molecules are not competitive to that of their chemically

synthesized counterparts. As these molecules have emerged as potential agents in many industrial and environmental processes as well as in biomedical and therapeutic applications, it is essential to make them cost competitive. Genetically engineered hyper producing organisms giving high yields can bring the real breakthrough in the production process. This is possible only if the genetics of the microbial surfactant production is known in details. It is therefore desirable that the future research on biosurfactants be focused on the development and use of hyperproducers. The detailed knowledge of the genetics of microbial surfactant production should be used to produce organisms giving higher production with better product characteristics. With a better knowledge of the genes involved in this process, biosurfactant production can be realized in non-pathogenic industrial strains. The knowledge of expression of genes of a particular biosurfactant producer in a particular habitat will also throw light upon substrate dependence of production and preference for a particular substrate. Detailed description of the genetics of production of the newly identified biosurfactants like flavolipids, tensin and lokisin is not available. Efforts should be made by investigators to develop high yielding strains of microorganisms producing strong biosurfactants such as arthrofactin and lichenysin. A few marine biosurfactants have been discovered (Kalinovskaya *et al.* 2004) and there is high possibility of finding many other novel surface active compounds from the marine sources. Many of these are expected to possess interesting properties as pharmaceuticals and biomedical agents. The knowledge of molecular genetics of microbial surfactant production and its subsequent use to produce hyperproducers will determine the fate of biosurfactant industry.

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References

Andersen, J.B., Koch, B., Nielsen, T.H., Sørensen, D., Hansen, M. *et al*. (2003) Surface motility in *Pseudomonas* sp. DSS73 is required for efficient biological containment of the root-pathogenic microfungi *Rhizoctonia solani* and *Pythium ultimum*. *Microbiology* **149**, 37–46

Arima, K., Kakinuma, A. and Tamura, G. (1968) Surfactin, a crystalline peptide lipid surfactant produced by *Bacillus subtilis*: isolation, characterization and its inhibition of fibrin clot formation. *Biochemical Biophysical Research Communications* **31**, 488 -494

Askolin, S., Nakari-Setälä, T. and Tenkanen, M. (2001) Overproduction, purification, and characterization of the *Trichoderma reesei* hydrophobin HFBI. *Applied Microbiology Biotechnology* **57**, 124-130

Bach, H., Berdichevsky, Y. and Gutnick, D. (2003) An exocellular protein from the oil-degrading microbe *Acinetobacter venetianus* RAG-1 enhances the emulsifying activity of the polymeric bioemulsifier emulsan. *Applied and Environmental Microbiology* **69**, 2608-2615

Banat, I. (2000). Potential commercial applications of microbial surfactants. *Applied Microbiology Biotechnology* **53**, 495-508

Benincasa, M., Contiero, J., Manresa, M.A. and Moreaes, I.O. (2002) Rhamnolipid production by *Pseudomonas aeruginosa* LBI growing on soapstock as the sole carbon source. *Journal of Food Engineering* **54**, 283-288

Bodour, A.A., Guerrero-Barajas, C., Jiorle, B.V., Malcomson, M.E., Paull, A.K., Somogyi, A., Trinh, L.N., Bates, R.B. and Maier, R.M. (2004) Structure and characterization of flavolipids, a novel class of biosurfactants produced by *Flavobacterium* sp. strain MTN11. *Applied and Environmental Microbiology* **70(1)**, 114-120

Braun, P.G., Hildebrand, P.D., Ells, T.C. and Kobayashi, D.Y. (2001) Evidence and characterization of a gene cluster required for the production of viscosin, a lipopeptide biosurfactant, by a strain of *Pseudomonas fluorescens*. *Canadian Journal of Microbiology* **47**, 294-301

Burger, M.M., Glaser, L. and Burton, R.M. (1963) The enzymatic synthesis of a rhamnose-containing glycolipid by extracts of *Pseudomonas aeruginosa. Journal of Biological Chemistry* **238**, 2595–2601

Cameotra, S. and Makkar, R. (2004) Recent applications of biosurfactants as biological and immunological molecules. *Current Opinion in Microbiology* **7**, 262-266

Cirigliano, M.C. and Carman, G.M. (1985) Purification and characterizafton of liposan, a bioemulsifier from *Candida lipolytica. Applied and Environmental Microbiology* **50**, 846-850

Cosby, W.M., Vollenbroich, D., Lee, O.H. and Zuber, P. (1998) Altered *srf* expression in *Bacillus subtilis* resulting from changes in culture pH is dependent on the Spo0K oligopeptide permease and the ComQX system of extracellular control. *Journal of Bacteriology* **180**, 1438-1445

Daniels, R., Vanderleyden, J. and Michiels, J. (2004) Quorum sensing and swarming migration in bacteria. *FEMS Microbiology Reviews* **28**, 261-289

Das, P., Mukherjee, S. and Sen, R. (2008). Antimicrobial potential of a lipopeptide biosurfactant derived from a marine *Bacillus circulans*. *Journal of Applied Microbiology* (doi:10.1111/j.1365-2672.2007.03701.x)

Desai, J.D. and Banat, I.M. (1997) Microbial production of surfactants and their commercial potential. *Microbiology Molecular Biology Reviews* **61**, 47-64

Deshpande, M. and Daniels, L. (1995) Evaluation of sophorolipid biosurfactant production by *Candida bombicola* using animal fat. *Bioresource Technology* **54**, 143-150

Deziel, E., Lepine, F., Milot, S. and Villemur, R. (2003) *rhl*A is required for the production of a novel biosurfactant promoting swarming motility in *Pseudomonas aeruginosa*: 3-(3-hydroxyalkanoyloxy) alkanoic acids (HAAs), the precursors of rhamnolipids. *Microbiology* **149**, 2005-2013

Dubern, J.F., Lagendijk, E.L., Lugtenberg, B.J.J. and Bloemberg, G.V. (2005) The heat shock genes *dna*K, *dna*J, and *grp*E are involved in regulation of putisolvin biosynthesis in *Pseudomonas putida* PCL1445. *Journal of Bacteriology* **187**, 5967-5976

Dubern, J.F., Lugtenberg, B.J.J. and Bloemberg, G.V. (2006) The ppuI-rsaL-ppuR quorum sensing system regulates biofilm formation of *Pseudomonas putida* PCL 1445 by controlling biosynthesis of the cyclic lipopeptides putisolvins I and II.

Journal of Bacteriology **188 (8)**, 2898-2906

Dubey, K. and Juwarkar, A. (2001) Distillery and curd whey wastes as viable alternative sources for biosurfactant production. *World Journal of Microbiology and Biotechnology* **17**, 61-69

Franzetti, A., Bestett, G., Caredda, P., La Colla P. and Tamburini, E. (2008) Surface-active compounds and their role in the access to hydrocarbons in *Gordonia* strains. *FEMS Microbiology Ecology* **63(2)**, 238-248

Guerra-Santos, L.H., Kappeli, O. and Fiechter, A. (1984) *Pseudomonas aeruginosa* biosurfactant production in continuous culture with glucose as carbon source. *Applied and Environmental Microbiology* **48**, 301–305

Guerra-Santos, L.H., Kappeli, O. and Fiechter, A. (1986) Dependence of *Pseudomonas aeruginosa* continuous culture biosurfactant production on nutritional and environmental factors. *Applied Microbiology Biotechnology* **24**, 443–448

Gurjar, M., Khire, J.M. and Khan, M.I. (1995) Bioemulsifier production by *Bacillus stearothermophilus* VR-8 isolate. *Letters in Applied Microbiology* **21(2)**, 83-86

Haba, E., Espuny, M.J., Busquets, M. and Manresa, A. (2000) Screening and production of rhamnolipids by *Pseudomonas aeruginosa* 47T2 NCIB 40044 from waste frying oils. *Journal of Applied Microbiology* **88**, 379-387

Hasumi, K., Takizawa, K., Takahashi, F., Park, J.K. and Endo, A. (1995) Inhibition Acyl-CoA: Cholestrol acyltransferase by isohalobacillin, a complex of novel cyclic acylpeptides produced by *Bacillus* sp. A1238. *Journal of Antibiotics*. **48**, 1419-1424

He, H., Shen, B., Korshalla J. and Carter, G.T. (2001) Circulocins, new antibacterial lipopeptides from *Bacillus circulans*, J2154. *Tetrahedron* **57**, 1189-1195

Hisatsuka, K., Nakahara, T., Sano, N. and Yamada, K. (1971) Formation of rhamnolipid by *Pseudomonas aeruginosa* and its function in hydrocarbon fermentation. *Agricultural and Biological Chemistry* **35**, 686-692

Horowitz, S. and Currie, J.K. (1990) Novel dispersants of silicon and aluminum nitride. *Journal of Dispersion Science and Technology* **11**, 637-59

Ito, S., Kinta, M. and Inoue, S. (1980) Growth of yeasts on n-alkanes: Inhibition by a lactonic sophorolipid produced by *Torulopsis bombicola. Agricultural Biological Chemistry* **44**, 2221-2223

Itoh, S. (1971) Rhamnolipids produced by *Pseudomonas aeruginosa* grown on n-paraffin. *Journal of Antibiotics* **24**, 855-859

Itoh, S. and Suzuki, T. (1974) Fructose-lipids of *Arthrobacter, Corynebacteria, Nocardia* and *Mycobacteria* grown on fructose. *Agricultural Biological Chemistry* **38**, 1443-1449

Kalinovskaya, N.I., Ivanova, E.P., Alexeeva, Y.V., Gorshkova, N.M., Kuznetsova, T.A. *et al*. (2004) Low-molecular-weight, biologically active compounds from marine *Pseudoalteromonas* sp. *Current Microbiology* **48**, 441-446

Kitamoto, D., Akiba, S., Hioki, C. and Tabuchi, T. (1990a) Extracellular accumulation of mannosylerythritol lipids by a strain of *Candida antarctica. Agricultural Biological Chemistry* **54**, 31-36

Kobayashi, T., Ito, S. and Okamoto, K. (1987) Production of Mannosylerythritol by *Candida* sp. KSM-1529. *Agricultural Biological Chemistry* **51**, 1715-171

Koch, B., Nielsen, T.H., Sorensen, D., Andersen, J.B. and Christophersen, C. (2002) Lipopeptide production in *Pseudomonas* sp. DSS73 is regulated by components of

sugar beet seed exudate via the Gac two-component regulatory system. *Applied and Environmental Microbiology* **68,** 4509-4516

Kuiper, I., Lagendijk, E.L., Pickford, R., Derrick, J.P., Lamers, G.E.M., Thomas-Oates, J.E., Lugtenberg, B.J.J. and Bloemberg, G.V. (2004) Characterization of two *Pseudomonas putida* lipopeptide biosurfactants, putisolvin I and II, which inhibit biofilm formation and break down existing biofilms. *Molecular Microbiolgy* **51 (1)**, 97-113

Lee, S.C., Kim, S.H., Park, I.H., Chung, S.Y. and Choi, Y.L. (2007) Isolation and structural analysis of bamylocin A, novel lipopeptide from *Bacillus amyloliquefaciens* LP03 having antagonistic and crude oil-emulsifying activity. *Archives of Microbiology* **188**, 307–312

Li, H., Tanikawa, T., Sato, Y., Nakagawa, Y and Matsuyama, T. (2005) *Serratia marcescens* gene required for surfactant serrawettin W1 production encodes putative aminolipid synthetase belonging to nonribosomal peptide synthetase family. *Microbiology and Immunology* **49**, 303-10

Liu, L., Nakano, M., Lee, O.H. and Zuber, P. (1996) Plasmid-amplified *com*S enhances genetic competence and suppresses *sin*R in *Bacillus subtilis*. *Journal of Bacteriology* **178**, 5144-5152

Luttinger, A., Hahn, J. and Dubnau, D. (1996) Polynucleotide phosphorylase is necessary for competence development in *Bacillus subtilis*. *Molecular Microbiology* **19**, 343-356

Makkar, R.S. and Cameotra, S.C. (1999) Biosurfactant production by microorganisms on unconventional carbon sources. *Journal of Surfactants and Detergents* **2(2)**, 237–241

Marahiel, M.A., Konz, D. and Dokel, S. (1999) Molecular and biochemical characterization of the protein template controlling biosynthesis of the lipopeptide lichenysin of *Bacillus licheniformis*. *Journal of Bacteriology* **181**, 133-140

Matsuyama, T., Kameda, K. and Yano, I. (1986) Two kinds of bacterial wetting agents: aminolipid and glycolipid. *Proceedings of Japan Society of Mass Spectrometry* **11**, 125-128

Matsuyama, T., Keneda, K., Ishizuka, I., Toida, T. and Yano, I. (1990) Surface-active novel glycolipid and linked 3-hydroxy fatty acids produced by *Serratia rubidaea*. *Journal of Bacteriology* **172(6)**, 3015-3022

Matsuyama, T., Sogawa, M. and Nakagawa, Y. (1989) Fractal spreading growth of *Serratia marcescens* which produces surface-active exolipids. *FEMS Microbiology Letters* **61**, 243-246

Menkhaus, M., Ullrich, C., Kluge, B., Vater, J., Vollenbroich, D. *et al*. (1993) Structural and functional organization of the surfactin synthetase multienzyme system. *Journal of Biological Chemistry* **268**, 7678-7684

Mercade, M.E., Manresa, M.A., Robert, M., Espuny, M.J., de Andres. C. and Guinea, J. (1993) Olive oil mill effluent (OOME). New substrate for biosurfactant production. *Bioresource Technology* **43**, 1-6

Morikawa, M., Daido, H., Takao, T., Murata, S., Shimonishi, Y. and Imanaka, T. (1993) A new lipopeptide biosurfactant produced by *Arthrobacter* sp. strain MIS38. *Journal of Bacteriology* **175(20)**, 6459-6466

Morita, T., Konishi, M., Fukuoka, T., Imura, T. and Kitamoto, D. (2007) Microbial conversion of glycerol into glycolipid biosurfactants, mannosylerythritol lipids, by

a basidiomycete yeast, *Pseudozyma antarctica* JCM 10317^{T}. J*ournal of Bioscience and Bioengineering* **104 (1)**, 78–81

Mukherjee, S., Das, P. and Sen, R. (2006) Towards commercial production of microbial surfactants. *Trends in Biotechnology* **24**, 509-514

Mulligan, C.N. (2005) Environmental applications for biosurfactants. *Environmental Pollution* **133**, 183-198

Nakar, D. and Gutnick, D.L. (2001) Analysis of the *wee* gene cluster responsible for the biosynthesis of the polymeric bioemulsifier from the oil-degrading strain *Acinetobacter lwoffii* RAG-1. *Microbiology* **147**: 1937-1946

Nakar, D. and Gutnick, D.L. (2003) Involvement of a protein tyrosine kinase in production of the polymeric bioemulsifier emulsan from the oil-degrading strain *Acinetobacter lwoffii* RAG-1. *Journal of Bacteriology* **185**, 1001–1009

Neu, T.R. and Poralla, K. (1990) Emulsifying agents from bacteria isolated during screening for cells with hydrophobic surfaces. *Applied Microbiology Biotechnology* **32**, 521–525

Nitschke, M. and Pastore, G.M. (2003) Cassava flour wastewater as a substrate for biosurfactant production. *Applied Biochemistry Biotechnology* **106**, 295–302

Nitschke, M. and Pastore, G.M. (2006) Production and properties of a surfactant obtained from *Bacillus subtilis* grown on cassava wastewater. *Bioresource Technology* **97**, 336–341

Nitschke, M., Costa, S.G.V.A.O., Haddad, R., Gonsalves, L.A.G., Eberlin, M.N. *et al*. (2005) Oil wastes as unconventional substrates for rhamnolipid biosurfactant production by *Pseudomonas aeruginosa* LBI. *Biotechnology Progress* **21**, 1562 -1566

Ochsner, U.A., Fiechter, A. and Reiser, J. (1994) Isolation, characterization, and expression in *Escherichia coli* of the *Pseudomonas aeruginosa rhl*AB genes encoding a rhamnosyltransferase involved in rhamnolipid biosurfactant synthesis. *Journal of Biological Chemistry* **269**, 19787-19795

Ochsner, U.A., Fiechter, A., Reiser, J. and Witholt, B. (1995) Production of *Pseudomonas aeruginosa* rhamnolipid biosurfactants in heterologous hosts. *Applied and Environmental Microbiology* **61**, 3503-3506

Ochsner, U.A., Hembach, T. and Fiechter, A. (1996) Production of rhamnolipid biosurfactants. *Advances in Biochemical Engineering and Biotechnology* **53**, 89-118

Panilaitis, B., Castro, G.R., Solaiman D. and Kaplan, D.L. (2006) Biosynthesis of emulsan biopolymers from agro-based feedstocks. *Journal of Applied Microbiology* doi:10.1111/j.1365-2672.2006.03078.x

Patel, R.M. and Desai, A.J. (1997) Biosurfactant production by *Pseudomonas aeruginosa* GS3 from molasses. *Letters in Applied Microbiology* **25**, 91–94

Pearson, J.P., Gray, K.M., Passador, L., Tucker, K.D. and Eberhard, A. (1994) Structure of the autoinducer required for the expression of *Pseudomonas aeruginosa* virulence genes. *Proceedings of the National Academy of Sciences of United States of America* **91**, 197-201

Persson, A., Osterberg, E. and Dostalek, M. (1988) Biosurfactant production by *Pseudomonas fluorescens* 378: growth and product characteristics. *Applied Microbiology Biotechnology* **29**, 1-4

Pesci, E.C., Pearson, J.P., Seed, P.C. and Iglewski, B.H. (1997) Regulation of *las* and

rhl quorum sensing in *Pseudomonas aeruginosa*. *Journal of Bacteriology* **179**, 3127-3132

Peypoux, F., Bonmatin, J.M. and Wallach, J. (1999) Recent trends in the biochemistry of surfactin. *Applied Microbiology Biotechnology* **51**, 553-563

Rahim, R., Ochsner, U.A., Oliveira, C., Graninger, M., Messner, P. *et al*. (2001) Cloning and functional characterization of the *Pseudomonas aeruginosa rhl*C gene that encodes rhamnosyltransferase 2, an enzyme responsible for di-rhamnolipid biosynthesis. *Molecular Microbiology* **40**, 708–718

Rahman, M.S., Ano, T. and Shoda, M. (2006) Second stage production of iturin A by induced germination of *Bacillus subtilis* RB14. *Journal of Biotechnology* **125(4)**, 513-515

Raza, Z.A., Khan, M.S. and Khalid, Z.M. (2007) Evaluation of distant carbon sources in biosurfactant production by a gamma ray-induced *Pseudomonas putida* mutant. *Process Biochemistry* **42**, 686–692

Robert, M., Mercade, M.E., Bosch, M.P., Parra, J.L., Espuny, M.J., Manresa, M.A. and Guinea, J. (1989) Effect of the carbon source on biosurfactant production by *Pseudomonas aeruginosa* 44Ti. *Biotechnology Letters* **11**, 871–874

Rodrigues, L., Banat, I.M., Teixeira, J. and Oliveira, R. (2006) Biosurfactants: potential applications in medicine. *Journal of Antimicrobial Chemotherapy* **57**, 609-618

Ron, E.Z. and Rosenberg, E. (2001) Natural roles of biosurfactants. *Environmental Microbiology* **3**, 29-236

Roongsawang, N., Hase, K., Haruki, M., Imanaka, T., Morikawa, M. *et al*. (2003) Cloning and characterization of the gene cluster encoding arthrofactin synthetase from *Pseudomonas* sp. MIS38. *Chemistry and Biology* **10**, 869–880

Rosenberg, E., Bekerman, R., Segal, G. and Ron, E.Z. (2005) The AlnB protein of the bioemulsan alasan is a peroxiredoxin. *Applied Microbiology Biotechnology* **66**, 536–541

Sen, R. (1997) Response surface optimization of the critical media components for production of surfactin. *Journal of Chemical Technology Biotechnology* **68**, 263–270

Sen, R. and Swaminathan, T. (1997) Application of response-surface methodology to evaluate the optimum environmental conditions for the enhanced production of surfactin. *Applied Microbiology Biotechnology* **47**, 358–363

Sen, R. and Swaminathan, T. (2004) Response surface modeling and optimization to elucidate the effects of inoculum age & size on surfactin production. *Biochemical Engineering Journal* **21**, 141–148

Shabtai, Y. (1990) Production of exopolysaccharides by *Acinetobacter* strains in a controlled fed-batch fermentation process using soap stock oil (SSO) as carbon source. *International Journal of Biological Macromolecules*. **12**, 145-152

Singh, P. and Cameotra, S.S. (2004) Potential applications of microbial surfactants in biomedical sciences. *Trends in Biotechnology* **22**, 142-146

Sunaga, S., Li, H., Sato, Y., Nakagawa, Y. and Matsuyama, T. (2004) Identification and characterization of the *psw*P gee required for the parallel production of prodigiosin and serrawettin W1 in *Serratia marcescens*. *Microbiology and Immunology* **48**, 723-728

Syldatk, C., Lang, S., Matulovic, U. and Wagner, F. (1985a) Chemical and physical characterization of four interfacial-active rhamnolipids from *Pseudomonas* sp. DSM

2874 grown on n- alkanes. *Z Naturforsch* **40**, 51-60

Syldatk, C., Lang, S., Wagner, F., Wray, V. and Witte, L. (1985b) Production of four interfacial active rhamnolipids from n-alkanes or glycerol by resting cells of *Pseudomonas* sp. DSM 2874. *Z Naturforsch* **40**: 61-67

Thaniyavarn, J., Roongsawang, N., Kameyama, T., Haruki, M., Imanaka, T., Morikawa, M. and Kanaya, S. (2003) Production and characterization of biosurfactants from *Bacillus licheniformis* F2.2. *Bioscience Biotechnology Biochemistry* **67**(6), 1239-1244

Toren, A., Orr, E., Paitan, Y., Ron, E.Z. and Rosenberg, E. (2002) The active component of the bioemulsifier alasan from *Acinetobacter radioresistens* KA53 is an OmpA-like protein. *Journal of Bacteriology* **184**, 165-170

Tran, H., Ficke, A., Asiimwe, T., Höfte, M. and Raaijmakers, J.M. (2007) Role of the cyclic lipopeptide massetolide A in biological control of *Phytophthora infestans* and in colonization of tomato plants by *Pseudomonas fluorescens*. *New Phytologist* doi: 10.1111/j.1469-8137.2007.02138.x

Trischmann, J.A., Jensen, P.R. and Fenical, W. (1994) Halobacillin: a cytotoxic cyclic acylpeptide of the iturin class produced by a marine *Bacillus*. *Tetrahedron Letters* **35**, 5571-5574

Tsuge, K., Akiyama, T. and Shoda, M. (2001) Cloning, sequencing and characterization of the iturin A operon. *Journal of Bacteriology* **183**, 6265-6273

Uchida, Y., Tsuchiya, R., Chino, M, Hirano, J. and Tabuchi,, T. (1989) Extracellular accumulation of mono- and di-succinoyl trehalose lipids by a strain of *Rhodococcus erythropolis* grown on n-alkanes. *Agricultural Biological Chemistry* **53**, 757-763

Vance-Harrop, M.H., de Gusmão, N.B. and de Campos-Takaki, G.M. (2003) New bioemulsifiers produced by *Candida lipolytica* using D-Glucose and Babassu oil as carbon sources. *Brazilian Journal of Microbiology* **34**, 120-123

Venkataramana, K. and Karanth, N.G. (1989) Factors affecting biosurfactant production using *Pseudomonas aeruginosa* CFTR-6 under submerged conditions. *Journal of Chemical Technology Biotechnology* **45**, 249–257

Winson, M.K., Camara, M., Latifi, A., Foglino, M., Chhabra, S.R. *et al*. (1995) Multiple N-acyl-l-homoserine lactone signal molecules regulate production of virulence determinants and secondary metabolites in *Pseudomonas aeruginosa*. *Proceedings of the National Academy of Sciences of United States of America* **92**, 9427-9431

Wu, J.Y., Yeh, K.L., Lu, W.B., Lin, C.L. and Chang, J.S. (2008) Rhamnolipid production with indigenous *Pseudomonas aeruginosa* EM1 isolated from oil-contaminated site. *Bioresource Technology* **99**, 1157-1164

Yakimov, M.M., Abraham, W.-R., Meyer, H., Giuliano, L. and Golyshin, P.N. (1999) Structural characterization of lichenysin A components by fast atom bombardment tandem mass spectrometry. *Biochimica Biophysica Acta* **1438**, 273-80

Yakimov, M.M., KroÈger, A., Slepak, T.N., Giuliano, L., Timmis, K.N. and Golyshin, P.N. (1998) A putative lichenysin A synthetase operon in *Bacillus licheniformis*: initial characterization. *Biochimica Biophysica Acta* **1399**, 141-53

Yakimov, M.M., Timmis, K.N., Wray V. and Fredrickson, H.L. (1995) Characterization of a new lipopeptide surfactant produced by thermotolerant and halotolerant subsurface *Bacillus licheniformis* BAS50. *Applied and Environmental Microbiology* **61**, 1706-1713

Yu, G.Y., Sinclair, J.B., Hartman, G.L. and Bertagnolli, B.L. (2002) Production of

iturin A by *Bacillus amyloliquefaciens* suppressing *Rhizoctonia solani*. *Soil Biology and Biochemistry* **7**, 955-963

ZOSIM, Z., ROSENBERG, E. AND GUTNICK, D.L. (1986) Changes in emulsification specificity of the polymeric bioemulsifier emulsan: effects of alkanols. *Colloid and Polymer Science* **264**, 218-223