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DYNAMICS OF DEOXYNIVALENOL AND ZEARALENONE PRODUCTION BY *FUSARIUM GRAMINEARUM* UNDER LABORATORY CONDITIONS

ABSTRACT: Toxicological investigations encompassed two cultures of *Fusarium graminearum*: (i) D2 isolate, originating from air was obtained on Sabouraud medium during a routine control of laboratory sterility conditions at the Department of Microbiology of the Center for Bio-Ecology in 2006, and (ii) GZ-LES control isolate, a well known producer of zearalenone (ZON) and deoxynivalenol (DON), was isolated from maize kernel collected at Leskovac in 1975. Preliminary analysis of fungal potential for the production of DON and ZON were performed by the modified rapid screening method of Filtenborg et al. (1983). Dynamics of DON and ZON biosynthesis was tested under different conditions of isolate cultivation: (i) in a basic liquid semi-synthetic medium with 2% yeast extract and 15% sucrose, pH 6.5 (YES), (ii) in broth with same concentrations of yeast extract and sucrose supplemented with 0.23 mg/l $ZnSO_4$ x 5 H_2O, pH 6.5 (YES^{Zn}) and (iii) on natural solid substrates such as wet sterilized maize and rice kernels. The quantitative determination of DON and ZON was performed in both liquid and natural solid substrates with thin-layer chromatographic methods (TLCs).

The maximum yield of DON was recorded after three weeks of cultivation on maize kernels at 27±1°C. Contrary to the D2 isolate, which did not show the potential for the DON biosynthesis, the control isolate GZ-LES produced 645.6 ppb of the same type B trichothecene under previously mentioned conditions. The ZON biosynthesis by the isolate D2 (1.2 ppb) was observed after 2 weeks of the stationary cultivation in YES and YES^{Zn} at room temperature (17—19°C). The same isolate produced 0.74 ppb and 17.35 ppb ZON on maize and rice kernels after only 7 and 28 days of cultivation at the room temperature ranging from 17 to 19°C and from 15 to 23°C, respectively.

KEY WORDS: *Fusarium graminearum*, DON, ZON

INTRODUCTION

Fusarium graminearum, beside *F. culmorum*, is the main causal organism of fusarium head blight (FHB) or scrib, a disease that leads to severe losses of

yield and quality of cereal grain. During the development of FHB these pathogens commonly contaminate grain with mycotoxins deoxynivalenol (DON) and zearalenone (ZON). These mycotoxins that can be the cause of health--threatening toxicoses (M a r a s a s et al., 1984), represent at least two biochemical origins. ZON with the estrogenic activity in mammals is biosynthesized through a polycetidic pathway, while 8-ketotrichothecenes DON and nivalenol (NIV) are derived from the condensation of three mevalonate units (B l a c k - w e l l et al., 1985).

F. graminearum isolates can be broadly divided into two chemotypes based on the production of host specific virulence factors DON and NIV (I l - g e n et al., 2008). According to K i m et al. (2003) small cereal grains grown in Korea produced either DON or NIV, whereas isolates from corn grown in the United States produced DON only. In England and Wales (J e n n i n g s et al., 2004) DON is predominant chemotype (75% of tested *F. graminearum* cultures), as well as, in Argentina where only chemotype IA (DON and 3-acetyl-DON) was observed (M o l t o et al., 1997).

There are only a few reports about simultaneous presence of DON and ZON in Serbian crops (J a j i ć et al., 2007; B a g i et al., 2008). In order to determine the occurrence of DON and ZON, this study was carried out with *F. graminearum* isolates cultivated on different substrates under laboratory conditions.

MATERIAL AND METHODS

Microorganisms. Two isolates of *F. graminearum* were under present investigation. Isolate D2, originating from air, was obtained on Sabouraud medium during a routine control of laboratory sterility conditions at the Department of Microbiology of the Center for Bio-Ecology in 2006, and control isolate GZ-LES, a well known producer of DON and ZON (J a j i ć et al., 2007), was isolated from maize kernel collected at Leskovac in 1975. Stock cultures of the fungi were maintained on the potato-sucrose agar at 4—6°C.

Preliminary analysis of fungal potential to produce deoxynivalenol (DON) and ZONralenone (ZON) were performed according to the rapid screening method of F i l t e n b o r g et al. (1983) modified by B o č a r o v - S t a n č i ć et al. (in press) on the following media: YESA (2% yeast extract, 15% sucrose and 2% agar, pH 6.5), $YESA^{Zn}$ (2% yeast extract, 15% sucrose, 0.23 mg/l $ZnSO_4$ x 5 H_2O, and 2% agar, pH 6.5), PPSA (2% peptone-1, 5% sucrose and 2% agar, pH 6.5), $PPSA^{Zn}$ (2% peptone-1, 5% sucrose, 0.23 mg/l $ZnSO_4$ x 5 H_2O and 2% agar, pH 6.5) and PDA (potato-dextrose agar, pH 6.9).

Liquid media and conditions for the toxin production. The *F. graminearum* isolate D2 and the control isolate GZ-LES were grown in the yeast extract-sucrose broth (YES, pH 6.5) (S a m s o n and v a n R e e n e n - H o - e k s t r a, 1988) as well as in yeast extract-sucrose medium supplemented with 0.23 mg/l $ZnSO_4$ x 5 H_2O (YES^{Zn}, pH 6.5) (M ü h l e n c o e r t, 2004). Both media (YES and YES^{Zn}) contained 2% yeast extract and 15% sucrose. Media (250 mL each) were poured into 500 mL Erlenmeyer flasks and were inoculated with five fragments (5 x 5 mm) of the fungus, that were grown on po-

tato-sucrose agar (PSA) in Petri dishes at 27±1°C for seven days. After inoculation of the media, the Erlenmeyer flasks were kept stationary at the room temperature (17—19°C) for 14 days. The pH value was measured after cultivation of the isolate. The samples for the analysis were taken every week during the cultivation period. All cultivations were performed in two replications.

Solid media and conditions for the toxin production. Both isolates of *F. graminearum* were grown on wet (>45%) sterilized maize and rice kernels (50 g per Roux bottle). Each substrate was inoculated with five fragments (5 x 5 mm) of the fungus that was grown on the potato-sucrose agar (PSA) in Petri dishes at 27±1°C for seven days. Inoculated maize samples were cultivated during a 4-week period under room (17—19°C) and chamber (27±1°C) conditions. On other hand, during 14, 21 and 28 days the inoculated rice samples were cultivated under room (15—23°C) and chamber conditions (27±1°C). All cultivations were performed in two replications.

Determination of fusariotoxins. Qualitative and quantitative DON determinations in filtrates of fungal cultures were carried out by applying the thin-layer chromatographic (TLC) method developed by Cvetnic et al. (2005), and ZON after Bočarov-Stančić et al. (2003). In accordance with these methods, liquid cultures of fungus were filtered after a stationary cultivation. Furthermore, after the cultivation on maize and rice kernels, the samples were dried for 24 h or more at 60°C, until constant weight was achieved. After a pulverization of dried samples, the DON determination was done according to the TLC method of Pepeljnjak and Babić (1991), and ZON after Balzer et al. (1978). Thin-layer chromatography was performed with toluene/ethyl acetate/formic acid developing saturated solvent (5:4:1, v/v/v). Only in case of the ZON, determination in benzene/glacial acetic acid developing solvent (90+10 v/v) was used parallely. After developing plates and air drying in the dark fume extractor the plates were examined under long wave UV light (366 nm). DON and ZON were visualized by the use of 20% $AlCl_3$ in 60% ethanol. All analyses were done in three replications.

RESULTS AND DISCUSSION

Results of the present investigation are shown in Tables 1 — 4.

Tab. 1. Fusariotoxins production by *F. graminearum* isolates on different agar media and temperatures

Medium	Aver. temp. (°C)	Days	Intensity of fusariotoxins production			
			D2 isolate		GZ-LES control islate	
			DON	ZEN	DON	ZEN
YESA	19.0	7	–	+	–	++
		14	–	+	–	++
		21/28	–/–	+++/+++	–/–	+++/+++
	24.9	7	–	+	–	+
		14	–	+	–	++/+++
		21/28	–/–	++/+++	+/+	+++/+++

YESA^{Zn}	19.0	7	–	–	–	+
		14	–	+	–	+++
		21/28	–/–	+++/+++	–/–	+++/++++
	24.9	7	–	+	–	–
		14	–	+++	–	+
		21/28	n.a.	n.a.	+/+	+/+
PPSA	19.0	7	–	–	–	–
		14	–	+	–	–
		21/28	–/–	++/+++	–/–	+++/+++
	24.9	7	–	–	–	–
		14	–	+	–	–
		21/28	–	+++/++++	+/+	++/++
PPSA^{Zn}	19.0	7	–	–	–	–
		14	–	++	–	–
		21/28	–/–	++/++++	–/+	+++/++++
	24.9	7	–	–	+	–
		14	–	++	+	–
		21/28	n.a.	n.a.	+/+	–/–
PDA	19.0	7	–	–	–	–
		14	–	–	–	–
		21/28	–/–	–/–	–/–	–/–
	24.9	7	–	–	–	–
		14	–	–	–	–
		21/28	–/–	–/–	–/+	–/–

Legend: n.a. — not analyzed; – not detected; + low intensity; ++ moderate intensity; +++ high intensity; ++++ very high intensity

Screening of the fusariotoxin production under different conditions of fungal cultivation *in vitro* (Table 1) revealed that only *F. graminearum* GZ-LES had the capability to produce DON. The biosynthesis of this type B trichothecene was observed in almost all cases at higher temperature (average 24.9°C). The exception was cultivation of this isolate on PPSA^{Zn} when DON was recorded after prolonged cultivation (28 days) at lower temperature (average 19.0°C). Dynamics of the DON production was as follows: early detection (after 7 days) on PPSA^{Zn}, after 21 days on PPSA, YESA, and YESA^{Zn}, and after 4 weeks on PDA.

ZON was biosynthesized on agar media by both tested isolates of *F. graminearum* isolates (Table 1) but under different cultivation conditions. PDA was not applicable for testing the ZON production. ZON was observed on PPSA^{Zn} and PPSA after 2 weeks of D2 isolate cultivation, regardless of the applied temperature, while in the case of GZ-LES isolate after 3 weeks cultivation at a lower temperature (average 19.0°C). After 7 days, ZON was biosynthesized on YESA^{Zn} by the D2 isolate at higher temperatures (average 24.9°C), and by GZ-LES at lower temperatures (average 19.0°C), respectively. The best results were achieved on YESA where both isolates of *F. graminearum* produced ZON after the 7-day cultivation regardless of the temperature conditions. A prolonged cultivation of fungi resulted in higher ZON yields.

Tab. 2 — Quantity of fusariotoxins produced by D2 isolate of *F. graminearum* stationary cultivated in liquid media

Temperature (°C)	Days	Medium	pH value	Toxin yield (mg/l)	
				DON	ZON
17—19	7	YES	—	n.d	n.d.
		YES^{Zn}	—	n.d	n.d.
	14	YES	4.97	n.d	1.20
		YES^{Zn}	4.73	n.d	1.50
	21	YES	4.90	n.d	1.50
		YES^{Zn}	4.50	n.d	1.50
	28	YES	5.20	n.d	0.90
		YES^{Zn}	4.60	n.d	1.20

Legend: n.d. — not detected (< 0.037 and < 0.097 mg/l of ZON and DON, respectively)

After the incubation period, a decrease of pH value was determined in both tested liquid media (Table 2).

Investigations of the DON and ZON production in liquid media (YES and YES^{Zn}) revealed that the control isolate GZ-LES *F. graminearum* did not biosynthesize any of the tested fusariotoxins after 28 days of cultivation at room temperature (15—23°C). The explanation for this finding is that the applied temperature was maybe too high for the ZON production, but, on the other hand, too low for the DON biosynthesis under stationary cultivation conditions.

In the case of D2 isolate, it was not surprising that it did not produce DON (Table 2), because it also was not a producer of the same fusariotoxin on agar media of the same composition (Table 1). The ZON biosynthesis by the same culture was recorded after 14 days of cultivation in both tested liquid media (YES and YES^{Zn}) at lower room temperatures (17—19°C). The maximal yield of ZON (1.5 mg/l) was observed after 21 day cultivation in both applied liquid media (Table 2). The supplementation of the trace element Zn to YES (0.23 mg/l $ZnSO_4$ x 5 H_2O) resulted in a more outstanding decrease of the initial pH 6.5 to the final pH 4.6, as well as, in higher ZON yields after 14 (1.50 mg/l in comparison to 1.20 mg/l) and 28 days of cultivation (1.20 mg/l in comparison to 0.90 mg/l).

The use of different liquid media for testing the toxin production by isolates of *F. graminearum* is reported also by other authors (Miller and Greenhalgh, 1985; Pestka et al., 1985). Pestka et al. (1985) obtained 5.50 mg/l of DON after 20 days by the use of the glucose-yeast extract-peptone nutrient medium for cultivation of the strain R6576.

The results of fusariotoxin yields and dynamics of biosynthesis by isolates of *F. graminearum* cultivated on natural solid substrates (Table 3. and 4) show significant differences regarding the temperature conditions and the type of substrate.

Tab. 3. Quantity of fusariotoxins production by two isolates of *F. graminearum* cultivated on maize kernels

Temperat. (°C)	Days	Moisture (%)	Toxin yield (mg/kg)			
			D2 isolate		GZ-LES	
			DON	ZON	DON	ZON
17—19	7	46.2	n.d.	0.74	0.58	n.d.
	14	48.0	n.d.	0.94	1.44	0.37
	21	50.0	n.d.	1.29	n.d.	0.37
	28	46.8	n.d.	1.84	n.d.	0.37
27±1	7	43.0	n.d.	n.a.	1.44	n.d.
	14	50.6	n.a.	n.a.	1.44	n.d.
	21	48.4	n.a.	n.a.	1.73	n.d.
	28	49.8	n.a.	n.a.	2.02	n.d.

Legend: n.d. — not detected (d55of ZON and DON, respectively); n.a. — not analyzed

Tab. 4. Quantity of fusariotoxins production by two isolates of *F. graminearum* cultivated on rice kernels

Temperat. (°C)	Days	Moisture (%)	Toxin yield (mg/kg)			
			D2 isolate		GZ-LES	
			DON	ZON	DON	ZON
15—23	28	42.3	n.d.	17.35	n.a.	n.a.
27±1	14	37.9	n.a.	n.a.	302.5	n.d.
	21	—	n.a.	n.a.	645.6	1.26
	28	—	n.a.	n.a.	400.0	3.13

Legend: n.d. — not detected (dof ZON and DON, respectively); n.a. — not analyzed

DON production was observed only in the GZ-LES isolate, regardless of the applied temperature and on both types of cereal substrate (Tables 3. and 4). Much higher quantity of this type B trichothecene was detected after 28 days of cultivation on rice grain at 27±1°C (400.0 mg/kg) than on maize kernels (2.02 mg/kg). Biosynthesis of the maximum DON quantities on maize kernels was detected after cultivation for two weeks at room temperature (17—19°C), and four weeks at 27±1°C, 1.44 mg/kg and 2.02 mg/kg, respectively (Table 3). On rice kernels maximal yield of DON (645.6 mg/kg) was achieved after three weeks of cultivation at 27±1°C (Table 4). Other authors also established that a higher incubation temperature (28°C) favored the DON production (Greenhalgh et al., 1983; Liorens et al., 2004).

After 28 days of cultivation at room temperature the D2 isolate of *F. graminearum* produced more ZON on rice grain, then on corn kernels (Tables 3. and 4). During the cultivation on maize kernels, the yield of this estrogenic substance succesively increased from initial 0.74 mg/kg (after 7 days) to final 1.84 mg/kg (after 28 days) (Table 3).

In contrast to the D2 isolate the GZ-LES isolate of *F. graminearum* biosynthesis of ZON was detected after longer cultivation (14 days) on maize kernels at room temperature (17—19°C) and its yield did not change during

further cultivation; it was constantly 0.37 mg/kg. During cultivation at higher temperature (27±1°C), isolate GZ-LES did not produce ZON at all on the same substrate (Table 3), while on rice grains the biosynthesis of the same mycotoxin (1.26 mg/kg) was detected after three weeks (Table 4). The ZON yield increased during cultivation period at 27±1°C (Table 4). The highest quantities of ZON that can be obtained at lower temperatures, such as 19.5°C or room temperature ranging from 17 to 21°C, are indicated by other authors too (Greenhalgh et al., 1983; Lori et. al., 1990).

CONCLUSIONS

The temperature and nutrient media affected significantly the mycotoxin production, although the tested isolates (D2 and GZ-LES) responded differently to the same cultivation conditions.

The best medium for screening ZON, regardless of the temperature conditions, was YESA, and for DON screening it was $PPSA^{Zn}$ and higher temperatures (about 25°C).

In liquid media (YES and YES^{Zn}) at room temperature (17—19°C) only the ZON biosynthesis occurred by *F. graminearum* D2 isolate.

Higher incubation temperatures (27±1°C) favored the DON production on wet cereal kernels, while lower temperatures (17 to 21°C) favored the ZON biosynthesis.

The isolate *F. graminearum* GZ-LES evidently belongs to the DON chemotype contrary to D2, because the last one did not possess the capability to biosynthesize DON.

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REFERENCES

Bagi, F., Balaž, F., Jajić, I., Stojšin, V. (2008): *Mycotoxin content in different parts of Fusarium infected wheat heads.* Cereal Research Communications, 36 (Suppl. B): 341—342.

Balzer, I., Bogdanić, Č., Pepeljnjak, S. (1978): *Rapid thin layer chromatographic method for determining aflatoxin B_1, ochratoxin A, and zearalenone in corn.* J. Assoc. Offic. Anal. Chem. 61: 584—585.

Blackwell, B., Miller, D. J., Greenhalgh, R. (1985): *^{13}C NMR study of the biosynthesis of toxins by Fusarium graminearum.* The Journal of Biological Chemistry, 260 (7): 4243—4247.

Bočarov-Stančić, A., Laco, D., Tomašević-Čanović, M., Adamović, M., Daković, A. (2003): Toksigenost izolata *Fusarium* spp. sa zrna pšenice kontaminiranog zearalenonom. *Toxicity of Fusarium isolates from wheat grain contaminated with zearalenone.* X Simpozijum "Tehnologija hrane za životinje" (sa međunarodnim učešćem), 19—23. 10. 2003, Vrnjačka Banja. Zbornik radova, 299—305.

Bočarov-Stančić, A. S., Lević, J. T., Dimić, G. R., Stanković, S. Ž., Salma, N. M. (2009): *Investigation of the toxigenic potential of fungal species by the use of simple screening method.* Zbornik Matice srpske za prirodne nauke — Proc. Nat. Sci., Matica Srpska Novi Sad (u štampi).

Cvetnic, Z., Pepeljnjak, S., Sevgic, M. (2005): *Toxigenic potential of Fusarium species isolated from non-harvested maize.* Arh Hig Rada Toksikol. 56 (3): 275—280.

Filtenborg, O., Frisvald, J. C., Svensen, J. A. (1983): *Simple screening method for toxigenic molds producing intracellular mycotoxins in pure culture.* Appl. Envuron. Microbiol, 45: 581—585.

Greenhalgh, R., Neish, G. A., Miller, J. D. (1983): *Deoxynivalenol, acetyl deoxynivalenol, and zearalenone production by Canadian isolates of Fusarium graminearum on solid substrates.* Appl. Environ. Microbiol., 46 (3): 625—629.

Ilgen, P., Maier, F. J., Schäfer, W. (2008): *Trichothecenes and lipases are host induced and secreted virulence factors of Fusarium graminearum.* Cereal Research Communications, 36 (Suppl. B): 421—428.

Jajić, I. M., Bočarov-Stančić, S. A., Bijelić, M. B. (2007): *Investigation of the capability of Fusarium isolates from corn for biosynthesis of fusariotoxins.* Zbornik Matice srpske za prirodne nauke — Proc. Nat. Sci., Matica Srpska Novi Sad, 113, 125—134.

Jennings, P., Coates, M. E., Walsh, K., Turner, J. A., Nicholson, P. (2004): *Determination of deoxynivalenol- and nivalenol-producing chemotypes of Fusarium graminearum isolated from wheat crops in England and Wales.* Plant Pathology, 53 (5): 643—652.

Kim, H.-S., Lee, T., Dawlatana, M., Yun, S.-H., Lee, Y.-W. (2003): *Polymorphism of trichothecene biosynthesis genes in deoxynivalenol- and nivalenol--producing Fusarium graminearum isolates*. Mycological Research, 107:190—197.

Liorens, A., Mateo, R., Hinojo, M. J., Valle-Algarra, F. M., Jiménez, M. (2004): *Influence of environmental factors on the biosynthesis of type B trichothecenes by isolates of Fusarium spp. from Spanish crops.* International Journal of Food Microbiology, 94: 43—54.

Lori, G. A., Henning, C. P., Violante, A., Alippi, H. E., Varsavsky, E. (1990): *Relation between the production of deoxynivalenol and zearalenone and mycelial growth of Fusarium graminearum on solid natural substrates.* Mycologia, 6 (2): 76—82.

Marasas, W. F. O., Nelson, P. E., Toussoun, T. A. (1984): *Toxigenic Fusarium species. Identity and mycotoxicology.* The Pennsylvania State University Press, University Park and London.

Miller, J. D., Greenhalgh, R. (1985): *Nutrient effects on the biosynthesis of trichothecenes and other metabolites by Fusarium graminearum.* Mycologia, 77 (1): 130—136.

Molto, G. A., Gonzalez, H. H. L., Resnik, S. L., Pereyra-Gonzalez, A. (1997): *Production of trichothecenes and zearalenone by Fusarium isolates from Argentinian maize.* Food Additives and Contaminants, 14 (3): 263—268.

Mühlencoert, E. (2004): *Ochratoxin A production by Aspergillus ochraceus.* Doctoral dissertation, Technical Universty, Munchen, Germany.

Pepeljnjak, S., Babić, A. (1991): *Detekcija trihotecenskih mikotoksina T-2, HT-2, DON i DAS tankoslojnom hromatografijom i biološkim metodama*, Prehrambeno--tehnol. biotehnol. Rev. 29: 65—70.

Pestka, J. J., El-Bahrawy, A., Hart, L. P. (1985): *Deoxynivalenol and 15-monoacetiyl deoxynivalenol production by Fusarium graminearum R6576 in liquid media.* Mycopathologia, 91 (1): 23—28.

Samson, R. A., van Reenen-Hoekstra, E. S. (1988): *Introduction to Foodborne Fungi*. Centralbureau voor Schimmelcultures, Institute of the Royal Netherlands Academy of Art and Sciences, Baarn, Delft.

ДИНАМИКА ПРОИЗВОДЊЕ ДЕОКСИНИВАЛЕНОЛА И ЗЕАРАЛЕНОНА КОД ИЗОЛАТА *FUSARIUM GRAMINEARUM* У ЛАБОРАТОРИЈСКИМ УСЛОВИМА

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Резиме

Испитивањем су били обухваћени новоизолована култура *F. graminearum* (D2) неиспитаног токсиколошког профила и контролни изолат исте врсте гљиве (GZ-LES) добро познат произвођач деоксиниваленола (DON) и зеараленона (ZON). Прелиминарне анализе DON-а и ZON-а су извршене према модификованој методи Filtenborg-а и сар. (1983). Динамика биосинтезе DON-а и ZON-а је праћена гајењем изолата гљива у/на четири различита типа подлоге: течној полусинтетичкој подлози са 2% екстракта квасца и 15% сахарозе (YES pH 6,5), подлози истог састава са додатком 0,23 mg/l $ZnSO_4$ x 5 H_2O (YES^{Zn} pH 6,5) и стерилисаним влажним зрнима кукуруза и пиринча.

Квантитативно одређивање DON-а у култури изолата гљива гајених у течној подлози је извршено танкослојном хроматографијом према Цветнићу и сар. (2005), а у чврстој подлози применом поступка аутора Пепељњака и Бабића (1991). Потенцијал за биосинтезу ZON-а код изолата гајених у течној подлози је одређиван поступком танкослојне хроматографије према Бочаров--Станчић и сар. (2003), а изолата гајених на чврстој подлози према Балзеру и сар. (1978).

Максимална концентрација DON-а (645,6 ppb) је детерминисана после три недеље култивације контролног изолата *F. graminearum* GZ-LES на зрну кукуруза и при 27±1°C. За изолат исте врсте гљиве D2 је утврђено да не поседује способност биосинтезе DON-а, с обзиром да није производио овај трихотецен типа Б ни у једном од тестираних услова култивације. Производња ZON-а је констато-

вана код изолата D2 (1,2 ppb, односно 1,5 ppb) после две недеље стационарне култивације у течним подлогама (YES и YES^{Zn}) на собној температури од 17 до 19°C. На природним чврстим супстратима (зрно кукуруза и пиринча) исти изолат је биосинтетисао ZON већ после седам дана култивације на зрну кукуруза и собној температури од 17 до 19°C (0,74 ppb) или после 28 дана култивације на зрну пиринча и собној температури од 15 до 23°C (17,4 ppb).