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Response of *Paenibacillus polymyxa* to Iron: Alternations in Cellular Chemical Composition and the Production of Fusaricidin Type Antimicrobial Compounds

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ABSTRACT

In this work, growth, cellular chemical composition and production of fusaricidin type antimicrobial compounds by P. polymyxa SQR-21 were compared in tryptone broth supplemented with four concentrations of iron (25, 50, 100 and 200 µM). The data revealed that the growth of P. polymyxa SQR-21 was increased by 3-8% with the increase in concentration of ferric ion (Fe^{3+}). The production of fusaricidin type compounds was increased by 33-49% only up to 50 µM Fe^{3+} and the highest level of Fe^{3+} was inhibitory. Increase in the liquid culture Fe^{3+}concentration increased the intracellular protein (2%), intracellular carbohydrate (14%), extracellular protein (7%) and polysaccharide contents (18%) while the intracellular lipid contents were increased (11%) only up to 50 µM Fe^{3+}. In addition, the regulatory effects of Fe^{3+} were also reflected by the increase in total RNA contents and relative expression of the fusaricidin synthetase gene (FusA) by 3-13 and 35-56%, respectively, up to 50 µM Fe^{3+}, after that a continuous decrease was observed.

Key words: Chemical composition, Fusaricidin, Iron; *Paenibacillus polymyxa*, RNA

INTRODUCTION

The *Paenibacillus polymyxa*, formerly *Bacillus polymyxa* (Ash et al., 1991), is a Gram-positive, aerobic or facultative anaerobic, rod-shaped, endospore-forming bacterium that is commonly found in many mineral deposits and in the rhizosphere (Raza et al., 2009; Zhang et al., 2008). *P. polymyxa* has become important biological control microorganism and has been top ranked among the applied microorganisms in commerce by US Environmental Protection Agency (EPA) in 2002 (Bent, 2002). As a member of the rhizosphere community, *P. polymyxa* may antagonize plant pathogenic microorganisms and, therefore, minimize the damage to the roots. The bacterium has long been known for its ability to produce two groups of antimicrobial compounds. One group comprises the antibiotics, active against bacteria, such as polymyxins, polypeptins etc. (Ito and Koyama, 1972a; 1972b). The other group is made up of the peptide antibiotics active against fungi and Gram-positive bacteria, such as gavaserin, saltavalin and fusaricidins A, B, C, and D (Pichard et al., 1995; Beatty and Jensen, 2002). In addition, there are many reports where the nature of the inhibitory agent is undefined (Dijksterhuis et al., 1999). In this work, *P. polymyxa* strain SQR-21 was used, found to produce fusaricidin type of antifungal compounds, composed of a group of

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cyclic depsipeptides with molecular masses of 883, 897, 948 and 961 Da, and an unusual15-guanidino-3-hydroxypentadecanoic acid moiety bound to a free amino group (Raza et al., 2009). *P. polymyxa* SQR-21 was evaluated for all the possible antimicrobial volatile and non-volatile metabolites and it was found that this strain did not produce any volatile antimicrobial compound, except fusaricidin type antifungal compounds. The fusaricidin production was optimized under different conditions, including temperature, pH and C sources, etc. (Raza et al., 2009). The fusaricidin biosynthetic gene cluster spans 32.4 kb, including an open reading frame and encodes a six-module nonribosomal peptide synthetase (Li and Jensen, 2008). Fusaricidin has excellent germicidal activity against plant pathogenic fungi such as *Fusarium oxysporum, Aspergillus niger, Aspergillus oryzae* and *Penicillum thomii* and particularly fusaricidin B has germicidal activity against *Candida albicans* and *Saccharomyces cerevisiae* (Kajimura and Kaneda, 1997; Raza et al., 2008; Ryu et al., 2006). Fusaricidin also has excellent germicidal activity against Gram-positive bacteria, including *Staphylococcus aureus* and *Leptosphaeria maculans* (Dijksterhuis et al., 1999; Beatty and Jensen, 2002). According to the recent reports on the excellent germicidal activity of fusaricidin against pathogenic Gram-positive bacteria and plant pathogenic fungi, fusaricidin seems to have great potential for industrial uses and thereby is in increasing demand. Therefore, it is desirable to find the ways to increase the production of fusaricidin.

In liquid culture, antibiotic production by many organisms is influenced by carbon and nitrogen sources, inorganic compounds and growth phase (Espeso and Penalva, 1992; Milner et al., 1995). Among these, iron has its own importance. Iron is known to be a "key" metal for secondary metabolite production in bacteria. It is perhaps the most important micronutrient used by bacteria and is required as a cofactor for a large number of enzymes and iron-containing proteins (Leong et al., 1990). The effect of iron on antibiotic production has been widely studied. In *Streptomyces* sp., iron is a requirement for the production of actinomycin, neomycin, streptomycin and chloramphenicol (Weinberg, 1970). An antifungal antibiotic, azasteroidal, from *Geotrichum flavo-brunneum* was enhanced by 10% with the addition of 0.2% iron (Boeck et al., 1975). The bacterial culture of *Nocardia mediterranea* A TCC 13685 was used for the production of antitubercular antibiotic rifamycin by submerged fermentation and it was observed that 100 ppm of iron had stimulatory effects on the rifamycin production (Mukhtiar, 2000). In addition, ferric iron (0.25-1.0 mM) enhanced zwittermicin A accumulation and disease suppression (Milner et al., 1995) and Kanosamine accumulation was enhanced by the addition of ferric iron to rich medium (Milner et al., 1996). However, there is no information regarding the effect of iron on fusaricidin production by *P. polymyxa*. A study was planned to determine the effect of ferric ion (Fe^{3+}) on the growth and fusaricidins production by *P. polymyxa* strain SQR-21. In addition, to get more knowledge about metabolic effects of iron in *P. polymyxa*, intra and extracellular chemical composition and total RNA contents were measured and fusaricidin gene expression was evaluated by reverser transcriptase and Real Time PCR assay.

MATERIAL AND METHODS

Bacteria and fungi strains

A chitinase deficient and fusaricidin producing strain of *Paenibacillus polymyxa* SQR-21 and a tested pathogenic strain *Fusarium oxysporum* f. sp. *cucumerinum* (*F. oxysporum*) were provided by the Soil-Microbe-Interaction Laboratory, Nanjing Agriculture University, Nanjing, China. The bacterial culture was maintained on potato dextrose agar (PDA) plates and was stored at -80°C in tryptic soya broth (TSB) containing 20% glycerol for further use. The fungal pathogenic strain was maintained by cultivation on PDA plates for three days at 28°C and then the plates were sealed with parafilm and stored at 4°C. The pathogen was subcultured onto a fresh PDA plate after one month.

Media preparation and antifungal activity assay

Liquid-culture experiments were performed in 100 ml of tryptone broth (tryptone; 10, NaCl; 5 and sucrose; 10 g/L; pH 7.2) in 500 ml Erlenmeyer flasks. Initial Fe^{3+} contents in tryptone broth were 10 µM, determined by Spectra AA, 220 FS atomic absorbance spectrometry. After sterilization, the cultures were supplemented with $FeCl_3$; four concentrations of Fe^{3+} (25, 50, 100 and 200 µM) were considered. Each experiment had three replicates, including the control cultures without supplemented Fe^{3+}. For the isolation of antifungal compounds, SQR-21 strain was pre-inoculated in tryptone broth overnight at 37°C. After adding $FeCl_3$, tryptone broth was inoculated with 100 µL of over night culture of SQR-21 and incubated in a shaking

incubator (170rpm, 37°C). After four days, OD_{600} and pH were determined and liquid cultures were centrifuged at 12000 × g for 10 min to remove the cells. The supernatants were pooled and active compounds were extracted twice with an equal volume of n-butanol. The extracts were concentrated by using a rotary evaporator and the residues were dissolved in methanol. These extracts were used to determine the antifungal activity by agar diffusion assay using *F. oxysporum* as test pathogen. After three days, the inhibition zone was measured. To represent the cellular data (protein, carbohydrate, lipid and RNA contents) on dry weight basis, the dry weights of lyophilized cell pellets were measured.

Cellular chemical composition

The bacterial liquid culture samples (2 ml) were centrifuged (12,000 x g) for 10 min. The cell pellets were suspended in 2 ml of deionized water for washing and then centrifuged three times. These pellets were used for the determination of total intracellular protein, carbohydrate and lipid contents separately. For total protein contents, the rinsed cells were resuspended in deionized water and incubated in 1 N NaOH at 90°C for 10 min to solubilize cellular protein. Proteins were measured by the method of Bradford (1976) with bovine serum albumin standards ranging from 10-100 µg/ml. Total carbohydrate contents were estimated in the rinsed-cell samples by the phenol-sulfuric acid method (Dubois et al., 1956). The lipid contents of bacterial cells were calculated by the phosphoric acid–vanillin reagent method of Izard and Limberger (2003) with Triolein standards ranging from 10 to 100 µg. The cell-free liquid culture was used for the estimation of extracellular protein and polysaccharide (EPS) contents by the above-described methods. For EPS estimation, the liquid culture was heated to boiling for 10 min to release the polysaccharides attached to the cells and to inactivate polysaccharide degrading enzymes then liquid culture was cooled and centrifuged to remove the cells. Before assaying the protein, the resulting EPS solution was dialyzed using a membrane of 1000 MWCO against ultra pure water for two days at 4 °C to remove the small molecules and entrained media residues. Protease activity of the liquid culture was assayed as described by Tseng and Mount (1974). One unit of activity was defined as the activity, which resulted an increase in OD of 0.1 OD units per 30 min per ml of enzyme. Cellulase activity was determined by the DNS method (Berlin et al., 2005). One unit cellulase activity was defined as the amount of enzyme that produced 1 µmol reduced sugar per hour.

RNA extraction and primers design

Total RNA was isolated by using the Trizol reagent method (Invitrogen™, Shanghai) according to manufacturer's instructions. To remove the contaminating DNA, 10U DNase1 (Takara, Dalian) along with 20U RNase inhibitor (Takara, Dalian) (37°C, 40 min) were used in the reaction mixture of 50 µl containing 20-50 µg RNA. RNA was estimated by determining the absorbance at 260 nm. Specific primers for fusaricidin synthetase gene (*fusA*) (111bp) and 16S rRNA gene (16s) (210bp) were designed by using the premier 5 software (PREMIER biosoft international). The designed primers were as follows:

fusA1, 5' GCAGAGGATGATAGTGTTGGTC 3',
fasA2, 5' CAGCACATCATGCGTTCC 3'.
16s1, 5' CATTCATCGTTTACGGCGT 3' and
16s2, 5' TGTTAATCCCGAGGCTCACT 3'.

Reverse transcription and Real Time PCR assay

For the synthesis of first stand cDNA, 3µg of total RNA, 200U of RevertAid™ M-MuLV reverse transcriptase (Fermantas), 20U RNase inhibitor (TaKaRa, Dalian), 0.2 µg of Random hexamer primer and 1mM dNTP in the total volume of 20µl were used. RT was performed using the following parameters; 5 min at 65°C, 2 min on ice, 60 min at 42°C and 5 min at 95°C. Target genes from cDNA were amplified separately using 3µl aliquots of RT product as template and 30 pmol of each primer pair. Reaction mixtures for PCR contained 2.5 U Taq polymerase (TaKaRa, Dalian), 20 nmol of dNTP and 100 nmol Mg^{2+}. The PCR conditions were as follows; 5 min at 95°C, 30 cycles; each including 30s at 94°C, 30s at 58°C and 1 min at 72°C and in the end 2 min at 72°C. Amplified products were checked for band intensity and cDNA quality in 2% agarose (w/v). Singleplex relative Real Time PCR was performed using an iCycler MyiQ™ single color Real Time PCR detection system (BioRad). Reactions were performed in a 20 µl volume reaction mixture containing 1 mM primers, 3µl cDNA and 10µl of SYBR®*Premix Ex Taq*™ (Perfect Real Time) (TaKaRa, Dalian), including TaKaRa *Ex Taq*™ HS and SYBR® Green I, dNTP and buffer. The PCR protocol included; 10 min at 95°C followed by 40 cycles with 95°C for 30 s, 58°C for 30 s and 72°C for 1 min. The detection of the fluorescent product was carried out at the end of the 72°C

extension period (2 min). After the PCR, these samples were heated from 58 to 95°C. When the temperature reached the Tm of each fragment, there was a steep decrease in fluorescence of the product. The 2 µl cDNA of each treatment were mixed together to prepare relative standards. The whole experiment was repeated twice.

Statistical Analysis

For the statistical analysis, Duncan's multiple-range test was applied when one-way ANOVA revealed significant differences ($P \leq 0.05$). All the statistical analysis was performed with SPSS BASE ver.11.5 statistical software (SPSS, Chicago, IL).

RESULTS

The results regarding the effect of different ferric ion concentrations (0, 25, 50, 100, 200 µM Fe^{3+}) on optical density (OD) and antifungal activity (Fig. 1) revealed that OD of liquid culture (LSD = 0.09) of *P. polymyxa* SQR-21 and antifungal activity (LSD = 1.80) against *F. oxysporum* were increased by increasing the concentration of Fe^{3+} after four days incubation. The maximum OD was determined at 200 µM Fe^{3+} while maximum antifungal activity was determined at 50 µM Fe^{3+}. The increase in OD over untreated control was 3, 4, 5 and 8% at 25, 50, 100, 200 µM Fe^{3+}, respectively.

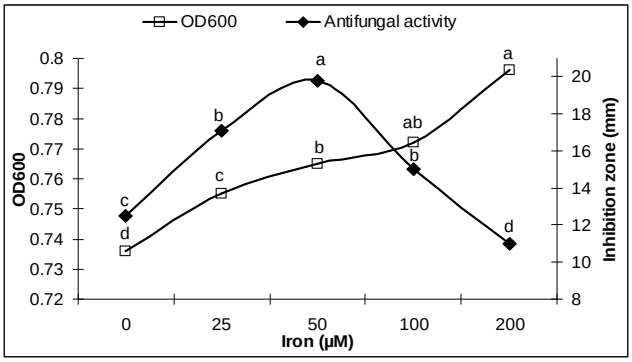

Figure 1 - Effect of different Fe^{3+} concentrations (0, 25, 50, 100 and 200 µM Fe^{3+}) on OD_{600} and antifungal activity of *P. polymyxa* SQR-21 against *Fusarium oxysporum* after four days incubation. Letters indicate a significant difference with means of three replicates at $P \leq 0.05$ level.

The increase in the antifungal activity over untreated control was 33, 49 and 25% at 25, 50 and 100 µM Fe^{3+}, respectively while at the highest level of Fe^{3+} (200 µM), 12% decrease in antifungal activity was measured. Initially, the tryptone medium has pH 7.2 but after four days incubation, *P. polymyxa* cells increased the pH of liquid culture by 15-16% at all levels of Fe^{3+} (LSD = 0.56) and among all levels of Fe^{3+}, the differences were non-significant (Fig. 2). The liquid culture, used to extract the antifungal compounds and to measure the OD, was also used to estimate the extra and intracellular chemical composition.

The data regarding the intracellular chemical composition depicted that increase in the concentration of Fe^{3+} in the liquid culture increased the intracellular protein (LSD = 3.49) and carbohydrate contents (LSD = 7.02) (Fig. 3) while the intracellular lipid contents (LSD = 5.67) (Fig. 4) were increased only at 25 and 50 µM Fe^{3+}. The increase in intracellular protein contents was 0.6, 1 and 2% at 25, 50, 100 µM Fe^{3+}, respectively and in the intracellular carbohydrate contents, the increase was 1, 5, 10 and 14% as compared with control, at 25, 50, 100, 200 µM Fe^{3+}, respectively. The increase in the intracellular lipid contents at 25 and 50 µM Fe^{3+} was 11 and 2%, respectively while decrease in the lipid contents was 4 and 12% at 100 and 200 µM Fe^{3+}, respectively. All the Fe^{3+} levels showed more protein contents over control but with the increase in Fe^{3+} concentration from 100 to 200 µM, decrease in the intracellular protein contents was measured.

The data regarding the extracellular chemicalcomposition revealed that all levels of Fe^{3+} increased the extracellular protein (LSD = 7.08) (Fig. 5) and EPS contents (LSD = 39.19) (Fig. 5).

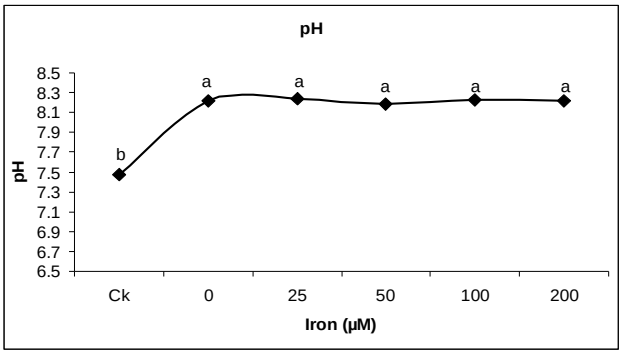

Figure 2 - Effect of different Fe^{3+} concentrations (0, 25, 50, 100 and 200 µM Fe^{3+}) on final pH of *P. polymyxa* SQR-21 liquid culture after four days incubation. Means sharing same letter do not differ significantly ($P \leq 0.05$).

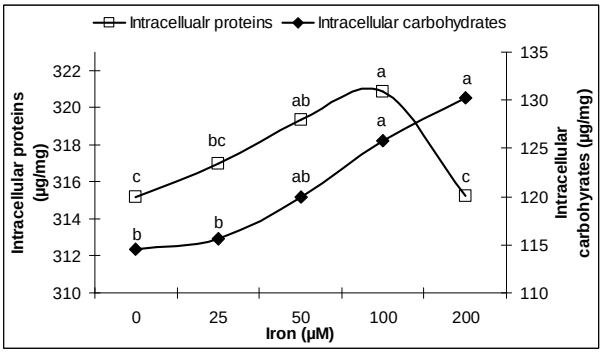

Figure 3 - Effect of different Fe^{3+} concentrations (0, 25, 50, 100 and 200 µM Fe^{3+}) on intracellular protein and carbohydrate contents of *P. polymyxa* SQR-21 after four days incubation. Means sharing same letter do not differ significantly ($P \leq 0.05$).

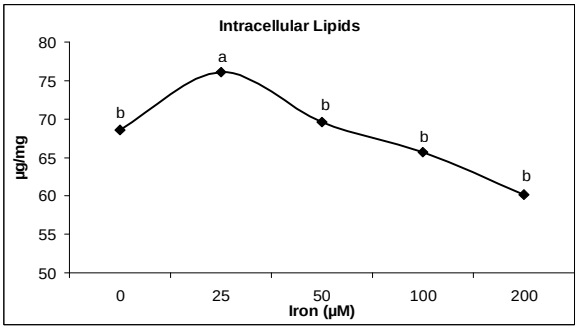

Figure 4 - Effect of different Fe^{3+} concentrations (0, 25, 50, 100 and 200 µM Fe^{3+}) on intracellular lipid contents of *P. polymyxa* SQR-21 after four days incubation. Means sharing same letter do not differ significantly ($P \leq 0.05$).

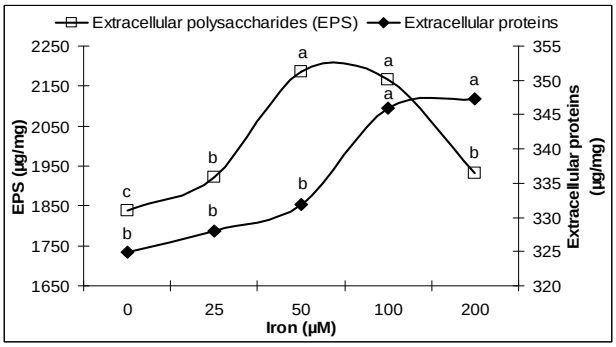

Figure 5 - Effect of different Fe^{3+} concentrations (0, 25, 50, 100 and 200 µM Fe^{3+}) on extracellular protein and polysaccharide contents of *P. polymyxa* SQR-21 after four days incubation. Means sharing same letter do not differ significantly ($P \leq 0.05$).

The increase in extracellular protein contents was 1, 2, 6 and 7% and increase in EPS contents was 1, 5, 10 and 14% over untreated control at 25, 50, 100, 200 µM Fe^{3+}, respectively. The protease activity of liquid culture of *P. polymyxa* (LSD = 3.24) (Fig. 6) was increased by 6, 11, 12 and 13% while cellulase activity (LSD = 0.31) (Fig. 6) was increased by 4, 9, 15 and 20% over control at 25, 50, 100, 200 µM Fe^{3+}, respectively. The results of total RNA contents (Fig. 7) and the relative expression of fusaricidin synthetase gene (*fusA*) of *P. polymyxa* SQR-21(Fig. 7) revealed that total RNA contents (LSD = 4.37) and the relative expression of *fusA* gene (LSD = 0.22) was increased with the increase in concentration of Fe^{3+} up to 50 µM Fe^{3+} in the liquid culture. After RNA extraction, DNase treatment was carried out to degrade the genomic DNA and it was confirmed by RT-PCR. No DNA contamination was observed after DNase treatment as shown in Fig. 8. Total RNA contents were measured prior to cDNA synthesis. The intensity of amplified *fusA* gene bands was increased in gene expression with the increase in concentration of Fe^{3+} in liquid culture up to 50 µM Fe^{3+} (Fig. 8). For RT- and Real Time-PCR, 16S rRNA was used as the positive control. The increase in total RNA contents was 3 and 13% and increase in the relative expression of *fusA* gene was 35 and 51% over untreated control at 25 and 50 µM Fe^{3+}, respectively. The decrease in RNA contents was 4 and 23% while in relative expression of *fusA* was 29 and 61% as compared with control at 100 and 200 µM Fe^{3+}, respectively.

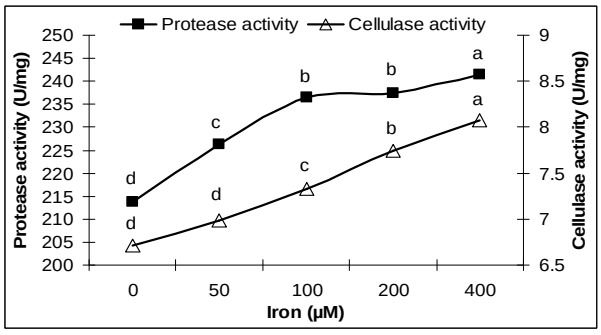

Figure 6 - Effect of different Fe^{3+} concentrations (0, 25, 50, 100 and 200 µM Fe^{3+}) on protease and cellulase activity in the liquid culture of *P. polymyxa* SQR-21 after four days incubation. Means sharing same letter do not differ significantly ($P \leq 0.05$).

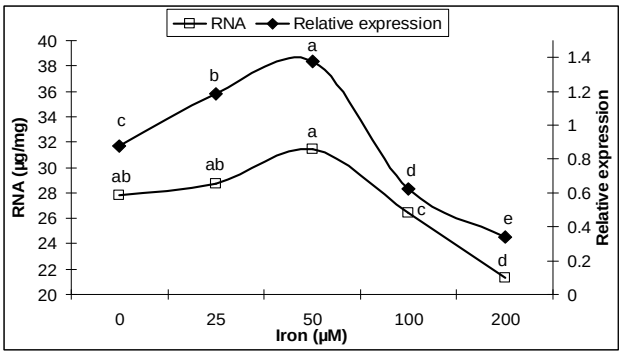

Figure 7 - Effect of different Fe^{3+} concentrations (0, 25, 50, 100 and 200 µM Fe^{3+}) on total RNA contents and relative expression of fusaricidin synthetase gene (*fusA*) in *P. polymyxa* SQR-21. Bars with the same letter are not significantly different at P≤0.05.

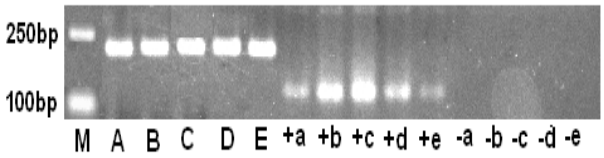

Figure 8 - RT-PCR products originating from cDNA, after extraction of total RNA of *P. polymyxa* SQR-21 grown in submerged culture treated with four concentrations of Fe^{3+} (a = 0, b = 25, c = 50, d = 100, e = 200 µM Pb^{+2}), M = DNA marker, A to E= positive control 16S rRNA gene, +a to +e = *fusA* gene (+RT), -a to –e = negative controls for *fusA* gene (-RT).

DISCUSSION

Iron is a basic requirement for the normal metabolism of bacterial cell and variations in its concentration in the surrounding environment cause significant effect on usual cell processes and metabolic products. The results of this work revealed that all levels of Fe^{3+} increased the growth while increase in the antibiotic production was only observed up to 50 µM Fe^{3+}. Intra and extracellular protein and carbohydrate contents were increased with the increase in Fe^{3+} concentration in the liquid culture while intracellular lipid contents were decreased. Total RNA contents and relative expression of *fusA* gene were increased up to 50 µM Fe^{3+}.

The increase in Fe^{3+} concentration increased the pH of liquid culture although *P. polymyxa* has been found to produce organic acids such as acetic, formic and oxalic acid (Sharma and Rao, 2001). The acid production by bacteria is associated normally with low nutrient availability as in minimal medium that is low in nutrition. In this work, tryptone broth used had sufficient nutrition for bacterial growth and Fe^{3+} levels did not influence the acid production by *P. polymyxa*. The slight increase in the growth up to 4% while increase in the antifungal activity up to 49 and in the relative expression of *fusA* up to 56% at 50 µM Fe^{3+} reflected that Fe^{3+} played a regulatory role, directly or indirectly, in the production of fusaricidin. At higher concentrations, Fe^{3+} led to a strong decrease in the antibiotic production without affecting slight increase in the bacterial growth, suggesting a specific Fe^{3+} effect on bacterial secondary metabolism. The mechanisms that how Fe^{3+} increased the growth and antifungal activity yet has not been elucidated. Different results have been reported regarding the effect of iron on the growth and antibiotic production. For example, Wensinck et al. (1967) reported the iron growth-limiting factor of *Pseudomonas aeruginosa* using synthetic media solidified with agar. In addition, the elemental Fe had a marked effect on the growth and bulbiformin production by *Bacillus subtilis.* The optimum requirement of Fe was 20 ppm for both, the growth and antibiotic activity (Mahmood, 1970). Iron was required for the biosynthesis of cephamycin C in *Streptomyces clavuligerus* and higher concentrations

of iron increased the cephamycin C production two times. The iron content of the chemically defined medium was shown to be sub-optimal for antibiotic production and the addition of 130 µg/ml iron almost doubled the cephamycin C levels (Rollins et al., 1989). In addition to positive effects of iron on antibiotic production, negative effects have also been reported. Like, *Escherichia coli* AY25 showed a 95% decrease in microcin yield when grown in minimal medium containing 10 µM iron (high iron) as compared to 0.2 µM (low iron). Studies with *Escherichia coli* mutant deficient in iron-regulated proteins (*fur*) suggested that factors other than *Fur* could mediate iron regulation of microcin synthesis (Salomon and Farias, 1994). An iron concentration above 1-2 mM inhibited tetracycline production in *S. aureofaciens* ATCC 10762 (Bechet and Blondeau, 1998), in contrast with the 0.4 ppb (approximately) of iron required for blocking the tetracycline biosynthesis in the *S. aureofaciens* strain studied by Zenaitis and Cooper (1994). In the present case, different results were obtained as higher levels of iron (>50µM Fe^{3+}) increased the growth and other cellular parameters (except intracellular lipids) but inhibited the antibiotic production, which reflected the concentration dependent specific role of iron on antibiotic production. Whether the effect of iron is direct on genetic or biochemical regulation of fusaricidin or whether their effects are mediated through interactions between these, or other ions. In addition, the location of the regulation by iron remains to be determined and experiments at the molecular level are required to characterize the impact of iron on the fusaricidin production.

In the present study, Fe^{3+} might be interfering with the secondary metabolism more general to enzymes or cellular processes. The Fe^{3+} might stimulate the synthesis of the prepeptide or the activation of the appropriate prepeptide maturation enzymes and the transport out of the cell. Recently, Ca^{2+} binding sites were predicted to be present in *NisP* peptidase, which cleaved the leader peptide from the precursor nisin (Siezen et al., 1995). Since the precursor was devoid of antibacterial activity (Meer et al. 1993), so Fe^{3+} might activate the leader peptidase. Lubbe et al. (1984) reported that the complete cephamycin pathway benefited from the higher iron concentration. Three of the enzymes common to the cephalosporin C and cephamycin C biosynthetic pathways are known to require iron in their catalytic activities, namely isopenicillin N synthase (IPNS), deacetoxycephalosporin C synthase (DAOCS), and deacetoxycephalosporin C hydroxylase (DAOCH).

The increase in concentration of antibiotic in the medium could be a consequence of the increase in cell wall permeability of SQR-21 promoted by Fe^{3+}, which was in agreement with Petit-Glatron et al. (1993) who studied the capacity of the cell wall to concentrate Ca^{2+} and proposed that the increased concentration of Ca^{2+} in the microenvironment of the cell wall could play an important role in the last step of the secretion. Another possibility could be that the Fe^{3+} activated the enzymes whose activity resulted in a change in the regulatory functions of the cell in favor at low concentration and against at higher concentration of different secondary metabolites, especially fusaricidins. As iron increased the intra and extracellular protein and carbohydrate contents, Fe^{3+} seemed increasing the enzymes production mainly involved in the fusaricidin synthesis, growth or other activities related to the different cellular processes, which resulted in decrease in the intracellular lipid contents and residual energy was being used for the protein and carbohydrate synthesis. These results were further supported by the increase in protease and cellulase activity with the increase in Fe^{3+} concentration. The presence of different metal ions such as Ca^{2+}, Pb^{2+} and Mg^{2+} as well as minerals such as iron, aluminium and calcium oxides during the growth influence the types and quantity of polysaccharides, proteins and enzymes secreted by the bacteria (Reed, 1987). The EPS of *P. polymyxa* were composed of varying concentrations of glucose, fructose, galactose, mannose and xylose (Haggag, 2007). The EPS aids in the biological uptake of the metals by chelating and binding them to the cell wall (Deo and Natarajan, 1998). It is preliminary but the first report of the effect of iron on the fusaricidin production. Although Fe^{3+} exerted negative effects on intracellular lipid contents but it increased the intra and extracellular protein and carbohydrate contents and antibiotic production in the liquid culture. These results provide useful information about the effects of Fe^{3+} on the overall metabolic processes of *P. polymyxa* mainly fusaricidin production, which could help in the fermentation technology for maximum antibiotic production. Do Fe^{3+} directly affect the affinity of a protein for its target or substrate? Is Fe^{3+} a 'chemical switch' or it encodes specificity? It would be interesting to see what function Fe^{3+} plays in fusaricidins production and other cellular processes in *P. polymyxa* at different concentrations.

ACKNOWLEDGEMENTS

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RESUMO

Tipo compostos do fusaricidin do produto das tensões do polymyxa de Paenibacillus que é ativo de encontro a uma variedade larga das bactérias e de fungos gram-positive. O crescimento, a composição química celular e a produção do fusaricidin datilografam compostos antimicrobial pelo P. o polymyxa SQR-21 foi comparado no caldo de carne do tryptone suplementado com as quatro concentrações (25, µM 50, 100 e 200) do ferro. Os dados revelaram que o crescimento do P. o polymyxa foi aumentado por 3-8% com o aumento na concentração do íon férrico (Fe^{3+}) e o tipo produção do fusaricidin dos compostos foi aumentado 33-49% somente até 50 pelo µM Fe^{3+} quando o nível o mais elevado de Fe^{3+} era inhibitory. O aumento na concentração de Fe^{3+} na cultura líquida aumentou a proteína intracellular (2%) e os índices de hidrato de carbono (14%) e a proteína extracellular (7%) e os índices do polysaccharide (18%) quando os índices intracellular do lipid eram (11%) somente até 50 o µM aumentado Fe^{3+}. Além, os efeitos regulatory de Fe^{3+} foram refletidos também pelo aumento em índices totais do RNA e na expressão relativa do gene do synthetase do fusaricidin (*FusA*) por 3-13 e por 35-56% respectivamente, até 50 o µM Fe^{3+}, em seguida que uma diminuição contínua estêve observada.

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