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RESEARCH ARTICLE

Mutagenecity of Enniatin A1 and B1 Mycotoxins in Ames Salmonella Microsome Test [1]

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Abstract

Mycotoxins are secondary metabolites produced by microfungi like *Aspergillus, Penicillium, Fusarium, Alternaria*. The enniatins are cyclic peptide mycotoxins and have produced by several strains of *Fusarium* sp. They have got biological activities like acting as enzyme inhibitors, antifungal and antibacterial agents. In this research the mutagenic effects of enniatin A1 and B1 were investigated with Ames assay. Ames is a test system which can detect mutations at celluler level. In this test *Salmonella typhimurium* TA98 and *Salmonella typhimurium* TA100, both in the presence and absence of S9 metabolic activation was used. Five different concentrations of Enniatin A1 and B1 (12.5 µM, 25 µM, 50 µM, 100 µM, 200 µM) were exposed to these strains. In all test strains (*Salmonella typhimurium* TA98 and *Salmonella typhimurium* TA100) Enniatin A1 and B1 did not show mutagenic effect.

Keywords: *Ames test, Enniatin A1, Enniatin B1, Salmonella typhimurium*

Enniatin A1 ve B1 Mikotoksinlerinin Ames Salmonella Mikrozom Testi ile Mutajenitesi

Özet

Mikotoksinler *Aspergillus, Penicillium, Fusarium, Alternaria* gibi bazı mikrofunguslar tarafından üretilen sekonder metabolitlerdir. Enniatinler birkaç *Fusarium* suşu tarafından üretilir ve siklik peptid mikotoksinlerdir. Enniatinler antifungal, antibakteriyel ajan ve enzim inhibitörü olarak hareket etmek gibi biyolojik aktivitelere sahiptir. Bu çalışmada Enniatin A1 ve B1'in mutajenik etkileri Ames testiyle araştırıldı. Ames hücresel seviyedeki mutasyonları belirleyen bir test sistemidir. Bu testte *Salmonella typhimurium* TA98 ve *Salmonella typhimurium* TA100 suşları metabolik enzim olan S9'lu ortamda ve S9'suz ortamda kullanıldı. Enniatin A1 ve B1'in 5 farklı konsantrasyonu (12.5 µM, 25 µM, 50 µM, 100 µM, 200 µM) bu suşlara uygulandı. Bütün test suşlarında(*Salmonella typhimurium* TA98 ve *Salmonella typhimurium* TA100) Enniatin A1 ve B1 mutajenik etki göstermedi.

Anahtar sözcükler: *Ames testi, Enniatin A1, Enniatin B1, Salmonella typhimurium*

INTRODUCTION

Enniatins are fungal metabolites produced by several species of Fusarium, and were first isolated in 1947 by Gäumann et al.[1,2]. Enniatins are members of a family of fungal N-methylated cyclic hexadepsipeptides and consist of three residues of D-2-hydroxyisovaleric acid alternating with three of N-methyl branched-chain L-amino acids such as valine, leucine, or isoleucine. Enniatin A contains only N-methyl isoleucine residues, whereas other enniatin homologues contain other patterns of branched-chain amino acids [3]. Enniatins are non-ribosomal, cyclic hexadepsipeptides with general cation chelating, ionophore [3], and they have got biological activities like acting as enzyme inhibitors, e.g. acyl-CoA-cholesterol-acyl transferase [4] and cyclic nucleotide phosphodiesterase [5], antifungal and antibacterial agents, and immunomodulatory substances [6-8]. They are also anthelmintic, cytotoxic and phytotoxic activities [2,9-11]. Although they can accumulate in Fusarium-infected grain, enniatins have not been associated with any animal disease outbreaks nor been shown to cause disease in experimental animals [3], but Wätjen et al.[5] said

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that Enniatins have important impact on human health and they can cause outbreaks in humans and animals [12]. They are also known as phytotoxins and are associated with plant diseases characterized by wilt and necrosis [13]. Enniatins exert potent cytotoxic activity against cancer cell lines [5,14], and can act also as potent inhibitors of an ABC membrane transporter related to multidrug-resistance [15]. These properties make them candidate compounds for further development as drugs, particularly as potent cytotoxic effects against several cancer cell lines [2,16].

The aim of this study was to investigate the mutagenic effects of enniatin A1 and B1 using Ames test. *Salmonella typhimurium* TA98 and TA100 was used both in the presence and absence of a liver microsome (S9).

MATERIAL and METHODS

Mutagenic effects of Enniatin A1 and B1 were investigated with Ames test system. The Ames test was performed with or without S9 mix using the incorporation method of exposure in accordance to the work of Maron and Ames [17].

Sample Preparation and Doses

Enniatin A1 and B1 were disolved in the Dimethyl Sulfoxide (DMSO). Each of them stored in DMSO for best to best dissolve. The stock was stored room temperature. Non-toxic doses of the compounds for standard test strains was determined and the five non-cytotoxic dose (200-100-50-25-12,5 µM) of each substances were studied.

The Bacterial Strains

The tester strains used were histidine requiring *Salmonella typhimurium* TA98 and TA100. The culture stocks were stored at -80°C. While the TA98 strains were used for determining the frameshift, TA100 was used to determine the base pair exchange [18]. The tester strain was freshly prepared by pre-culturing for 12 h at 37°C in nutrient broth No. 2 and suspension was used for the assay. To carry out experiments healthy, strains have been checked at regular intervals whether the strains have the original mutations.

Procedure in Presence of Liver Microsome (S9)

100 µL of the test solution for each concentration, 300 µL biotin/histidine solution and 100 µL of a cell suspension from an overnight culture ((1-2) × 10^9 cells/mL) were added to 3 mL of top agar (kept at 45°C) and vortexed the mixture. Experiments with S9, 500 µL of S9 mix was added. The entire mixture was transferred on the minimal agar plate. Samples were tested on triplicate plates in independent parallel experiments. The plates were incubated at 37°C for 48-72 h and then bacterial colonies on each plate were counted.

Positive Control

100 µL 2 Amino flourene, 300 µL biotin/histidine solution and 100 µL of a cell suspension from an overnight culture ((1-2) × 10^9 cells/mL) were added to 3 mL of top agar (kept at 45°C) and vortexed the mixture. Experiments with S9, 500 µL of S9 mix was added. 2AF was used as positive controls with metabolic activation. The entire mixture was transferred on the minimal agar plate. Samples were tested on triplicate plates in independent parallel experiments. The plates were incubated at 37°C for 48-72 h and then bacterial colonies on each plate were counted.

Solvent Control

100 µL of dimethylsulfoxide (DMSO), 300 µL biotin/histidine solution and 100 µL of a cell suspension from an overnight culture ((1-2) × 10^9 cells/mL) were added to 3 mL of top agar (kept at 45°C) and vortexed the mixture. Experiments with S9, 500 µL of S9 mix was added. The entire mixture was transferred on the minimal agar plate. Samples were tested on triplicate plates in independent parallel experiments. The plates were incubated at 37°C for 48-72 h and then bacterial colonies on each plate were counted.

Spontaneous Control

300 µL biotin/histidine solution and 100 µL of a cell suspension from an overnight culture [(1-2) × 10^9 cells/mL] were added to 3 mL of top agar (kept at 45°C) and vortexed the mixture. Experiments with S9 500 µL of S9 mix was added. The entire mixture was transferred on the minimal agar plate. Samples were tested on triplicate plates in independent parallel experiments. The plates were incubated at 37°C for 48-72 h and then bacterial colonies on each plate were counted.

Procedure in Absence of Liver Extract (S9)

All the steps in this stage are the same as previous part. But, here, liver microsome extract (S9) was not used. 4-nitrophenyldiamine was used as a positive control for TA98, Sodium Azide was used for TA100.

RESULTS

Mutagenecity Enniatin A1 and B1were investigated with Ames test. In the Ames assay *S. typhimurium* TA98 and *S. typhimurium* TA100 strains were used: Strain TA98 detects frame-shift mutations whereas TA100 detects base-pair substitutions. The test results of Enniatin A1 and B1 are summarised in *Table 1* and *Table 2*. Analyzing test results, all doses of Enniatin A1 and B1, in the presence and absence of S9 enzyme was determined to be non-mutagenic. Results were compared with the number of colonies of spontaneous control group.

Table 1. *Results of mutagenecity of Enniatin A1 with Ames test in different S. typhimurium strains (TA98 and TA100) in the presence or absence of S9-mix*

Tablo 1. *Enniatin A1'in Ames testi ile farklı S. typhimurium (TA98 ve TA100) suşlarında S9 enzimi varlığında ve yokluğunda mutajenite sonuçları*

Strain	TA98		TA100	
	-S9	+S9	-S9	+S9
Spontaneous control	34±3	29±5	152±12	252±8
Solvent control (DMSO)	30±2	35±5	149±6	270±20
200 µM Enniatin A1	33±3	28±4	146±4	281±4
100 µM Enniatin A1	38±8	29±5	141±11	257±11
50 µM Enniatin A1	37±4	39±4	144±20	271±13
25 µM Enniatin A1	37±7	29±10	153±16	243±26
12,5 µM Enniatin A1	32±5	28±6	176±11	247±20
Positive control				
4 Nitro-o-phenilenediamine	1235±35	-	-	-
Sodium azide	-	-	1720±30	-
2 Aminoflourene	-	1240±20	-	2327±16

Table 2. *Results of mutagenecity of Enniatin B1 with Ames test in different S. typhimurium strains (TA98 and TA100) in the presence or absence of S9-mix*

Tablo 2. *Enniatin B1'in Ames testi ile farklı S. typhimurium (TA98 ve TA100) suşlarında S9 enzimi varlığında ve yokluğunda mutajenite sonuçları*

Strain	TA98		TA100	
	-S9	+S9	-S9	+S9
Spontaneous control	34±3	29±5	152±12	252±8
Solvent control (DMSO)	30±2	35±5	149±6	270±20
200 µM Enniatin B1	36±5	34±3	136±10	243±24
100 µM Enniatin B1	34±3	36±3	150±18	245±22
50 µM Enniatin B1	33±5	28±2	183±13	275±16
25 µM Enniatin B1	33±6	34±3	167±20	267±10
12,5 µM Enniatin B1	34±6	29±3	160±15	240±5
Positive control				
4 Nitro-o-phenilenediamine	1235±35	-	-	-
Sodium azide	-	-	1720±30	-
2 Aminoflourene	-	1240±20	-	2327±16

DISCUSSION

Enniatins are non-ribosomal, cyclic hexadepsipeptides with general cation chelating ionophore and antibiotic activities [3]. Enniatins have got various biological activities [5,14,15]. These properties make them candidate compounds for further development as drugs [2,16]. For these reasons; evaluating the mutagenic, cytotoxic, antimicrobial effects of enniatin A1 and B1 is important.

Studies on the mutagenic effects of enniatins are found of limitedly. Behm et al.[16], investigated mutagenic effects of Enniatin B with *Salmonella* Ames test system. They used *S. typhymurium* TA98, 100, 102 and 104 strains with five different concentrations (100 nM, 1 µM, 10 µM, 30 µM, 100 µM). No mutagenecity of Enniatin B was detected in their research [16]. Our results support the findings of Behm et al.[16]. Studies of the cytotoxic effects of enniatins have been evaluated by MTT assay by Lu et al.[12]. They reported that ENs cytotoxicity depend on their concentrations, and also on their combination with other mycotoxins [12]. The effect of EN A_1, B, and B_1 on cell viability determinated in rat hepatoma (H4IIE), human hepatoma (HepG2), and rat glioma (C6) cell lines using the tetrazolium salt (MTT) assay. The enniatins showed a moderate toxicity in C6 glioma and HepG2 hepatoma cells [5].

Antimicrobial effect of Enniatin were studied against *Escherichia coli*, *Enterococcus faecium*, *Salmonella enterica*, *Shigella dysenteriae*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Clostridium perfringens*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* by Meca et al.[19]. Enniatin B was found no toxic, at tested concentrations on the strains *S. aureus* CECT 240, *E. coli* CECT 4782, and *S. dysenteriae* CECT 584 but all the others strains tested showed inhibitory activity dependent on the quantity utilized [19].

In conclusion the Ames test showed us that Enniatin A1 and B1 did not posses mutagenic activity. Further mutagenicity tests should perform to reach whole decision about their safety.

REFERENCES

1. Gäumann E, Roth S, Ettlinger LE, Plattner PA, Nagel U: Enniatin, ein neues, gegen Mykobakterien wirksames Antibiotikum. *Experientia,* 3, 202-203, 1947.

2. Föllmann W, Behm C, Degen GH: The emerging Fusarium toxin enniatin B: *in-vitro* studies on its genotoxic potential and cytotoxicity in V79 cells in relation to other mycotoxins. *Mycotox Res,* 25, 11-19, 2009.

3. Desjardins AE, Proctor RH: Molecular biology of Fusarium mycotoxins. *Int J Food Microbiol,* 119 (1-2): 47-50, 2007.

4. Tomada H, Huang XH, Cao J, Nishida H. Nagao R, Okuda S, Tanaka H, Omura S, Arai H, Inoue K: Inhibition of acyl-Coa: Cholesterol achyltransferase avtivity by cyclodepsipeptide antibiotics. *J Antibiot,* 45, 1626-1632, 1992.

5. Wätjen W, Debbab A, Hohlfeld A, Chovolou Y, Kampkötter A, Edrada RA, Ebel R, Hakiki A, Mosaddak M, Totzke F, Kubbutat MHG, Proksch P: Enniatins A1, B and B1 from an endophytic strain of *Fusarium tricinctum* induce apoptotic cell death in H4IIE hepatoma cells accompanied by inhibition of ERK phosphorylation. *Mol Nutr Food Res,* 53, 431-440, 2009.

6. Fairlie DP, Abbenante G, March DR: Macrocyclic peptidomimetics - forcing peptides into bioactive conformations. *Curr Med Chem,* 2, 654-686, 1995.

7. Fusetani N, Sugawara T, Matsunaga S, Hirota H: Orbiculamide A: A novel cytotoxic cyclic peptide from a marine sponge *Theonella* sp. *J Am Chem Soc,* 113, 7811-7812, 1991.

8. Hyun U, Lee DH, Lee C, Shin CG: Apoptosis induced by enniatins H and MK1688 isolated from *Fusarium oxysporum* FB1501. *Toxicon,* 53, 723-

728, 2009.

9. Jestoi M: Emerging Fusarium-mycotoxins fusaproliferin, beauvericin, enniatins, and moniliformin-A review. *Crit Rev Food Sci Nutr,* 48, 21-49, 2008.

10. Firakova S, Proksa B, Sturdikova M: Biosynthesis and biological activities of enniatins. *Pharmazie,* 62, 563-568, 2007.

11. Scherkenbeck J, Jeschke P, Harder A: PF1022A and related cyclodepsi-peptides. *Curr Top Med Chem,* 2, 759-777, 2002.

12. Lu H, Fernández-Franzón M, Font G, Ruiz MJ: Toxicity evaluation of individual and mixed enniatins using an *in vitro* method with CHO-K1 cells. *Toxicol In Vitro,* 27 (2): 672-680, 2013. DOI: 10.1016/j.tiv.2012.11.009.

13. Gäumann E, Naef-Roth St, Kern H: Zur phytotoxischen wirksamkeit der enniatine. *Phytopathologische Zeitschrift,* 40, 45-51, 1960.

14. Dornetshuber R, Heffeter P, Kamyar MR, Peterbauer T, Berger W, Lemmens-Gruber R: Enniatin exerts p53-dependent cytostatic and p53-independent cytotoxic activities against human cancer cells. *Chem Res Toxicol,* 20, 465-473, 2007.

15. Hiraga K, Yamamoto S, Fukuda H, Hamanaka N, Oda K: Enniatin has a new function as an inhibitor of the Pdr5p, one of the ABC transporter in *Saccharomyces cerevisiae*. *Biochem Biophys Res Commun,* 25, 328 (4): 1119-1125, 2005.

16. Behm C, Degen GH, Föllmann W: The Fusarium toxin enniatin B exerts no genotoxic activity, but pronounced cytotoxicity *in vitro. Mol Nutr Food Res,* 53, 423-430, 2009.

17. Maron DM, Ames BN: Revised methods for the mutagenicity test. *Mutation Res,* 113, 173-215, 1983.

18. Uysal A, Durak Y, Aladağ MO: Investigation of mutagenic effects of some plant growth regulators on Salmonella/microsome test system. *Fresenius Environ Bull,*19 (10): 2170-2175, 2010.

19. Meca G, Sospedra I, Valero MA, Mañes J, Font G, Ruiz MJ: Antibacterial activity of the enniatin B, produced by Fusarium tricinctum in liquid culture, and cytotoxic effects on Caco-2 cells. *Toxicol Mech Methods,* 21 (7): 503-512, 2011.