Multiple metabolic pathways for metabolism of L-tryptophan in *Fusarium graminearum*

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2 *graminearum*

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18

19 **Abstract**

20 *Fusarium graminearum* is a plant pathogen that can cause the devastating cereal grain
21 disease fusarium head blight (FHB) in temperate regions of the world. Previous
22 studies have shown that *F. graminearum* can synthesize indole-3-acetic acid (auxin)
23 using L-tryptophan (L-TRP)-dependent pathways. In the present study, we have taken
24 a broader approach to examine the metabolism of L-TRP in *F. graminearum* liquid
25 culture. Our results showed that *F. graminearum* was able to transiently produce the
26 indole tryptophol when supplied with L-TRP. Comparative gene expression profiling
27 between L-TRP-treated and control cultures showed that L-TRP treatment induced the
28 up-regulation of a series of genes with predicted function in the metabolism of L-TRP
29 via anthranilic acid and catechol towards the tricarboxylic acid cycle. It is proposed
30 that this metabolic activity provides extra energy for 15-acetyldeoxynivalenol
31 production, as observed in our experiments. This is the first report of the use of
32 L-TRP to increase energy resources in a *Fusarium* species.

33

34 **Keywords:** L-tryptophan metabolism, *Fusarium graminearum*, tryptophol, global
35 gene expression profiling.

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40 **Résumé**

41

42 *Fusarium graminearum* est un phytopathogène causant la fusariose, une maladie
43 dévastatrice des céréales à petits grains dans les régions tempérées du globe. Des
44 études précédentes ont montrées que *F. graminearum* peut synthétiser l'acide
45 indole-3-acétique (auxine) en utilisant des voies de synthèse dépendantes du
46 L-tryptophane (L-TRP). Dans la présente étude, nous avons étendu notre approche
47 pour examiner le métabolisme du L-TRP dans des cultures liquides de *F.*
48 *graminearum*. Nos résultats ont montré que *F. graminearum* peut produire de façon
49 transitoire l'indole tryptophol à partir du L-TRP. Une comparaison des profils
50 globaux d'expression génique entre des cultures traitées au L-TRP et les contrôles a
51 montré que le traitement au L-TRP induit une expression accrue d'une série de gènes
52 avec fonction prédite dans le métabolisme du L-TRP via l'acide anthranilique et le
53 catéchol et vers le cycle de l'acide tricarboxylique. Il est proposé que cette activité
54 métabolique produise de l'énergie supplémentaire pour la synthèse de
55 15-acetyldeoxynivalenol, tel qu'observé dans nos expériences. C'est la première
56 évidence de l'utilisation du L-TRP pour augmenter ses ressources énergétiques par
57 une espèce *Fusarienne*

58

59 **Mots-clés:** métabolisme du L-tryptophane, *Fusarium graminearum*, tryptophol, profil
60 global d'expression génique.

61 Introduction

62 *Fusarium graminearum* Schwabe (Hypocreales: Nectriaceae) is a plant pathogen that
63 can cause a devastating disease known as fusarium head blight (FHB) in cereal crops
64 around the world (Parry et al. 1995). *F. graminearum* produces mycotoxins, including
65 deoxynivalenol (DON) and its acetylated derivatives (3- and 15-acetyldeoxynivalenol;
66 3- and 15-ADON), which accumulate during infection, and result in significant
67 contamination of harvested grain and reduction in grain quality (McMullen et al.
68 1997).

69 Many fungal species can biosynthesize the hormone indole-3-acetic acid (IAA),
70 which plays a critical role for some of those species as a virulence factor during plant
71 infection (Cohen et al. 2002). For most of the fungal species, biosynthesis of IAA
72 occurs via L-TRP-dependant pathway(s) and can be stimulated by external feeding
73 with L-TRP and its metabolite intermediates (Dewick, 2001; Tudzynski and Sharon
74 2002, Tsavkelova et al. 2012). It was recently shown that *F. graminearum* can produce
75 IAA; however the L-TRP-dependent tryptamine (TAM) and indole-3-acetonitrile
76 (IAN) pathways were being used rather than the indole-3-acetamide pathway as in
77 many other *Fusarium* species (DesRoches 2012; Luo et al. 2016). Although those
78 experiments clearly showed that *F. graminearum* can use TAM and IAN to produce
79 IAA, the results of treatment with L-TRP itself remained to be clarified. Using HPLC
80 analysis and gene expression profiling, we now describe a pathway used by *F.*
81 *graminearum* to metabolise L-TRP, at least in part via conversion to tryptophol (TOL),
82 towards production of energy.

Materials and Methods

Fusarium strains and culture conditions

F. graminearum virulent strains DAOM180378 and DAOM233423 (derived from GZ3639) were obtained from the Canadian Collection of Fungal Cultures (AAFC, Ottawa, ON). Mycelia from these two strains were cultured on Potato Dextrose Agar (PDA, Difco) plates. To collect macroconidia, sterile water was added to a *F. graminearum*-confluent PDA plate and the surface was gently scraped using the edge of a sterile microscope slide. The macroconidia suspension was filtered through 4 layers of cheesecloth (Fisher Healthcare, Houston, TX, USA), washed twice by centrifugation at 4,000 rpm for 10 min in an Eppendorf 5804R centrifuge using the S-4-72 swing-out rotor, and then pellets were resuspended in sterile distilled water. The concentration of the resulting macroconidia suspension was determined using a hemocytometer.

Feeding experiments and high performance liquid chromatography (HPLC) analysis

Liquid cultures of *F. graminearum* strain DAOM233423 were grown and sampled as described in Luo et al. (2016). Cultures (6-well culture trays, 4 ml/well) were treated with either 2 mM L-tryptophan (400µL of 20mM L-TRP stock solution) or the same volume of sterile water (control). For each treatment, three biological replicates were grown for every time point of collection; sampling was done at 2, 4, 6, 12 and 24 h. The culture supernatants were collected and filtered through 0.2 µm Nylon Syringe filters (Nalgene, Canada) and subjected to HPLC analysis.

105 Additional media treated with 2 mM L-TRP included 1st stage medium (Taylor et al.
106 2008), yeast extract peptone sucrose (YEPS: 1% w/v yeast extract, 2% w/v peptone, 2%
107 w/v sucrose), potato dextrose broth (PDB; Difco), Luria-Bertani broth (LB; Difco),
108 Synthetischer Nährstoffarmer Agar (SNA; Nirenberg 1976), and Czapek's Dox broth
109 (CDB, per l: NaNO₃ 2 g, K₂HPO₄ 1 g, KCl 0.5 g, FeSO₄ 0.01 g, MgSO₄·7H₂O 0.5 g,
110 sucrose 30 g). Samples were collected at 6, 12 and 24 h for HPLC analysis.
111 All HPLC analyses were carried out on an ATKA P-10 HPLC system (GE Healthcare,
112 Canada) equipped with an autosampler A-900, as described in Luo et al. (2016). Fifty
113 µl of the sample mixtures and reference compounds were separated through a 5
114 micron C18 Hypersil Reverse Phase Column (ThermoFisher Scientific Inc) in two
115 steps: first a gradient of 85:15 to 70:30 water:methanol, followed by a gradient of
116 55:45 to 45:55 water:methanol. Eluted compounds were quantified based on the area
117 under the curve (mAU*min) in reference to standard curves with reference
118 compounds (Sigma-Aldrich Co. LLC, Canada).

119 **Gene expression analysis**

120 Mycelia from *F. graminearum* strain DAOM180378 was grown as follows: 1·10⁶
121 macroconidia were inoculated into 50 mL of 1st stage medium (Taylor et al. 2008)
122 contained in 250 mL Erlenmeyer flask that was subsequently incubated on a rotary
123 shaker (200 rpm) at 28 °C in the dark for 72 h. The mycelia were homogenized in
124 their medium, then filtered through Miracloth (Calbiochem, Canada), rinsed with
125 sterile 0.9% saline solution and resuspended in 50 mL of 2nd stage medium (Taylor et
126 al. 2008). The 2nd stage medium, which provided conditions for induction of

mycotoxin production, was either supplemented with 2 mM L-TRP, or used as control without supplement. Three biological replicates were grown for each treatment; mycelia were collected at 6 h after treatment, filtered and immediately submerged into liquid nitrogen. The frozen mycelia were ground using a mortar and pestle and stored at -80 °C until used. RNA from the ground mycelia was extracted using TRIZOL reagent (Invitrogen, Life Technologies Co., Canada), following the manufacturer's protocol. RNA was purified further using the RNeasy Mini Kit (Qiagen Science, Canada) and the RNA cleanup protocol which included an RNase-free DNase I treatment. The integrity of the RNA used for microarray analysis was examined with a 2100 Bioanalyzer (Agilent Technologies Inc., Canada).

Comparative global gene expression profiling was performed using a custom-designed *F. graminearum* microarray (NCBI, GEO record# GPL11046) as described in Qi et al. (2012). For each treatment (L-TRP and control), hybridizations were done on three biological replicates, with two reverse-dye technical replicates for each biological sample. Microarray hybridization and analysis were performed as described in Qi et al. (2012). Hybridization features within the dataset that had Lowess A values above 7.5 in at least 2 of the 6 arrays were retained. The normalised data was analysed in Acuity 4.0 (Molecular Devices, Sunnyvale, CA) to identify candidate genes that were significantly differentially expressed, using the following cut-off parameters: t-test p-value < 0.05 and expression ratio fold changes ≥ 4 (i.e. $\log_2$ ratio ≥ 2.0 or -2.0 ; L-TRP vs control). The raw and normalised data used here are part of GEO accession #GSE100318, deposited at NCBI (National Center for

149 Biotechnology Information).

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152 **Results**

153 **Production of TOL by L-TRP-treated *F. graminearum* cultures**

154 *F. graminearum* has previously been shown to produce IAA under certain growth
155 conditions (DesRoches 2012; Luo et al. 2016; Qi et al. 2016,), using
156 L-TRP-dependent biosynthesis pathways. However, ambiguities persist in the
157 identification of the metabolites produced when the cultures are treated with L-TRP,
158 when compared with treatments with other potential intermediates in the
159 L-TRP-dependent biosynthesis of IAA, such as TAM, IAN and indole-3-acetaldehyde.
160 To address that issue, *F. graminearum* mycelia treated with 2 mM L-TRP were grown
161 under conditions known to be favourable for IAA production (DesRoches 2012), and
162 cultures sampled at five time points during a 24 h time course experiment. Fig. 1
163 shows representative examples of chromatograms obtained during the time course
164 treatment. It can be observed that L-TRP rapidly disappears from the medium, with
165 only about 0.8 mM of it still detected at 2 h and none detectable by 6 h. No production
166 of IAA was detected at any time point. TOL was detected in the first part of the time
167 course, with the highest concentration (0.25 mM) measured at the 2 h time point, then
168 not detectable at 6 h and later time points (Fig. 1 and Fig. 2). Our results suggest that
169 part of the L-TRP can be rapidly metabolised into the indole TOL, which is then also
170 rapidly metabolised by the fungus. Liquid cultures in different media were also tested
171 to determine if the fungus would produce IAA from L-TRP under different conditions,
172 to no avail. L-TRP was completely used in each medium tested except in LB; TOL
173 was also detected in 1st stage media; neither TOL nor IAA was detected in the other

174 media (data not shown).

175 As expected, presence of the trichothecene mycotoxin 15-ADON was observed in
176 increasing amounts during the time course (Fig. 1 and Fig. 2). When compared to
177 the control cultures (water treatment), L-TRP-treated filtrates had a statistically
178 significant ($P < 0.05$) larger amount of 15-ADON production at the 24 h time point
179 while fungal biomass was not significantly different between the two treatments (Fig.
180 3 A and B).

181

182 **Metabolism of L-TRP towards energy production in *F. graminearum***

183 The rapid disappearance of L-TRP and TOL, associated with the increased production
184 of 15-ADON in the L-TRP-treated cultures, suggested that L-TRP and/or TOL can be
185 used by the fungus to directly or indirectly produce an additional amount of
186 15-ADON. To identify candidate genes with enzymatic activity in the metabolism of
187 L-TRP and TOL, global gene expression profiling was performed to compare
188 L-TRP-treated cultures to control cultures, at 6 h after treatment. Analysis of the
189 microarray expression data generated a list of 153 genes that were up-regulated and
190 54 down-regulated in the L-TRP treatment¹. Of the 153 up-regulated genes, 73 were
191 annotated as conserved hypothetical or hypothetical proteins while 23 of the 54
192 down-regulated genes were similarly annotated.

193

194

195 ¹ See Supplementary Material, Table S1.

196 Among the up-regulated genes with known function, ten were predicted to be
197 involved in the metabolism of L-TRP and aromatic compounds, including the five
198 most up-regulated genes in our experiment¹ (Table 1, Fig. 4). The predicted function
199 of those up-regulated genes suggested that L-TRP was catabolized via catechol
200 towards the tricarboxylic acid cycle (TCA), to generate energy through oxidation.
201 Additional genes for catabolism of aromatic compounds towards the TCA cycle were
202 also induced by the L-TRP treatment (Table 1, Fig. 4).

203 Treatment with L-TRP significantly up-regulated many genes in other metabolic
204 pathways¹, including genes predicted to be involved in pyruvate metabolism
205 (FGSG_05168, FGSG_12368, FGSG_01531) and repression of galactose metabolism
206 (FGSG_07557, FGSG_12281). Up-regulated genes involved in catabolism of cysteine,
207 methionine, arginine and tyrosine (FGSG_12200, FGSG_09820, FGSG_12529 and
208 FGSG_03941) and other amino acids and amide compounds (FGSG_10537,
209 FGSG_08078), as well as in the regulation of nitrogen metabolism (FGSG_04830,
210 FGSG_02000 and FGSG_12211) were noted. Detoxification and transport were two
211 other categories including genes with strong up-regulation, however the annotation of
212 those genes is too generic to ascertain their roles.

213 The amplitude of the changes for genes with down-regulated expression was more
214 modest than that observed with the most up-regulated genes¹. Genes in the N-glycan
215 biosynthesis, metabolism and modification were repressed (FGSG_02920,

216

217 ¹ See Supplementary Material. Table S1.

218 FGSG_11578 and FGSG_02681), so were genes in glycerol and trealose catabolism
219 (FGSG_05622 and FGSG_13343) and genes with protein degradation functions. Two
220 tyrosinases, expected to contribute to the biosynthesis of melanin from tyrosine, were
221 also repressed.
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223 Discussion

224 Many fungal species can synthesize IAA from L-TRP. We have recently
225 demonstrated that *F. graminearum* can synthesize IAA using L-TRP-dependent
226 pathways and the intermediate pathway compounds TAM, IAN and
227 indole-3-acetaldehyde (DesRoches, 2012; Luo et al. 2016). In the present study, we
228 have shown that *F. graminearum* cultures treated with L-TRP produced TOL instead
229 of IAA. These results are supported by previous observations indicating that *F.*
230 *graminearum* produced TOL in addition to IAA when treated with IPA or
231 indole-3-acetaldehyde (IAAld) (DesRoches 2012; Luo et al. 2016). Fig. 5 illustrates
232 the proposed pathway for the conversion of L-TRP into TOL, based on this work and
233 previous studies. Conversion of L-TRP to TOL via IAAld has been observed in other
234 fungal species (Furukawa et al. 1996; Chen and Fink 2006); however *F. graminearum*
235 is the first *Fusarium* species shown to metabolise L-TRP into TOL instead of IAA.
236 In our experiments, at least 12% of the 2 mM L-TRP added to the *F. graminearum*
237 cultures was converted into TOL (ca 0.25 mM) at our earliest sampling time point; it
238 is possible that a larger amount of L-TRP was converted to TOL during the first 2 h of
239 treatment and that TOL had started to be metabolised by the first sampling point.
240 By comparison, treatments with 0.2 mM IAN or TAM in similar growth conditions
241 resulted in close to 100% conversion to IAA after 12 and 24 h respectively (Luo et al.
242 2016). Our results suggested that the conversion of L-TRP to TOL is much more rapid
243 than the conversion of other biosynthetic intermediates to IAA.
244 Comparative global expression profiling of *F. graminearum* cultures after 6 h of

245 treatment with L-TRP versus control has shown a strong induction of genes with
246 predicted functions for catabolism of L-TRP via anthranilic acid and catechol towards
247 the TCA cycle, suggesting that L-TRP is rapidly metabolised for energy production.
248 The observation that the L-TRP-treated cultures produced additional 15-ADON, when
249 compared to the water-treated cultures, supports our interpretation that L-TRP is being
250 metabolised towards the TCA cycle and the production of extra energy.

251 In addition to the up-regulation of genes associated with the metabolism of aromatic
252 compounds, there was up-regulation of genes associated with pyruvate metabolism
253 and down-regulation of genes associated with galactose, glycerol and trealose
254 metabolism. Those changes are consistent with the contribution of the TCA cycle to
255 the Krebs cycle and energy production via pyruvate (Fernie et al. 2004). Catabolism
256 of amino acids may also contribute to energy production via pyruvate. Besides genes
257 involved in amino acid catabolism, nitrogen metabolism regulators and protein
258 degradation genes were also up-regulated, possibly to maintain nitrogen balance.

259 This is the first report that L-TRP can be metabolised for energy in *Fusarium* species.
260 Induction of a similar degradation pathway following treatment with L-TRP has been
261 shown in *Trichosporon cutaneum* and *Aspergillus niger* (Rao et al. 1967; Anderson
262 and Dagley, 1981). Kamath and Vaidyanathan (1990) have shown that *Aspergillus*
263 *niger* can also degrade indole compounds to anthranilate and catechol. It is possible
264 that the degradation pathway induced by L-TRP in *F. graminearum* cultures
265 contributed to the rapid disappearance of TOL in addition to L-TRP. These results are
266 consistent with our previous study showing that *F. graminearum* can use salicylic

267 acid as the main source of carbon (Qi et al. 2012); salicylic acid can be converted
268 directly into catechol by a salicylate 1-monooxygenase (H. Rocheleau, personal
269 communication).

270 Induction of additional genes for metabolism of aromatic compounds was also
271 observed in our global expression profiling experiment. This is consistent with
272 findings by Anderson and Dagley (1980), who have observed that treatment of *T.*
273 *cutaneum* cultures with salicylic acid, anthranilate or 3-hydroxybenzoic acid induced
274 enzymatic activities necessary for their metabolism, as well as for metabolism of
275 additional aromatic compounds.

276 It has been shown that infection by *F. graminearum* leads to an increased biosynthesis
277 of L-TRP and derived compounds in wheat and *Brachypodium distachyon*
278 (Paranidharan et al. 2008; Kumaraswamy et al. 2011; Pasquet et al. 2014). It is
279 tempting to speculate that the ability of *F. graminearum* to quickly metabolise L-TRP
280 and related compounds may provide an advantage for the fungus during infection, and
281 to propose that the production of L-TRP by the host is stimulated by the fungus itself.
282 Further experiments will be required to investigate this aspect.

283 In conclusion, *F. graminearum* can convert exogenous L-TRP into TOL. However the
284 exogenous L-TRP and/or the biosynthesized TOL were ultimately metabolised by *F.*
285 *graminearum* as an energy resource.

286

287

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296 **References**

- 297 Anderson, J. J. and Dagley, S. 1981. Catabolism of tryptophan, anthranilate, and 2,
298 3-dihydroxybenzoate in *Trichosporon cutaneum*. J. Bacteriol. **146**: 291-297.
- 299 Chen, H. and Fink, G. R. 2006. Feedback control of morphogenesis in fungi by
300 aromatic alcohols. Gene. Dev. **20**: 1150-61.
- 301 Cohen, B. A., Amsellem, Z., Maor, R., Sharon, A. and Gressel, J. 2002.
302 Transgenically enhanced expression of indole-3-acetic acid confers hypervirulence to
303 plant pathogens. Phytopathol. **92**: 590-596.
- 304 DesRoches, C. 2012. Investigating the Genes and Pathways Involved in the
305 Biosynthesis of Indole-3-acetic Acid in *Fusarium graminearum*, [Master Thesis].
306 University of Ottawa.
- 307 Dewick, P. M. 2001. Medicinal natural products: a biosynthetic approach, John Wiley
308 & Sons, Ltd., Sussex, England. pp. 8-34.
- 309 Fernie, A.R., Carrani, F., Sweetlove, L.J. 004. Respiratory metabolism: glycolysis, the
310 TCA cycle and mitochondrial electron transport. Curr. Opinion Plant Biol. **7**: 254-261.
- 311 Furukawa, T., Koga, J., Adachi, T., Kishi, K. and Syono, K. 1996. Efficient
312 conversion of L-tryptophan to indole-3-acetic acid and/or tryptophol by some species
313 of Rhizoctonia. Plant Cell Physiol. **37**: 899-905.
- 314 Kamath, A. V. and Vaidyanathan, C. S. 1990. New pathway for the biodegradation of
315 indole in *Aspergillus niger*. Appl. Environ. Microbiol. **56**: 275-280.
- 316 Kumaraswamy, G. K., Bollina, V., Kushalappa, A. C., Choo, T. M., Dion, Y., Rioux,
317 S., Mamer, O. and Faubert, D. 2011. Metabolomics technology to phenotype
318 resistance in barley against *Gibberella zeae*. Eur. J. Plant Pathol. **130**: 29-43.
- 319 Luo, K., Rocheleau, H., Qi, P., Zheng, Y., Zhao, H. and Ouellet, T. 2016.
320 Indole-3-acetic acid in *Fusarium graminearum*: Identification of biosynthetic
321 pathways and characterization of physiological effects. Fungal Biol. **120**: 1135-1145.
- 322 McMullen, M., Jones, R. and Gallenberg, D. 1997. Scab of wheat and barley: a
323 re-emerging disease of devastating impact. Plant Dis. **81**: 1340-1348.

- 324 Paranidharan, V., Abu-Nada, Y., Hamzehzarghani, H., Kushalappa, A. C., Mamer, O.,
325 Dion, Y., Rioux, S., Comeau, A. and Choiniere, L. 2008. Resistance-related
326 metabolites in wheat against *Fusarium graminearum* and the virulence factor
327 deoxynivalenol (DON). *Botany*. **86**: 1168-1179.
- 328 Parry, D. W., Jenkinson, P. and McLeod, L. 1995. *Fusarium* ear blight (scab) in small
329 grain cereals—a review. *Plant Pathol.* **44**: 207-238.
- 330 Pasquet, J., Chaouch, S., Macadré, C., Balzergue, S., Huguet, S., Martin-Magniette,
331 M., Bellvert, F., Deguerce, X., Thareau, V. and Heintz, D. 2014. Differential gene
332 expression and metabolomic analyses of *Brachypodium distachyon* infected by
333 deoxynivalenol producing and non-producing strains of *Fusarium graminearum*.
334 *BMC Genomics*. **15**: 629.
- 335 Qi, P., Johnston, A., Balcerzak, M., Rocheleau, H., Harris, L. J., Long, X., Wei, Y.,
336 Zheng, Y. and Ouellet, T. 2012. Effect of salicylic acid on *Fusarium graminearum*, the
337 major causal agent of fusarium head blight in wheat. *Fungal Biol.* **116**: 413-426.
- 338 Qi, P., Balcerzak, M., Rocheleau, H., Leung, W., Wei, Y., Zheng, Y. and Ouellet, T.
339 2016. Jasmonic acid and abscisic acid play important roles in host–pathogen
340 interaction between *Fusarium graminearum* and wheat during the early stages of
341 *Fusarium* head blight. *Physiol. Mol. Plant Pathol.* **93**: 39-48.
- 342 Rao, P. S., Moore, K. and Towers, G. 1967. The conversion of tryptophan to 2,
343 3-dihydroxybenzoic acid and catechol by *Aspergillus niger*. *Biochem. Bioph. Res.*
344 *Commun.* **28**: 1008-1012.
- 345 Taylor, R. D., Saparno, A., Blackwell, B., Anoop, V., Gleddie, S., Tinker, N. A. and
346 Harris, L. J. 2008. Proteomic analyses of *Fusarium graminearum* grown under
347 mycotoxin-inducing conditions. *Proteomics*. **8**: 2256-2265.
- 348 Tsavkelova, E., Oeser, B., Oren-Young, L., Israeli, M., Sasson, Y., Tudzynski, B. and
349 Sharon, A. 2012. Identification and functional characterization of
350 indole-3-acetamide-mediated IAA biosynthesis in plant-associated *Fusarium* species.
351 *Fungal Genet. Biol.* **49**: 48-57.
- 352 Tudzynski, B. and Sharon, A. 2002. Biosynthesis, biological role and application of
353 fungal phytohormones. *Industrial Applications*, Springer: 183-211.

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Table 1. Upregulated genes in *F. graminearum* L-TRP-treated cultures that have a predicted function associated with L-TRP and aromatic compound metabolism.

Gene	Predicted function	Catabolic step [#]	FC mean ⁺
<i>FGSG_04828</i>	Tryptophan 2,3 dioxygenase	A	178.7
<i>FGSG_04829</i>	Kynureninase	B	25.1
<i>FGSG_09061</i>	2,3-dihydroxybenzoic acid decarboxylase	C	643.1
<i>FGSG_11347</i>	Catechol-1,2-dioxygenase	D	119.8
<i>FGSG_03667*</i>	Catechol-1,2-dioxygenase	D	57.5
<i>FGSG_13983</i>	Phenol 2-monooxygenase	E	166.3
<i>FGSG_03657*</i>	Salicylate 1-monooxygenase	F	37.5
<i>FGSG_09063</i>	Salicylate 1-monooxygenase	F	124.3
<i>FGSG_13157</i>	Maleylacetate reductase	G	4.7
<i>FGSG_13141</i>	1,4-Benzoquinone reductase	H	23.3

[#] Catabolic steps are illustrated in Fig. 4.

⁺ FC: Expression fold change, L-TRP treatment vs control treatment, means of 3 biological replicates.

*Biochemical activity was recently confirmed in *F. graminearum* (H. Rocheleau, personal communication)

Figure captions

Fig. 1. HPLC chromatograms of filtrates from *F. graminearum* liquid cultures treated with 2mM L-TRP. Treated cultures were collected at 2, 4, 6 and 24 h. Under our separation conditions, the retention times for L-TRP, TOL and 15-ADON were around 17.68, 20.90 and 19.40 min, respectively. If present, IAA would have been detected around 21.10 min.

Fig. 2. Quantities of TOL and 15-ADON detected in liquid cultures of *F. graminearum* treated with 2mM L-TRP. Values are means of 3 biological replicates ($\pm$ SE).

Fig. 3. Effect of L-TRP treatment on fungal biomass (A) and accumulation of 15-ADON (B). *F. graminearum* cultures were treated for 24 h with either water (control) or 2 mM L-TRP. Values are means of 3 biological replicates ($\pm$ SE); the letters above the bars indicate statistical significance ($P < 0.05$).

Fig. 4. Possible catabolism pathway for L-TRP in *F. graminearum*.

Fig. 5. Proposed tryptophan-dependent enzymatic pathways for biosynthesis of TOL in *F. graminearum*. Filled arrows, conversions observed in or deduced from this study; dotted arrows, steps predicted from literature.

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183x99mm (300 x 300 DPI)

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70x39mm (300 x 300 DPI)

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107x74mm (300 x 300 DPI)

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224x133mm (300 x 300 DPI)

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194x151mm (96 x 96 DPI)

Table S1. Microarray profiling of gene expression in *F. graminearum* cultures treated with tryptophan, L-TRP. Up-regulated genes were selected as genes with ≥ 4 fold difference and down-regulated genes with ≤ -4 fold differences between treated and untreated samples. The values represent means of 3 biological replicates $\pm$ standard deviation.

Gene name	Predicted function	Mean	SD	Pathway
FGSG_12200	ACC deaminase	10.1	1.8	Amino acid metabolism
FGSG_08078	general amidase	10.0	1.4	Amino acid metabolism
FGSG_03941	bifunctional 4-hydroxyphenylacetate degradation enzyme	6.7	1.8	Amino acid metabolism
FGSG_10537	D-amino acid oxidase	5.8	1.6	Amino acid metabolism
FGSG_12529	arginase	4.8	1.2	Amino acid metabolism
FGSG_09820	cysteine dioxygenase type I	4.1	1.5	Amino acid metabolism
FGSG_04828	tryptophan 2,3 dioxygenase	178.7	1.4	Amino acid - L-TRP metabo
FGSG_04829	kynureninase	25.1	1.6	Amino acid - L-TRP metabo
FGSG_09061	2,3-dihydroxybenzoic acid decarboxylase	643.1	2.7	Aromatic compound metabo
FGSG_13983	phenol 2-monooxygenase	166.3	1.8	Aromatic compound metabo
FGSG_09063	salicylate 1-monooxygenase	124.3	1.9	Aromatic compound metabo
FGSG_11347	catechol-1,2-dioxygenase	119.8	1.5	Aromatic compound metabo
FGSG_03667	catechol-1,2-dioxygenase	57.5	2.0	Aromatic compound metabo
FGSG_03657	salicylate 1-monooxygenase	37.5	3.1	Aromatic compound metabo
FGSG_13141	1,4-benzoquinone reductase	23.3	1.1	Aromatic compound metabo
FGSG_13157	maleylacetate reductase	4.7	1.4	Aromatic compound metabo
FGSG_05401	beta-1,3-glucanase	4.7	1.4	Carbohydrate metabolism
FGSG_06465	haloacetate dehalogenase H-1	41.0	3.2	Carbon metabolism
FGSG_05168	Transcription co-factor Pirin	22.3	1.6	Carbon metabolism
FGSG_07557	transcription co-repressor GAL80	17.3	2.1	Carbon metabolism
FGSG_12368	ADH2 - alcohol dehydrogenase II	14.3	1.6	Carbon metabolism
FGSG_01531	CYB2 - lactate dehydrogenase cytochrome b2	7.4	2.3	Carbon metabolism
FGSG_05467	dTDP-glucose 4,6-dehydratase	6.1	1.6	Carbon metabolism
FGSG_06553	xylulose-5-phosphate/fructose-6-phosphate phosphoketolase	4.9	1.4	Carbon metabolism
FGSG_12281	lactose regulatory protein	4.4	1.0	Carbon metabolism
FGSG_03563	LSB3 - regulation of actin cytoskeletal organization	5.8	2.1	Cell structure
FGSG_10434	glutathione S-transferase GST-6.0	76.1	1.8	Detoxification

FGSG_11132	cyanamide hydratase	44.2	4.0	Detoxification
FGSG_00172	glutathione transferase omega 1	30.7	2.2	Detoxification
FGSG_07888	arylamine N-acetyltransferase 2	29.3	1.5	Detoxification
FGSG_12103	epoxide hydrolase	25.7	1.7	Detoxification
FGSG_11040	glutathione S-transferase	21.2	1.4	Detoxification
FGSG_05942	phenylcoumaran benzylic ether reductase	18.8	2.4	Detoxification
FGSG_03774	7alpha-cephem-methoxylase P8 chain	9.8	2.2	Detoxification
FGSG_12965	catalase isozyme P	5.9	1.3	Detoxification
FGSG_09991	gamma-glutamylcysteine synthetase	5.2	2.0	Detoxification
FGSG_04124	LYS7 - copper chaperone for superoxide dismutase Sod1p	4.4	1.6	Detoxification
FGSG_10124	NADPH quinone oxidoreductase homolog PIG3	5.9	1.9	Electron transport
FGSG_11295	enoyl-CoA hydratase precursor, mitochondrial	6.6	1.8	Lipid metabolism
FGSG_10374	putative fatty acid desaturase (mld)	4.2	2.3	Lipid metabolism
FGSG_03151	integral membrane protein	8.8	1.1	Membrane protein
FGSG_11381	integral membrane protein	7.9	1.4	Membrane protein
FGSG_04627	nik-1 protein (Os-1p protein)	7.1	1.4	Membrane protein
FGSG_07960	YTP1	4.5	1.1	Membrane protein
FGSG_03237	integral membrane protein	4.1	1.3	Membrane protein
FGSG_08079	<i>But1</i> (cytochrome P450)	64.3	1.2	Other metabolism
FGSG_03914	ycaC, hydrolase of unknown specificity	36.5	1.6	Other metabolism
FGSG_00828	7alpha-cephem-methoxylase P8 chain	24.6	2.9	Other metabolism
FGSG_03175	quinone reductase	20.9	2.4	Other metabolism
FGSG_09684	flavin oxidoreductase	16.9	1.3	Other metabolism
FGSG_04012	NADH oxidase	16.0	3.8	Other metabolism
FGSG_08452	RIB3 - 3,4-dihydroxy-2-butanone 4-phosphate synthase	12.3	2.0	Other metabolism
FGSG_08077	flavin oxidoreductase	11.0	1.5	Other metabolism
FGSG_06528	flavin oxidoreductase	10.0	2.0	Other metabolism
FGSG_07249	tox D gene	9.4	1.2	Other metabolism
FGSG_03710	NADH oxidase	6.2	2.0	Other metabolism
FGSG_10429	transcriptional activator CMR1	10.9	1.1	Protein degradation
FGSG_04817	serine protease	4.4	1.2	Protein degradation
FGSG_08453	COQ3 - enzyme of ubiquinone (coenzyme Q) biosynthesis	6.2	1.5	Redox
FGSG_02431	transcriptional regulator	9.0	1.5	Regulation
FGSG_04568	SIS2 protein (cycle-specific gene control)	9.0	1.2	Regulation
FGSG_12398	transcriptional activator Mut3p	8.3	1.3	Regulation
FGSG_02000	URE2 - nitrogen catabolite repression regulator	23.4	2.8	Regulation of metabolism

<i>FGSG_12211</i>	nitrogen metabolic regulation protein nmr	9.6	1.6	Regulation of metabolism
<i>FGSG_04830</i>	positive regulator of PUT (proline utilization) genes	5.5	2.1	Regulation of metabolism
<i>FGSG_10854</i>	antioxidant protein and metal homeostasis factor	4.0	1.2	Regulation of stress
<i>FGSG_03595</i>	ADP-ribosylation factor	10.1	1.2	Signaling
<i>FGSG_09863</i>	acyl-CoA cholesterol acyltransferase	10.9	1.3	Sterol metabolism
<i>FGSG_05921</i>	24-dehydrocholesterol reductase precursor	4.3	1.6	Sterol metabolism
<i>FGSG_03772</i>	aminotriazole resistance protein	54.1	1.6	Transport
<i>FGSG_00118</i>	neutral amino acid permease	16.2	1.9	Transport
<i>FGSG_04468</i>	neutral amino acid permease	13.4	1.4	Transport
<i>FGSG_08229</i>	monocarboxylate transporter	9.9	1.5	Transport
<i>FGSG_10506</i>	monocarboxylate transporter 2	9.1	2.1	Transport
<i>FGSG_07832</i>	CCC1 protein (involved in calcium homeostasis)	8.5	1.3	Transport
<i>FGSG_00226</i>	multidrug resistant protein	6.8	2.7	Transport
<i>FGSG_09701</i>	major facilitator MirA	5.5	1.7	Transport
<i>FGSG_00773</i>	copper transport protein	4.5	1.2	Transport
<i>FGSG_03893</i>	conserved hypothetical protein	88.6	5.3	Unknown
<i>FGSG_08588</i>	conserved hypothetical protein	73.5	2.1	Unknown
<i>FGSG_04616</i>	conserved hypothetical protein	63.0	1.2	Unknown
<i>FGSG_06540</i>	conserved hypothetical protein	56.2	2.0	Unknown
<i>FGSG_04845</i>	conserved hypothetical protein	56.1	2.0	Unknown
<i>FGSG_03666</i>	conserved hypothetical protein	54.5	1.9	Unknown
<i>FGSG_12397</i>	conserved hypothetical protein	50.9	2.4	Unknown
<i>FGSG_09062</i>	conserved hypothetical protein	45.0	1.7	Unknown
<i>FGSG_10433</i>	conserved hypothetical protein	39.5	1.5	Unknown
<i>FGSG_11311</i>	conserved hypothetical protein	35.8	1.4	Unknown
<i>FGSG_01773</i>	conserved hypothetical protein	30.0	5.0	Unknown
<i>FGSG_07991</i>	conserved hypothetical protein	28.7	1.1	Unknown
<i>FGSG_12049</i>	conserved hypothetical protein	27.9	2.1	Unknown
<i>FGSG_01866</i>	conserved hypothetical protein	27.2	1.7	Unknown
<i>FGSG_02210</i>	conserved hypothetical protein	23.8	1.4	Unknown
<i>FGSG_12330</i>	conserved hypothetical protein	22.1	3.7	Unknown
<i>FGSG_12857</i>	hypothetical protein	21.7	1.2	Unknown
<i>FGSG_10971</i>	conserved hypothetical protein	19.7	2.4	Unknown
<i>FGSG_03040</i>	conserved hypothetical protein	19.1	2.3	Unknown
<i>FGSG_10497</i>	conserved hypothetical protein	17.0	2.1	Unknown
<i>FGSG_01772</i>	conserved hypothetical protein	14.4	1.9	Unknown

<i>FGSG_15650</i>	hypothetical protein	13.9	3.2	Unknown
<i>FGSG_13368</i>	hypothetical protein	13.7	1.6	Unknown
<i>FGSG_03717</i>	conserved hypothetical protein	13.7	1.6	Unknown
<i>FGSG_08157</i>	conserved hypothetical protein	13.5	1.8	Unknown
<i>FGSG_02545</i>	conserved hypothetical protein	13.4	2.4	Unknown
<i>FGSG_11412</i>	conserved hypothetical protein	12.8	1.9	Unknown
<i>FGSG_03942</i>	conserved hypothetical protein	12.2	2.9	Unknown
<i>FGSG_09372</i>	conserved hypothetical protein	11.8	3.5	Unknown
<i>FGSG_12635</i>	hypothetical protein	11.5	1.5	Unknown
<i>FGSG_07378</i>	conserved hypothetical protein	11.2	2.5	Unknown
<i>FGSG_04377</i>	conserved hypothetical protein	10.8	1.9	Unknown
<i>FGSG_09677</i>	conserved hypothetical protein	10.7	2.1	Unknown
<i>FGSG_11312</i>	hypothetical protein	10.3	1.1	Unknown
<i>FGSG_11375</i>	fluconazole resistance protein (FLU1)	9.7	3.5	Unknown
<i>FGSG_00249</i>	conserved hypothetical protein	8.6	2.4	Unknown
<i>FGSG_07708</i>	conserved hypothetical protein	8.1	1.4	Unknown
<i>FGSG_02037</i>	conserved hypothetical protein	7.8	1.8	Unknown
<i>FGSG_08269</i>	conserved hypothetical protein	7.6	1.8	Unknown
<i>FGSG_10442</i>	conserved hypothetical protein	7.4	1.2	Unknown
<i>FGSG_03451</i>	conserved hypothetical protein	7.4	1.3	Unknown
<i>FGSG_06401</i>	conserved hypothetical protein	7.1	1.1	Unknown
<i>FGSG_05772</i>	conserved hypothetical protein	7.0	1.5	Unknown
<i>FGSG_10153</i>	conserved hypothetical protein	6.9	1.3	Unknown
<i>FGSG_09806</i>	conserved hypothetical protein	6.9	2.5	Unknown
<i>FGSG_02485</i>	conserved hypothetical protein	6.7	2.2	Unknown
<i>FGSG_02127</i>	conserved hypothetical protein	6.7	1.2	Unknown
<i>FGSG_10974</i>	conserved hypothetical protein	6.6	1.4	Unknown
<i>FGSG_09348</i>	conserved hypothetical protein	6.4	2.4	Unknown
<i>FGSG_02201</i>	conserved hypothetical protein	6.4	1.4	Unknown
<i>FGSG_03397</i>	Hypothetical protein	6.2	1.6	Unknown
<i>FGSG_06343</i>	conserved hypothetical protein	5.8	2.2	Unknown
<i>FGSG_08028</i>	conserved hypothetical protein	5.8	1.6	Unknown
<i>FGSG_12841</i>	hypothetical protein	5.8	1.6	Unknown
<i>FGSG_02997</i>	conserved hypothetical protein	5.7	1.4	Unknown
<i>FGSG_10809</i>	conserved hypothetical protein	5.7	1.8	Unknown
<i>FGSG_09570</i>	conserved hypothetical protein	5.5	1.4	Unknown

<i>FGSG_10430</i>	conserved hypothetical protein	5.4	1.4	Unknown
<i>FGSG_03970</i>	conserved hypothetical protein	5.3	2.0	Unknown
<i>FGSG_07880</i>	conserved hypothetical protein	5.1	1.3	Unknown
<i>FGSG_11062</i>	conserved hypothetical protein	5.0	1.6	Unknown
<i>FGSG_11308</i>	hypothetical protein	4.9	2.0	Unknown
<i>FGSG_05190</i>	conserved hypothetical protein	4.9	1.3	Unknown
<i>FGSG_03940</i>	conserved hypothetical protein	4.8	1.3	Unknown
<i>FGSG_08123</i>	conserved hypothetical protein	4.7	1.5	Unknown
<i>FGSG_00171</i>	conserved hypothetical protein	4.5	1.3	Unknown
<i>FGSG_13922</i>	conserved hypothetical protein	4.5	1.2	Unknown
<i>FGSG_07943</i>	conserved hypothetical protein	4.4	1.3	Unknown
<i>FGSG_12945</i>	conserved hypothetical protein	4.3	1.2	Unknown
<i>FGSG_13496</i>	conserved hypothetical protein	4.3	1.2	Unknown
<i>FGSG_02263</i>	conserved hypothetical protein	4.3	2.0	Unknown
<i>FGSG_02120</i>	hypothetical protein	4.1	1.6	Unknown
<i>FGSG_12788</i>	conserved hypothetical protein	4.1	1.7	Unknown
<i>FGSG_13455</i>	conserved hypothetical protein	4.1	1.1	Unknown
<i>FGSG_10152</i>	conserved hypothetical protein	4.0	1.1	Unknown
<i>FGSG_11528</i>	monophenol monooxygenase (tyrosinase)	-9.8	1.7	Amino acid metabolism
<i>FGSG_01988</i>	monophenol monooxygenase (tyrosinase)	-6.2	2.1	Amino acid metabolism
<i>FGSG_02681</i>	alpha-1,6-mannosyltransferase HOC1	-19.1	1.4	Carbohydrate metabolism
<i>FGSG_02920</i>	ROT2 - glucosidase II, catalytic subunit	-5.8	2.4	Carbohydrate metabolism
<i>FGSG_11578</i>	acetylxyln esterase	-4.6	1.0	Carbohydrate metabolism
<i>FGSG_02296</i>	aldehyde dehydrogenase	-8.0	N/A	Carbon metabolism
<i>FGSG_05622</i>	trehalase precursor	-4.5	1.6	Carbon metabolism
<i>FGSG_13343</i>	GUT1 - glycerol kinase	-4.1	1.6	Carbon metabolism
<i>FGSG_11498</i>	pisatin demethylase (cytochrome P450)	-5.6	2.4	Detoxification
<i>FGSG_02982</i>	pisatin demethylase (cytochrome P450)	-5.1	1.4	Detoxification
<i>FGSG_07896</i>	trichothecene 3-O-acetyltransferase (TRI101)	-4.2	1.1	Detoxification
<i>FGSG_05836</i>	membrane protein, peroxisomal	-4.9	1.1	Membrane protein
<i>FGSG_09042</i>	formamidase	-9.1	1.6	Nitrogen metabolism
<i>FGSG_03163</i>	monooxygenase	-12.7	1.0	Other metabolism
<i>FGSG_03816</i>	lactonohydrolase	-6.6	1.3	Other metabolism
<i>FGSG_12145</i>	3-hydroxybutyryl-CoA dehydratase	-5.9	1.7	Other metabolism
<i>FGSG_02615</i>	O-methyltransferase	-4.7	1.6	Other metabolism

<i>FGSG_09048</i>	7,8-diaminonanoate transaminase	-4.5	1.8	Other metabolism
<i>FGSG_03700</i>	O-methylsterigmatocystin oxidoreductase	-4.3	1.3	Other metabolism
<i>FGSG_00028</i>	metalloprotease MEP1	-15.2	4.0	Protein degradation
<i>FGSG_11164</i>	trypsin precursor	-8.3	1.1	Protein degradation
<i>FGSG_12371</i>	endopeptidase K	-6.4	2.6	Protein degradation
<i>FGSG_10595</i>	alkaline protease (oryzin)	-5.5	1.6	Protein degradation
<i>FGSG_10471</i>	helicase-like transcription factor protein	-4.1	1.2	Regulation
<i>FGSG_03985</i>	carnitine transporter	-5.3	1.6	Transport
<i>FGSG_12394</i>	neutral amino acid permease	-5.1	2.8	Transport
<i>FGSG_03778</i>	neutral amino acid permease	-4.7	1.2	Transport
<i>FGSG_04317</i>	multidrug resistant protein	-4.7	5.5	Transport
<i>FGSG_10935</i>	ATP-binding-cassette protein	-4.2	1.3	Transport
<i>FGSG_04745</i>	antifungal protein	-10.7	1.2	Unknown
<i>FGSG_03123</i>	conserved hypothetical protein	-9.9	2.3	Unknown
<i>FGSG_09119</i>	conserved hypothetical protein	-9.8	1.9	Unknown
<i>FGSG_08295</i>	conserved hypothetical protein	-8.4	2.1	Unknown
<i>FGSG_02679</i>	conserved hypothetical protein	-8.3	2.2	Unknown
<i>FGSG_05660</i>	Mx protein	-6.5	1.4	Unknown
<i>FGSG_09044</i>	conserved hypothetical protein	-6.3	1.2	Unknown
<i>FGSG_12146</i>	conserved hypothetical protein	-5.7	1.2	Unknown
<i>FGSG_02828</i>	conserved hypothetical protein	-5.6	1.6	Unknown
<i>FGSG_11099</i>	conserved hypothetical protein	-5.4	1.5	Unknown
<i>FGSG_03059</i>	conserved hypothetical protein	-5.2	1.4	Unknown
<i>FGSG_13202</i>	conserved hypothetical protein	-5.1	1.9	Unknown
<i>FGSG_07901</i>	conserved hypothetical protein	-5.1	1.3	Unknown
<i>FGSG_04801</i>	conserved hypothetical protein	-4.9	2.4	Unknown
<i>FGSG_02174</i>	conserved hypothetical protein	-4.8	1.2	Unknown
<i>FGSG_09045</i>	stage V sporulation protein K	-4.7	1.3	Unknown
<i>FGSG_02255</i>	conserved hypothetical protein	-4.6	3.2	Unknown
<i>FGSG_03911</i>	conserved hypothetical protein	-4.6	1.7	Unknown
<i>FGSG_04941</i>	conserved hypothetical protein	-4.6	1.6	Unknown
<i>FGSG_07903</i>	conserved hypothetical protein	-4.5	N/A	Unknown
<i>FGSG_11577</i>	conserved hypothetical protein	-4.4	1.5	Unknown
<i>FGSG_02365</i>	conserved hypothetical protein	-4.1	1.2	Unknown
<i>FGSG_07822</i>	conserved hypothetical protein	-4.1	1.9	Unknown
<i>FGSG_04015</i>	conserved hypothetical protein	-4.0	1.2	Unknown

<i>FGSG_01304</i>	conserved hypothetical protein	-4.0	1.8	Unknown
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Draft