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# OPTIMIZATION OF ENNIATINS PRODUCTION BY AN ENDOPHYTIC STRAIN FUSARIUM DIMERUM

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**ABSTRACT**

The goal of this study was to find suitable composition of cultivation media for enniatin production by isolated endophytic strain *Fusarium dimerum*. In order to find optimal cultivation media, mono- and di- saccharides, complex nitrogen sources and L-amino acids directed biosynthesis of enniatins were tested. Submerged cultivation experiments were carried out in cultivation flasks. Most promising medium for enniatin accumulation contained fructose, malt extract and peptone for bacteriology. Finally, quite expensive carbon source fructose was replaced by more available syrups. Optimization resulted in 4-times elevated enniatin biosynthesis by metabolites production microorganism. Moreover, this is the strain obtained from *Magnolia soulangeana*, which has similar metabolites spectrum as the isolated *Fusarium dimerum*. Comparison of these results with published ones revealed that this endophyte is a potential strain for enniatins biosynthesis in submerged cultivation in which the maximum accumulation 1.27 $g.L^{-1}$ of enniatin in culture medium was reached in a short period (96 h). The results proved that the endophytic strain *F. dimerum* may potentially be applied for efficient production of bioactive enniatins.

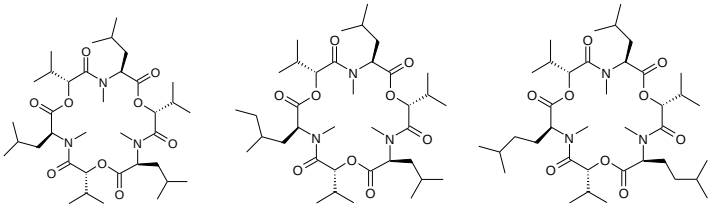
**Keywords:** Enniatins, production of secondary metabolites, *Fusarium dimerum*, optimization, endophytes

## INTRODUCTION

Enniatins are cyclic hexadepsipeptides composed of N-methyl L-amino acids and D-2-hydroxyisovaleric acid produced by microorganisms classified in *Fusarium*, *Alternaria*, *Verticillium* and *Halosarpheia* genera. These compounds are known for their ionophoric effect which is involved inter alia in their antibiotic, antifungal, phytotoxic and anthelmintic activity. They have been also investigated for their potentials as anti-HIV and anti-cancer agents, the latter due to their discovered new mechanisms of pro-apoptotic activity **(McKee *et al.*, 1997; Dornetshuber *et al.*, 2007; Meca *et al.* 2010)**. Enniatins were studied as potent cytotoxic and apoptic compounds in hepatocellular carcinoma, fibroblast-like foetal lung cancer and epidermoid carcinoma cell lines of human origin **(Dornetshuber *et al.* 2007; Uhlig *et al.*, 2009; Wätjen *et al.*, 2009)**. Moreover, enniatins at a non-toxic concentration are effective and specific inhibitors of Pdr5p a functional homologue of mammalian P-glycoprotein, one of the major efflux pumps involved in multidrug resistance **(Hiraga *et al.*, 2005; Yamamoto *et al.*, 2005)**. Additionally, they have chemically stable properties, serve as indicators of fungal contamination of food grains and are thus required as an analytical standards worldwide **(Sorensen *et al.*, 2008; Malachova *et al.*, 2011; Sifou *et al.*, 2011)**.

Plants used in a folk medicine are generally proposed for endophytes isolation. Magnolia bark was used in traditional Chinese medicine for thousands years. In recent studies focused on phylogenetic relationship, host affinity and fungal diversity endophytic microorganisms were isolated from *Magnolia grandiflora* **(Higgins *et al.*, 2006; Arnold and Lutzoni, 2007)** and *Magnolia lilifera* **(Promputtha *et al.*, 2007)**.

From a plant material (bark, leaves and buds) we isolated an endophytic fungal strain, later identified as *Fusarium dimerum* Penzig. The plant material was harvested from of an ornamental tree *Magnolia x soulangeana*. Enniatin B, B1 and G (Fig. 1) were separated from the hexane extract of *F. dimerum* cultivation broth **(Firáková *et al.*, 2008)**. Results of liquid culture media optimization for enniatin production by *Fusarium dimerum* are summarised in this paper.



Enniatin B  Enniatin B1  Enniatin G

**Figure 1** Structures of enniatins produced by endophytic fungi *Fusarium dimerum*.

## MATERIAL AND METHODS

### Strain and cultivation media

*F. dimerum* (culture collection of Department of Biotechnology, SUT Bratislava) isolated from the plant tissue of *Magnolia x soulangeana* Soul.-Bod. (*Magnoliaceae*) was used for production of secondary metabolites. Fungal strain spores were harvested with sterile Sabouraud maltose medium (SM, mycological peptone 10 $g.L^{-1}$, maltose 40 $g.L^{-1}$) from the slant agar and approximately $10^6$ spores were inoculated into 100 mL liquid SM media in 500 mL cultivation flasks. *F. dimerum* was grown for two days on a rotary shaker (3.7 Hz; 180 rpm) at 28 °C in the dark and a 10 mL portion of vegetative culture was inoculated into the flat bottom cultivation flasks (500 mL) with working volume 100 mL of liquid broth. All production media, pH 5.6 ± 0.2 (before sterilization) were incubated at 28 °C for 192 h on rotary shaker (180 rpm) in the dark. Experiments were conducted with three replicants.

Screening experiments for enniatins production were performed in SM medium, potato-dextrose medium (PD, potato infusions 200 $g.L^{-1}$, dextrose 20 $g.L^{-1}$, HiMedia, India) and in broth with malt extract (ME, malt extract 35 $g.L^{-1}$, peptone for bacteriology 7 $g.L^{-1}$, Imuna Pharm, Slovak Republic).

The series of feeding experiments aimed at the determination of the influence of amino acids on enniatins production were carried out in the liquid SM medium. *F. dimerum* was fed with L-amino acids: α-alanine, serine, threonine, leucine,

isoleucine (ICN Biochemicals, USA), cysteine, β-alanine (Merck, Germany), leucine + methionine (Merck, Germany), isoleucine + methionine (1 g.L$^{-1}$) in 24, 48 and 72 h since the inoculation.

Small scale cultivations focused on the selection of the most suitable nitrogen source were conducted in the liquid media containing glucose 40 g.L$^{-1}$ (Lachema, Czech Republic) and different nitrogen sources (7 g.L$^{-1}$): peptone for bacteriology, soya-peptone (Imuna Pharm, Slovak Republic), tryptone (Oxoid, England), peptone from casein, peptone from casein HCl hydrolysate (Serva, Germany), soya-meal, peanut-meal, fish-meal, corn steep, corn-gluten, corn-meal and oat-meal.

The effect of carbon sources was investigated in the media containing peptone for bacteriology (10 g.L$^{-1}$) supplemented with (40 g.L$^{-1}$) glucose, arabinose, fructose, maltose, saccharose, lactose and cellobiose (MicroCHEM, Slovak Republic), xylose (ICH SAS, Slovak Republic), galactose (Sigma, USA), or in the media with malt extract (Merck, Germany). Influence of concentration of carbon sources was studied in media containing peptone for bacteriology 10 g.L$^{-1}$ supplemented with glucose, xylose (40, 60, 80 g.L$^{-1}$) or fructose (40, 60, 80, 120, 150 g.L$^{-1}$) or combination of glucose (40 g.L$^{-1}$) + arabinose (40 g.L$^{-1}$). In the further experiments maltose and fructose syrups Fermentose 551, Fermentose 352, Glucoplus 111, Isosweet 119, and Mylose 451 (AmylumSlovakia, Slovak Republic) were used as the carbon source. Each medium contained 55 g.L$^{-1}$ of the syrup, peptone for bacteriology 15 g.L$^{-1}$ and malt extract 35 g.L$^{-1}$.

### Cultivation of *F. dimerum* and isolation of enniatins from culture media

To obtain an insight in the production kinetic of secondary metabolites the cultivation broth was analysed each 24 h since the beginning of cultivation. Dry weight, pH and concentration of saccharides in supernatant were determined for each sample. Reducing saccharides were determined by colorimetry (Meyer and Gibbons, 1951). Ethyl acetate was proved as the most suitable solvent for metabolites extraction. Each sample was extracted twice with ethyl acetate (2:1, v/v), the layers were separated by centrifugation (2800 rpm, 10 min), organic layer was dried with anhydrous $Na_2SO_4$ and filtrated. The organic solvent was evaporated in vacuum, the residue was weighted, dissolved in methanol and analysed by HPLC: model HP 1100 (Agilent Technologies, USA) equipped with degasser and autosampler; column 125 x 4 mm Hypersil ODS 5 µm (Merck, Germany); mobile phase acetonitrile: water (65:35 v/v), pH 2.4 - 2.7 adjusted with $H_3PO_4$; flow rate 0.7 mL min$^{-1}$ for 30 min; injection volume 10 µL, detection at 210 nm; data evaluation ChemStation software B 0103 (Agilent Technologies, USA). Enniatins concentration was assessed by the external standard method.

## RESULTS AND DISCUSSION

### Precursor directed enniatin biosynthesis

Threonine, α- and β-alanine, cysteine, serine, leucine, isoleucine and the latter two L-amino acids in combination with L-methionine (1 g.L$^{-1}$) were used in feeding experiments. Addition of amino acids in 24, 48 and 72 h to SM medium afforded various results (Fig. 2). Feeding of *F. dimerum* with leucine in 48 h and α-Ala, Cys, Leu + Met in 24, 48 h of cultivation inhibited production of enniatins. The highest stimulation of enniatin production (710 mg.L$^{-1}$) was achieved by addition of Ser to production media in 24 h with production peak in 120 h and dry weight of biomass 22 g.L$^{-1}$. Ile and Ile + Met added in 24 and 48 h enhanced production of enniatins up to 520 mg.L$^{-1}$ and 450 mg.L$^{-1}$, respectively, however, the maximum concentration of enniatin was shifted to 168 h.

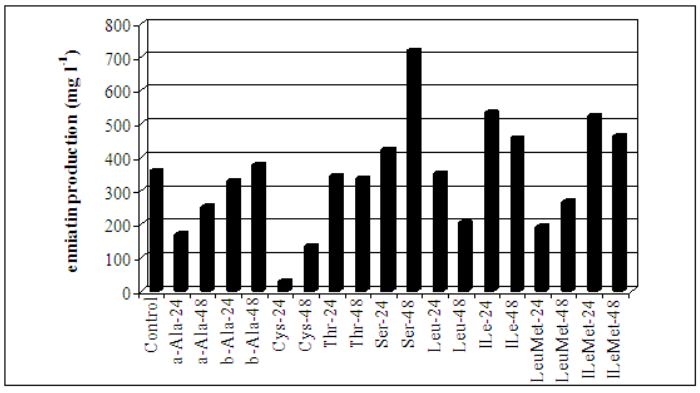



**Figure 2** Effect of L-amino acids on production of enniatins. Fusarium dimerum was cultivated in Sabouraud maltose medium supplemented in 24, 48 h with α-Ala, β-Ala, Thr, Cys, Ser, Leu, Ile, or Leu and Met or Ile and Met; control contained no amino acids.

### Effect of complex nitrogen sources on enniatin production

In our study peptone for bacteriology, soya-peptone, soya-meal, peanut-meal, fish-meal, corn steep, corn-gluten, corn-meal, oat-meal, tryptone, peptone from casein, peptone from casein hydrolysate (7 g.L$^{-1}$) together with glucose (40 g.L$^{-1}$) were used to increase enniatin production. In general, results of these experiments were ambiguous. The production was in all tested media lower than production in SM medium 350 mg.L$^{-1}$ (Tab. 1). If peptone for bacteriology, tryptone or peptone from casein hydrolysate were used, enniatin production reached 200 mg.L$^{-1}$ after 96 h, 170 mg.L$^{-1}$ after 144 h and 270 mg.L$^{-1}$ after 168 h, respectively, and peptone from casein only 100 mg.L$^{-1}$ after 168 h. Addition of soya-peptone, soya-meal, peanut-meal, fish-meal, and corn steep resulted in enhanced growth *F. dimerum* but enniatin production was more lower than the production achieved in amino acid supplemented medium.

**Table 1** Effect of selected nitrogen sources on enniatins production by *Fusarium dimerum*. Growth of the strain expressed as dry weight of biomass, residual saccharides and pH in the maximum value of enniatin (ENN) production.

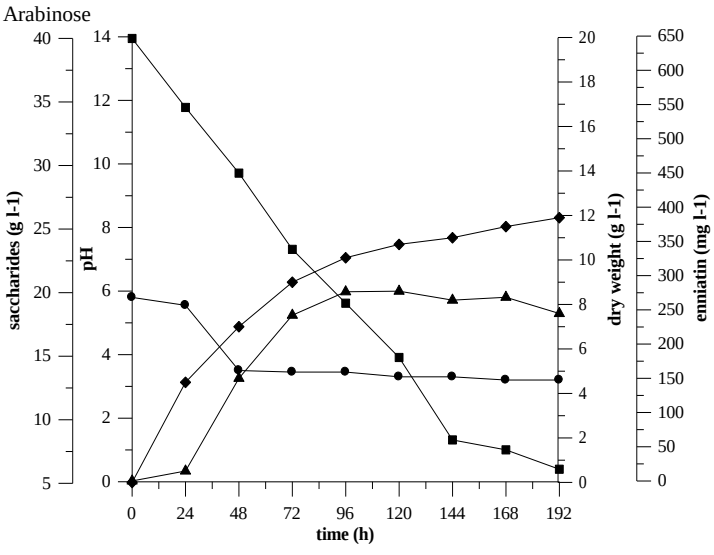
| Nitrogen source* | ENN (mg.L$^{-1}$) | Time (h) | Dry weight (g.L$^{-1}$) | Saccharides (g.L$^{-1}$) | pH |
|---|---|---|---|---|---|
| Soya-peptone | 23 | 168 | 14 | 0.5 | 4.3 |
| Soya-meal | 10 | 144 | 14 | 0.3 | 3.2 |
| Peanut-meal | 20 | 96 | 16 | 0.4 | 2.7 |
| Fish-meal | 7 | 96 | 18 | 1 | 4.1 |
| Corn-gluten | N.D. | 96 | 7 | 22 | 3.4 |
| Corn steep | 7 | 72 | 17 | 0.6 | 5.5 |
| Corn-meal | N.D. | 96 | 8 | 30 | 3.1 |
| Oat-meal | N.D. | 96 | 6 | 15 | 3.0 |
| Tryptone | 170 | 144 | 14 | 0.5 | 5.5 |
| Peptone for bacteriology | 200 | 96 | 16 | 0.4 | 3.4 |
| Peptone from casein | 94 | 168 | 10 | 7 | 3.8 |
| Peptone from casein HCl hydrol. | 270 | 168 | 12 | 3 | 6.5 |

**Legend:** N.D. – not detected

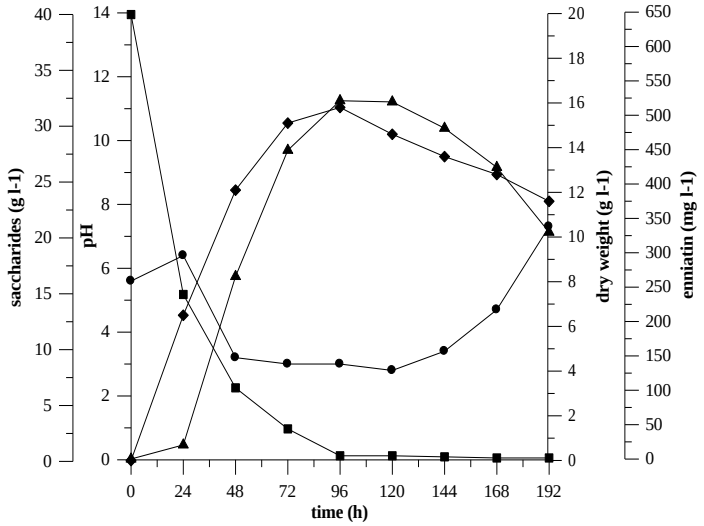
* Cells after inoculation were incubated at 28 °C for 192 hours on rotary shaker (3.7 Hz, 180 rpm) in dark in cultivation flasks containing 100 mL of media (nitrogen source 7 g.L$^{-1}$, glucose 40 g.L$^{-1}$), initial pH 5.6 ± 0.2 (before sterilization). All the parameters were determined every 24 h of fermentation.

### Effect of the carbon sources on enniatins production

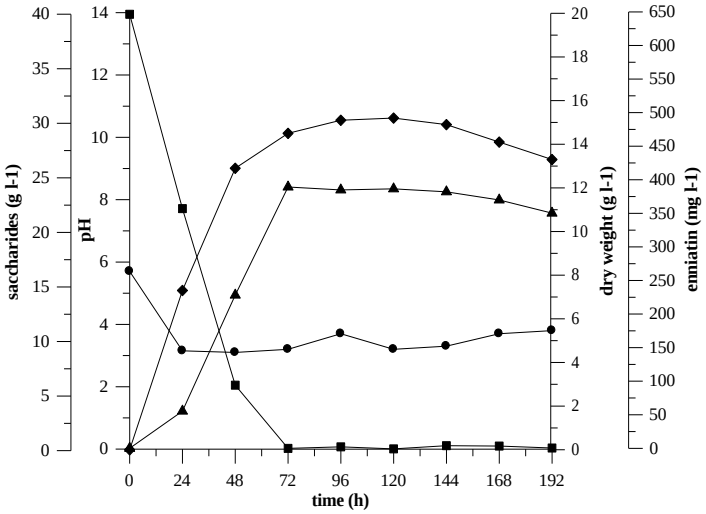
We examined the effect of various carbon sources on enniatin production. The highest production was observed with fructose (650 mg.L$^{-1}$) and cellobiose (520 mg.L$^{-1}$) in the cultivation media (Fig. 3, Fig. 4). Enniatins were detected in all media from an early stage of cultivation and the maximum of production was achieved after 96 h of cultivation.



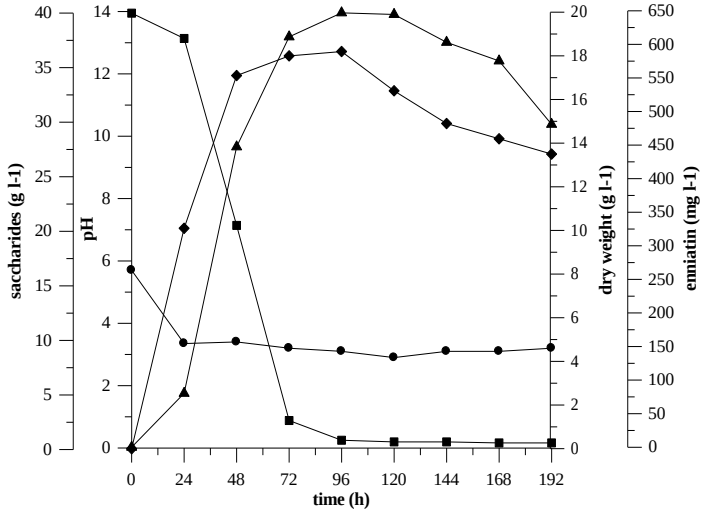
Cellobiose



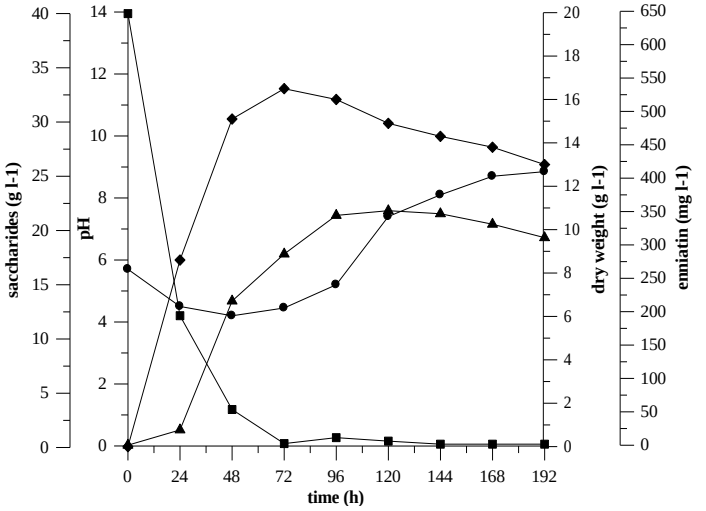

Glucose



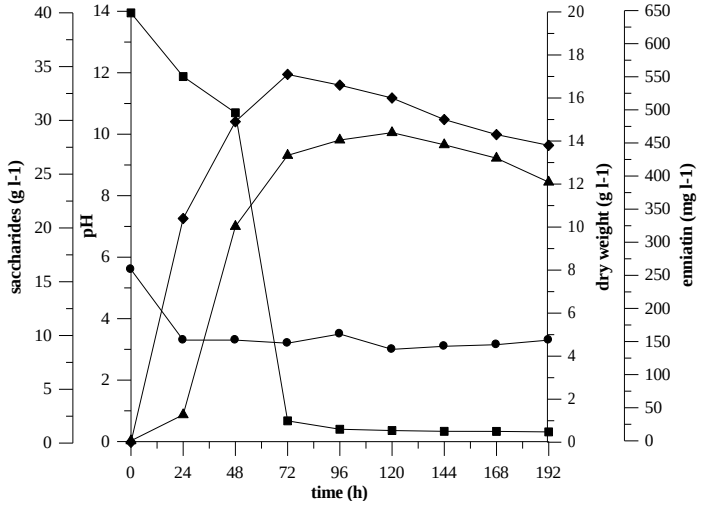

Fructose



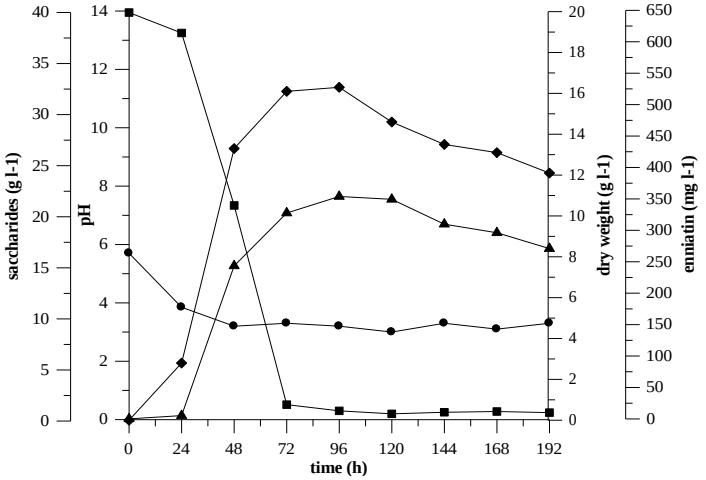

Maltose



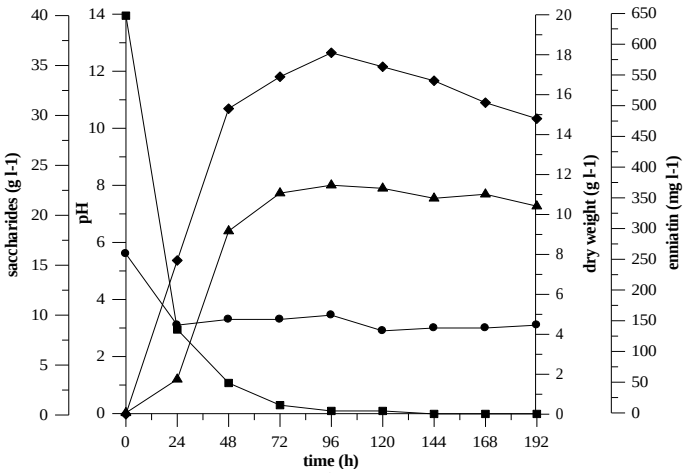

Galactose




Saccharose



Xylose




**Figures 3** Fermentation parameters of *Fusarium dimerum* grown in liquid media at 28 °C. Cultivation media contained pepton for bacteriology 10 g.L$^{-1}$, carbon source 40 g.L$^{-1}$. (♦) - dry weight, (■) - residual reducing compounds (saccharides), (●) - pH value, (▲) - enniatins production.

There was no wide variety in the starting and the final pH of the cultivation broth, 5.6 ± 0.2) and (3 - 4), respectively, for all saccharides except maltose which turned alkaline up to pH 8.9.

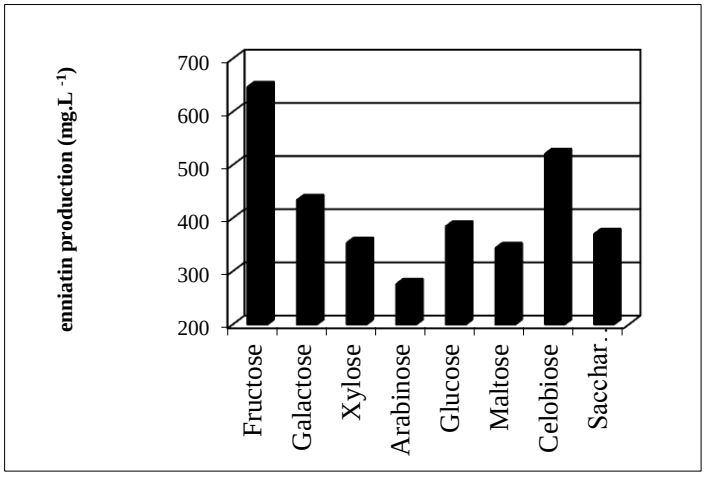



**Figure 4** Effect of saccharides on enniatin production after 96 h of the submersed cultivation. Liquid media contained pepton for bacteriology 10 g.L$^{-1}$, carbon source 40 g.L$^{-1}$.

Effect of carbon source concentration on production of enniatins was studied with glucose, arabinose, xylose and fructose. While the higher concentration of glucose and fructose enhanced production of enniatins, this effect was not observed with xylose. Increase of glucose concentration in medium from 40 g.L$^{-1}$ to 80 g.L$^{-1}$ doubled the production (up to 800 mg.L$^{-1}$). Xylose did not change the enniatin production, but stimulated the growth of *F. dimerum* (dry weight of biomass 24 g.L$^{-1}$). Combination of slowly utilized arabinose (40 g.L$^{-1}$) and rapidly utilized glucose (40 g.L$^{-1}$) led to only slightly promoted production of enniatins (600 mg.L$^{-1}$) and the peak production was shifted to 144 h of cultivation. Production of enniatin was mostly affected by concentration of fructose (Tab. 2). Yield of enniatins was elevated to almost 900 mg.L$^{-1}$, if the concentration of fructose was 150 g.L$^{-1}$. Biomass of *F. dimerum* was enhanced in the condition up to starting concentration of fructose 80 g.L$^{-1}$ however higher concentration of fructose slightly inhibited the growth of the strain (Tab. 2). After 192 h of cultivation all sources were depleted except fructose; if starting concentration was 80, 120 and 150 g.L$^{-1}$. Residual concentration of this saccharide was 13, 47 and 61 %, respectively.

Medium composed of malt extract afforded modest production of enniatins (380 mg.L$^{-1}$), but stimulated growth of mycelium. The combination of fructose (40 g.L$^{-1}$), peptone for bacteriology (15 g.L$^{-1}$) and malt extract (35 g.L$^{-1}$) in the cultivation media was used and resulted in the highest production of enniatins (1270 mg.L$^{-1}$) of all tested combinations (Tab. 2).

**Table 2** Influence of fructose concentration, malt extract and combination of fructose, malt extract and peptone for bacteriology on enniatins production by *Fusarium dimerum*. Growth of the strain expressed as dry weight of biomass, residual saccharides and pH in the maximum of enniatin production is presented.

| Carbon source* (g.L$^{-1}$) | ENN (mg.L$^{-1}$) | Time (h) | Dry weight (g.L$^{-1}$) | Saccharides (g.L$^{-1}$) | pH |
|---|---|---|---|---|---|
| Fructose 40 * | 611 | 72 | 18 | 3 | 3.2 |
| Fructose 60 * | 596 | 96 | 19 | 18 | 3.1 |
| Fructose 80 * | 698 | 96 | 19 | 40 | 3.1 |
| Fructose 120 * | 752 | 96 | 15 | 94 | 3.0 |
| Fructose 150 * | 883 | 96 | 14 | 106 | 3.0 |
| Malt extract 35*** | 379 | 96 | 17 | 5 | 3.5 |
| Fructose 40 ** | 814 | 96 | 19 | 1 | 3.5 |
| Fructose 40 + malt extract 35** | 1270 | 120 | 29 | 2 | 3.6 |

* Cells after inoculation were incubated at 28 °C for 192 h on rotary shaker (3.7 Hz, 180 rpm) in dark, in flasks containing 100 mL of cultivation media with initial pH 5,6 ± 0,2 (before sterilization). All parameters were assessed every 24 h from 0 – 192 h of fermentation. Composition of production medium: fructose and/or malt extract as shown in the table with * peptone for bacteriology 10 g.L$^{-1}$, ** peptone for bacteriology 15 g.L$^{-1}$, *** peptone for bacteriology 7 g.L$^{-1}$.

Consequently, fructose as relatively expensive carbon source, was replaced by cheaper fructose and maltose syrups (separately Fermentose 551, Fermentose 352, Glucoplus 111, Isosweet 119, and Mylose 451). The biosynthesis of enniatin on syrups in the cultivation medium was accompanied by enhanced growth of the microorganism (Tab. 3). The carbon source was completely utilized in 120 h of the cultivation and the peak of enniatin production was shifted to 144 – 168 h. All of the used syrups enhanced production of enniatin, but the most promising one was Glucoplus 111, with production of enniatins 800 mg.L$^{-1}$.

**Table 3** Effect of maltose and fructose syrups as carbon sources on enniatin production by *Fusarium dimerum*. Growth of the strain expressed as dry weight of biomass, saccharides and pH in the maximum of enniatin production is shown.

| Carbon source* (g.L$^{-1}$) | ENN (mg.L$^{-1}$) | Time (h) | Dry weight (g.L$^{-1}$) | Saccharides (g.L$^{-1}$) | pH |
|---|---|---|---|---|---|
| Glucoplus 111 | 799 | 144 | 30 | 0.9 | 3.8 |
| Isosweet 119 | 626 | 168 | 24 | 1.3 | 4.1 |
| Mylose 451 | 774 | 144 | 29 | 1.1 | 3.7 |
| Fermentose 551 | 738 | 168 | 30 | 1.0 | 3.8 |
| Fermentose 352 | 736 | 144 | 31 | 1.1 | 3.7 |

* Cells after inoculation were incubated at 28 °C for 192 h on rotary shaker (3.7 Hz, 180 rpm) in dark, in cultivation flasks with flat bottom containing 100 mL of cultivation media (peptone for bacteriology 15 g.L$^{-1}$ malt extract 35 g.L$^{-1}$, syrups individually 55 g.L$^{-1}$), initial pH 5,6 ± 0,2 (before sterilization). All parameters were assessed every 24 h up to 192 h of fermentation.

## CONCLUSION

Three culture media were tested in the screening submerged experiments for enniatin production by *F. dimerum*: sabouraud maltose, potato dextrose and malt extract broth. From these media PD broth was not suitable one because it supported neither the growth of *F. dimerum* nor biosynthesis of enniatins. Production of enniatins in the other two liquid media was almost the same, 350 mg.L$^{-1}$, 380 mg.L$^{-1}$, respectively. Audhya and Russell (1974) described production of enniatins by *Fusarium* sp. in the range 1300 – 1724 mg.L$^{-1}$after 21 – 34 days of cultivation on the solid agar medium. In the first series of experiments we achieved 4.5 times lower production but within 4 day long cultivation. Based on these results we supposed *F. dimerum* suitable for enniatin production and this encouraged us to optimize submerged cultivation conditions which are probably more promising than cultivation on solid agar media. Enniatins production displayed typical kinetics for secondary metabolite production. The highest concentration of enniatins in culture media extract was detected when carbon source in the media was completely depleted and the growth of microorganism ceased (dry weight of biomass was 17 – 19 g.L$^{-1}$). Low amounts of enniatins in media were observed in early stage of cultivation.

Various amino acid precursors were suitable for stimulation of enniatin production (Nilanonta et al., 2003; Supothina et al., 2004), therefore we added L-amino acids (α- and β-alanine, threonine, cysteine, serine, leucine, isoleucine and the latter two L-amino acids in combination with L-methionine (1 $g.L^{-1}$) to SM medium in 24 – 72. h of cultivation. In our previous studies we observed, that the most suitable medium for secondary metabolites production by fungi was SM broth, therefore this medium was chosen for the study. Supplementation of the medium with L-amino acids in 72 h did not substantially affected production of enniatins. At this time the growth of *F. dimerum* was in the exponential phase and the addition of selected amino acids had no influence on production of enniatins. Supplementation of SM media with Ser in 24. h of cultivation yielded in twice enniatin production (710 $mg.L^{-1}$). α-Ala added to the medium inhibited production of enniatins, on the other hand was not affected by β-Ala. Media enriched with Ile and Ile + Met added in 24 and 48 h slightly enhanced production of enniatins but resulted in new metabolite production. Addition of these amino acids in 24 and 48 h resulted in biosynthesis of a new metabolite manifested by a new peak eluting after the enniatins B, B1 and G. Addition of leucine + methionine to the medium in 24 h led to a new peak eluting in front of the group of enniatins B, B1 and G. Similar results were achieved by Peeters et al. (1984), Krause et al. (2001) and Nilanonta et al. (2003). Addition of branched amino acids as Leu and Val as well as Phe and Met to the cultivation media stimulated enniatin production by *Fusarium* sp. and *Verticillium hemipterigenum* and changed the array of biosynthesized enniatins.

Audhya and Russell (1974) studied also the influence of various inorganic nitrogen containing compounds, amino acids, urea, tryptone and enzymatic hydrolysate of casein (8 $g.L^{-1}$) in context of enniatin biosynthesis. These feeding experiments resulted in increased diversity of enniatins biosynthesized by *F. scirpi* and *F. sambucinum* together with enhanced production of these metabolites by *Verticillium hemipterigenum*. In our optimization of nitrogen source we chose complex nitrogen sources (peptones, meals). Considering these nitrogen sources contained wide variety of amino acids we supposed stimulation of enniatin production. The most suitable for nitrogen source was casein hydrolysate and peptone for bacteriology. Other sources in concentration 7 $g.L^{-1}$ were not suitable. We suppose inhibition of enniatin production in media containing soya pentone or meals can be due to their higher content of phosphates. Addition of $KH_2PO_4$ in range 1 – 10 $g.L^{-1}$ to SM medium decreased yields of enniatins and thus confirmed our assumption. Peptone for bacteriology was chosen for further study, because the maximum of production was reached early after the inoculation, the growth of microorganism was satisfactory and suitable for fermentation.

The importance of carbon source in antibiotics production has long been recognized and has been thoroughly discussed by Martin and Demain (1978, 1980) and Betina (1983). We tested monosaccharides (glucose, fructose, xylose, arabinose, galactose) and disaccharides (lactose, maltose, cellobiose, saccharose,) for enniatin production. Contrary to observation of Audhya and Russell (1974) in which lactose was the best carbon source for production of enniatin by *F. sambucinum*, in our experiments lactose stimulated neither growth of *F. dimerum* nor biosynthesis of enniatins. All saccharides except arabinose were completely utilised till 96 h of cultivation, when the peak of enniatin production was observed. Arabinose was slowly utilised by *F. dimerum* and the maximum production was achieved in 144. h of cultivation. The best source of carbon was fructose. Increasing concentration of fructose in medium stimulated also the production of enniatins but concentration up to 120 $g.L^{-1}$ slightly inhibited growth of *F. dimerum*. The production was also considerably promoted by the amount of nitrogen source in the liquid media. If the concentration of peptone for bacteriology increased from 10 $g.L^{-1}$ to 15 $g.L^{-1}$ in the medium containing 40 $g.L^{-1}$ of fructose enniatin production increased from 600 $mg.L^{-1}$ to 800 $mg.L^{-1}$, respectively. This finding indicates that the balanced ratio of C/N source is important for the production of enniatins.

Medium composed of malt extract afforded modest production of enniatins (380 $mg.L^{-1}$), but stimulated growth of mycelium. We expected combination of fructose with this complex source of carbon (mainly maltose), nitrogen (amino acids, amonnium salts) and other compounds as growth factors could improve the enniatin production. Supplementation media with malt extract resulted in the in the highest production of enniatins 1270 $mg.L^{-1}$ within 96. h cultivation. In an industrial biotechnological production the price of primary medium components is considered. Consequently, fructose as relatively expensive carbon source, was replaced by cheaper fructose and maltose syrups. Maltose syrups contained also di- and tri- saccharides, which had to be cleaved by *F. dimerum* therefore the maximum production was shifted to 144. – 168. h of cultivation. Although the yield of enniatins in the liquid production media with syrups was lower than the highest yield 1270 $mg.L^{-1}$ for above mentioned combination, we suppose that further optimization can offer a cost effective process of enniatin production.

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