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1 **Genomic analysis of the *Bacillus megaterium* Mes11: new**
2 **insights into nitroreductase genes associated with the**
3 **degradation of mesotrione.**

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50 **Highlights**

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52 The complete genome of the *Bacillus megaterium* Mes11 was sequenced

53 The strain transformed mesotrione alone or in herbicide mixtures

54 The NfrA2 nitroreductase played a key physiological role in mesotrione transformation

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102 **Abstract**

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104 Mesotrione is among the most recently marketed herbicides belonging to the triketone
105 family and applied on maize crops. A *Bacillus megaterium* Mes11 strain isolated from an
106 agricultural soil was able to completely transform 1 mM of mesotrione, alone or in a mixture
107 with 1 mM of nicosulfuron and/or S-metolachlor herbicides, in 5 h. Its complete genome
108 contains one circular chromosome (5,191,612 bp) and nine plasmids (from 45,372 to 169,684
109 bp). Genome analysis revealed that 10 genes encoded oxygen-insensitive nitroreductases of
110 which only one (NfrA2) was shown to be involved in the first step of mesotrione transformation
111 « *in cellulo* ». Indeed, quantitative reverse-transcription PCR analysis demonstrated that the
112 NfrA2 expression was concomitant with mesotrione transformation, showing a maximum 6-
113 fold increase after a 4 h exposure to this single molecule. As opposed to nicosulfuron, the
114 presence of S-metolachlor led to a significant 12 to 24-fold increase of NfrA2 expression after
115 the same time of exposure, in the binary and ternary mixtures, respectively. These results
116 highlight, for the first time, the physiological role of NfrA2 in mesotrione transformation « *in*
117 *cellulo* » and the environmental role of *B. megaterium* Mes11 for bioremediation of mesotrione-
118 contaminated ecosystems.

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120 **Keywords:** *Bacillus megaterium* Mes11; complete genome sequence; herbicide degradation;
121 mixtures; nitroreductases

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1. Introduction

Mesotrione (i.e. 2-(4-methylsulfonyl-2-nitrobenzoyl) cyclohexane-1,3-dione) is one of the new generation pesticides, chemically derived from a natural phytotoxin (leptospermone), and now becoming a major commercial herbicide (Casida and Durkin, 2017). Targeting a key enzyme involved in the plant carotenoid biosynthesis, the 4-hydroxyphenylpyruvate dioxygenase, by inhibition, its success was due not only to its high efficiency towards a wide range of broad-leaved and grass weeds, in particular those resistant to other herbicides, but also to its rather low recommended agronomic dose (applied at a rate of 150 g active ingredient ha⁻¹) conforming to the Integrated Pest Management (IPM), aiming to minimize pesticide use. It was also one of the first atrazine replacement herbicides, widely used worldwide (Mitchell et al., 2001). Belonging to the triketone family, mesotrione is a systemic post-emergence herbicide, typically used to protect maize crops.

Mesotrione fate has already been extensively studied in various matrices such as soil, water and crops (Barchanska, 2012; Dumas et al., 2017). Highly soluble, with a K_d value range from 0.13 to 5.0 L.kg⁻¹, this herbicide is classified as relatively mobile and bioavailable in soils, making biotransformation processes quite possible (Barchanska et al., 2016; Chaabane et al., 2008; Dyson et al., 2002; Gámiz et al., 2019).

The chemical mesotrione is distinguished by the presence of a nitro group on the benzoyl moiety. Indeed, a detailed analysis of the structure-activity relationships showed that an electron-withdrawing substituent at the 2-position was required for herbicidal activity, -NO₂ being one of the strongest and mesotrione the most efficient herbicide compared to other triketone derivatives (Beaudegnies et al., 2009; Lee et al., 1998). Nevertheless, this nitro group also constitutes a potential site of attack for its (bio)degradation, by reduction into hydroxylamino and then amino group.

Various bacterial strains able to transform mesotrione have already been isolated (Dumas et al., 2017). Although several mesotrione metabolic pathways have been observed for a few of the isolated strains (Dobrzanski et al., 2018; Olchanheski et al., 2014; Pileggi et al., 2012; Romdhane et al., 2016), they are most frequently well conserved and common to the majority of mesotrione-transforming strains (Dumas et al., 2017). Not surprisingly, the major pathway generally involves reduction of the nitro group of mesotrione into intermediate free or cyclized hydroxylamino derivatives, these being subsequently transformed to the final metabolite, 2-amino-4-methylsulfonylbenzoic acid (AMBA) after the hydrolysis of the β-diketone bond. AMBA then accumulates in the medium and has also been detected in the environment (Alferness and Wiebe, 2002; Dumas et al., 2017).

Type II and type I nitroreductases are the enzymes involved in such transformations of nitroaromatic compounds, such as mesotrione, by reduction *via* one or two-electron transfer, respectively (Bryant et al., 1981; Peterson et al., 1979). The type II nitroreductases, whose activity is oxygen-sensitive, catalyse the one-electron reduction of a substrate, using FMN or FAD as cofactor and forming a nitro anion radical. This radical undergoes futile cycling in the presence of oxygen giving super-oxide anions and regenerating the parent nitro-compound (Mason and Holtzman, 1975). The oxygen-insensitive type I nitroreductases are NAD(P)H-dependent, FMN binding proteins. These enzymes are responsible for catalytic reduction of the nitro group formally by addition of two electrons to give the nitroso intermediate. This, in turn is reduced further to hydroxylamino and amino intermediates accepting pairs of electrons from NAD(P)H (Bryant et al., 1981; Peterson et al., 1979). Therefore, nitroreductases are good candidates for bioremediation of nitroaromatic compounds and several enzymes have been characterized as able to degrade the toxic 2,4,6-trinitrotoluene (TNT) (Gumuscu and Tekinay, 2013; Hannink et al., 2001; Ramya Sree et al., 2019).

A *Bacillus megaterium* Mes11, among all the degrading-strains, was isolated from an agricultural soil and is able to completely and rapidly biotransform mesotrione in aerobic conditions (Batisson et al., 2009). The fact that nitroreductase is involved in this

207 biotransformation by *B. megaterium* Mes11 was first evidenced by a network analysis of
208 mesotrione-adaptative proteins which emphasizes a direct link with this type of enzyme (Bardot
209 et al., 2015). More recently, Carles et al. (2016) confirmed that two oxygen-insensitive
210 nitroreductases of the *B. megaterium* Mes11 strain, NfrA1 and NfrA2 (Genbank accession nos.
211 KT008921 and KT008916), expressed in a heterologous *E. coli* system and purified, were
212 involved in the first step of mesotrione transformation when exposed to this sole molecule.

213 To further investigate the role of nitroreductases in mesotrione transformation, a study of
214 their « *in cellulose* » expression was carried out by sequencing and analysing the whole genome
215 of the *Bacillus megaterium* Mes11. We also compiled an inventory of all the nitroreductase-
216 coding genes potentially involved in mesotrione-transformation. Moreover, as mesotrione is
217 usually applied agriculturally in mixture with the herbicides S-metolachlor (chloroacetanilide)
218 and nicosulfuron (sulfonylurea) in order to improve its efficiency and the range of weeds
219 treated, a focus was made on the impact of such mixtures on the nitroreductase expression. We
220 have already shown that when mixed, any of these herbicides could have an impact on their
221 individual toxicity and on the transformation kinetics of the others (Carles et al., 2018; Joly et
222 al., 2013). In this manuscript, the physiological activity of the various nitroreductases found in
223 *B. megaterium* to be involved in mesotrione-transformation was determined under multi-
224 contamination conditions to account for agricultural mixture exposure more realistically.

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227 **2. Materials and Methods**

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229 2.1. Bacterium, chemicals and media

230

231 *Bacillus megaterium* Mes11 was isolated from soil (Batisson et al., 2009). The analytical
232 grade herbicides (Mesotrione, nicosulfuron, S-metolachlor (Pestanal) and the Tryptic Soy broth
233 (TS) medium were purchased from Sigma Aldrich (France). The composition of Glucose-
234 mineral Salt Medium (GSM), pH 6.7, was previously reported by Carles et al. (2018).

235

236

237 2.2. Whole genome sequencing of *B. megaterium* Mes11 and annotation.

238

239 Pac-Bio RS technology (GATC Biotech) was used to sequence the *B. megaterium* Mes11
240 genome. The draft genome was annotated using RAST version 4.0 (1) and the NCBI
241 Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP;
242 <http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html>).

243 The *B. megaterium* Mes11 genome sequences have been deposited in GenBank under
244 accession numbers: CP048883 to CP048892.

245

246

247 2.3. Expression and purification of the plasmidic nitroreductase in *E. coli*

248

249 The PCR amplification was carried out using the primers NRK forward (5'-
250 ATGAATAATACAATTGAATTACTTTCT-3') and NRK reverse (5'-
251 TTTTTAGCAAACCCTTGTTTGTAA-3'). The amplicon was cloned into the expression
252 vector pEXP5-CT/TOPO (Invitrogen). The protein expression and mesotrione
253 biotransformation tests were carried out as described by Carles et al. (2016).

254

255

256 2.4. Culture of *B. megaterium* Mes11 with herbicides

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258 *Bacillus megaterium* Mes11 was grown in 750 mL-TS broth supplemented with 0.1 mM
259 mesotrione, under agitation (150 rpm) at 28 °C. After an overnight culture, the cells were
260 centrifuged (8,000 g, 5 min, 4 °C) and washed with a solution of NaCl (0.8 %). The pellet was
261 then resuspended in GSM (150 mL) supplemented with 1 mM of mesotrione, nicosulfuron and
262 S-metolachlor, either alone, in binary or ternary mixtures. Abiotic controls (medium without
263 inoculation and inoculation without herbicide) were also realized. For each condition, the
264 cultures were carried out in triplicate and incubated in the dark (28 °C, 150 rpm). Samples (1
265 mL) were regularly taken and centrifuged (12,500 g, 5 min). HPLC analyses were performed
266 on the supernatants and total RNA was recovered from the corresponding pellets (see below).
267 The herbicide biosorption was measured from a 1 mL culture pellet, stirred vigorously
268 overnight in 500 µL ethanol at room temperature before being centrifuged (12,500 g, 5 min).
269 The extraction process was repeated twice.

270

271 2.5. Analysis of the herbicides and their metabolites

272

273 The herbicide concentrations were measured by HPLC as described previously by Carles et
274 al. (2018). The mesotrione metabolites were identified and quantified by LC-MS and ¹H NMR,
275 respectively, as previously described by Carles et al. (2018).

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278 2.6. Reverse transcription- quantitative PCR (RT-qPCR) analysis

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280 Total RNA was extracted at T0, 2, 4 and 8 h as described by Dugat-Bony et al. (2011). The
281 DNase treatments (twice) and reverse transcription were performed with the DNaseI kit
282 (Ambion) and the SuperScript™ III Reverse Transcriptase kit (Invitrogen) using Random
283 Primers (Invitrogen), respectively. The qPCR reactions were performed in a total reaction
284 volume of 15 µL containing 7.5 µL of MESA GREEN qPCR MasterMix Plus for SYBR® Assay
285 Low ROX kit (Eurogentec), 0.3 µM of each primer and 1 µL of 0.1 X cDNA. The primers used
286 for *NfrA1* and *NfrA2* nitroreductase genes amplification were reported by Carles et al. (2016).
287 The qPCR was carried out in an Realplex2 egradient S Mastercycler (Eppendorf) as follows:
288 initial denaturation at 95 °C for 5 min, 40 cycles of denaturation at 95 °C for 30 s, primer
289 annealing at 57 °C for 15 s and elongation at 72 °C for 20 s. The 16S rRNA gene was used to
290 normalize the results using primers described by Borrel et al. (2012).

291

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293 2.7. Statistical analysis

294

295 R (v3.5.0) and RStudio (v1.1.456) were used for statistical analyses. Differences in *NfrA2*
296 expression fold-change were assessed in triplicate experiments using two-way ANOVA
297 followed by separate post hoc comparisons (Tukey's test, P < 0.05). The two-way ANOVA
298 includes the factors treatment (mesotrione; mesotrione + nicosulfuron; mesotrione + S-
299 metolachlor; mesotrione + nicosulfuron + S-metolachlor) and time (0, 2, 4 and 8 h). The
300 normality and homogeneity of variance were checked prior to ANOVA tests (Shapiro's and
301 Levene's tests, respectively, for P < 0.05).

302

303 3. Results

304

305 3.1. Analysis of the *B. megaterium* genome

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307 The PacBio sequencing of the *B. megaterium* Mes11 genome generated ca. 1,15 Gbp of
308 nucleotides and the average subread length was 12,649 bp. A total of 90,915 reads were
309 sequenced with an N50 of ca. 16.970 kb. After the *de novo* assembly, 10 contigs were produced

310 and the genome size of *B. megaterium* Mes11 was predicted to be 5.2 Mbp. It includes one
311 chromosome and 9 plasmids (pBMG1 to pBMG9). Their general features are summarized in
312 Fig. 1 and Table 1.

313 In our previous study, *in silico* analysis of the three available *B. megaterium* genomes
314 highlighted 9 chromosome encoded oxygen-insensitive nitroreductase genes also detected in
315 the Mes11 genome (Carles et al., 2016; Genbank accession nos. KT008914 – KT008922). The
316 analysis of the whole genome of *B. megaterium* Mes11 also revealed an additional
317 nitroreductase gene encoded by the pBMG7 plasmid (accession no. BMG_6397).

318

319 3.2. Mesotrione transformation by *B. megaterium* Mes11

320

321 The *B. megaterium* strain showed similar rapid mesotrione transformation kinetics whether
322 used alone or in binary or ternary mixtures with other herbicides (nicosulfuron and S-
323 metolachlor) (Fig. 2). A complete dissipation of the herbicide was observed within 5 h of
324 culture, whereas none was noted without the strain (abiotic control). Concomitantly to the
325 mesotrione dissipation, two transitory metabolites, the hydroxylamino derivative in equilibrium
326 with its cyclized form isoxazolol, appeared after 2 h of incubation and reached their maximum
327 concentration when there was no mesotrione left (Table 2). They were formed quantitatively
328 by the reduction of the nitro group of mesotrione before being transformed into AMBA. Mass
329 balance analyses showed that no loss of compound occurred during the mesotrione
330 transformation since values ranged from 86 % to 108 % at the end of the experiment (Table 2).
331 This is in agreement with the absence of significant mesotrione and metabolite adsorption
332 observed on bacterial cells (data not shown). The two other herbicides, nicosulfuron and S-
333 metolachlor, were not degraded during the 8 h of incubation monitored under these conditions.

334

335 3.3. “*In cellulo*” expression of the mesotrione-transforming nitroreductase

336

337 Since the newly-discovered plasmid encoded nitroreductase was unable to transform
338 mesotrione after heterologous expression and purification, reverse transcription - quantitative
339 PCR using the 16S rRNA gene for normalization (Borrel et al., 2012) was focused on the only
340 two nitroreductases previously described to do it *in vitro* (Carles et al., 2016) in order to confirm
341 their function « *in cellulo* » and in various multi-contamination contexts. Their transcription
342 level was determined during mesotrione biodegradation by *Bacillus megaterium* Mes11.

343 The qPCR results showed no induction of the NfrA1 expression but an induction of NfrA2
344 by mesotrione in the *B. megaterium* Mes11, whatever the herbicide exposure conditions. No
345 significant induction of its expression was observed when *B. megaterium* was exposed to
346 nicosulfuron alone (data not shown). Conversely, in the presence of mesotrione, alone or with
347 the other herbicides, the induction of NfrA2 expression was observed concomitantly to the
348 dissipation of mesotrione (Fig. 2). In comparison to the control culture (without any herbicide),
349 a NfrA2 expression was detected as soon as T0, showing similar values for all treatments (Table
350 2). It reached its maximum 4 h after induction before a decrease, probably due to the complete
351 dissipation of mesotrione after 5 h of culture (Fig. 2 and Table 2).

352 An effect of time (taking all the treatments together), treatments (taking all the times
353 together) and an interaction time*treatment for a given time (ANOVA, P < 0.001) was observed
354 on NfrA2 expression level. Thus, compared to the value at T0, the six-fold expression obtained
355 after 4 h of culture in the presence of mesotrione alone is unaffected by the addition of
356 nicosulfuron.

357 At the same time, when S-metolachlor was present in the mixture, a greatly increased
358 induction was, nevertheless, observed compared to the T0 expression (around 12 and 24 fold
359 for binary and ternary mixtures, respectively), highlighting a two- to four-fold stimulation
360 compared to mesotrione applied alone or with nicosulfuron. A slight stimulation of NfrA2
361 expression was also observed in the *B. megaterium* strain when the culture was exposed to the

362 S-metolachlor alone (about 4-fold at T4 h compared to the T0 expression) or in mixture with
363 nicosulfuron (about 2.5 at T8 h compared to the T0 expression) (data not shown). The up-
364 regulation of the *NfrA2* gene observed in the presence of S-metolachlor was synergized by the
365 presence of mesotrione with a 5 fold and 8.5 fold increase of the *NfrA2* expression with binary
366 and ternary herbicide mixtures, respectively.

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369 4. Discussion

370

371 The genome analysis of *Bacillus megaterium* Mes11 revealed one circular chromosome and
372 9 indigenous plasmids (Fig. 1). The high number of plasmids of this strain is within the range
373 of that already described for other *Bacillus megaterium* strains, harboring a highly variable
374 number of plasmids, from zero (DSM319 strain; Stahl et Esser, 1983; Eppinger et al., 2011) to
375 10 (strain NTC-2; Wang et al., 2020). The GC % of the *B. megaterium* Mes11 plasmids, varying
376 between 33.5 and 35.5 %, was lower than that of the chromosome (38.2 %) (Table 1). This is
377 in agreement with other studies which also revealed a lower GC content for plasmids in several
378 *B. megaterium* strains (Eppinger et al., 2011; Liu et al., 2011; Wang et al., 2020). Among the
379 4055 genes with predicted functions detected on the chromosome and the 520 genes harbored
380 by the nine plasmids (Table 1), 9 and 1 oxygen-insensitive nitroreductase were detected,
381 respectively. This plasmid-encoded nitroreductase blasted only with *Bacillus* genomes. Unless
382 already detected in the chromosome of several *Bacillus* strains (e.g. *B. subtilis* (McIntyre et al.,
383 2019), *B. intestinalis* (Xu et al., 2015), *B. pumilus* (Zhang et al., 2019)), this nitroreductase was
384 only recently detected in the chromosome of a *B. megaterium* strain (strain Ni2-3; Cai et al.,
385 2019) and on a plasmid of *B. megaterium* strain KNU-01 (Jo et al., unpublished).

386 Carles et al. (2016) tested the capacity of these nitroreductases to transform mesotrione *in*
387 *vitro* under a wide range of experimental conditions (pH, temperature, types of cofactor,
388 concentration of the cofactor). This study showed that after heterologous expression and
389 purification of the 9 chromosomic-encoded ones, only two of them (*NfrA1* and *NfrA2*) were
390 involved in the transformation of mesotrione. The plasmid encoded nitroreductase now
391 highlighted by the analysis of the complete *B. megaterium* Mes11 genome was also tested for
392 its capacity to transform mesotrione but not seen to do so for the various *in vitro* experimental
393 conditions tested. Thus, among the 10 nitroreductases found in the genome of *B. megaterium*
394 Mes11, we were only able to show the mesotrione-transformation capacity of the two
395 chromosomic-encoded enzymes *NfrA1* and *NfrA2* *in vitro* in our experimental conditions.

396 Moreover, comparing between these two nitroreductases, we showed that *NfrA1* was not
397 involved in the first step of mesotrione transformation “*in cellulo*” and that only *NfrA2* played
398 a key physiological role in *B. megaterium* Mes11. The mesotrione transformation kinetics by
399 this strain and that of metabolite production (Table 2) were similar to those previously observed
400 with purified nitroreductases (Carles et al., 2016).

401 Furthermore, a previous study has demonstrated the mesotrione transformation capacity of
402 the *B. megaterium* Mes11 strain in a multi-contamination context (Carles et al., 2018). Our
403 results corroborated this capacity “*in cellulo*”. Indeed, we showed that the *NfrA2* expression
404 was strongly increased during the mesotrione transformation and even more in the presence of
405 S-metolachlor coupled or not with nicosulfuron (Fig. 2 and Table 2). The 2 to 4-fold *NfrA2*
406 overexpression when S-metolachlor is present compared to mesotrione alone could not only be
407 attributed to its involvement in the mesotrione degradation since the mesotrione transformation
408 kinetics was similar in all conditions tested. We could thus stipulate that the stimulation of the
409 *NfrA2* expression by S-metolachlor could also be explained by the induction of oxidative stress
410 caused by this molecule which leads to its toxic effect for cells including lipid peroxidation and
411 the excessive production of reactive oxygen species (ROS) (Martins et al., 2011; Nykiel-
412 Szymańska et al., 2019; Machado and Soares, 2021). Defense mechanisms must be set up to
413 protect the cells from oxidative stress. These responses involve specific enzymes such as

414 disulfide reductase, superoxide dismutase, catalase or glutathione peroxidase. Nitroreductases
415 were shown to also harbour these enzymatic activities (Bang et al., 2012; de Oliveira et al.,
416 2009; Liochev et al., 1999; Mermoud et al., 2010; Paterson et al., 2002; Streker et al., 2005; Tam
417 et al., 2006), thus contributing to the cell defense by an up-regulation of the corresponding
418 genes (Mostertz, 2004; Roldán et al., 2008). The higher NfrA2 expression in presence of the
419 three herbicides studied could be explained by the synergistic action of the pesticides
420 (Cedergreen, 2014; Carles et al., 2018). To our knowledge, this is the first time that the
421 involvement of a nitroreductase has been demonstrated “*in cellulo*” during the degradation of a
422 pesticide, also revealing the influence of other molecules on the nitroreductase physiological
423 activity in a multi-contamination context.

424 Hence, our results provide new insight into the mesotrione transformation by *Bacillus*
425 *megaterium* Mes11 strain and highlighted the key physiological role of the NfrA2 enzyme in
426 this process. Nitroreductases being involved in reduction of various nitro-aromatic compounds
427 such as pesticides and antibiotics (Caballero et al., 2005; Symons and Bruce, 2006; Roldán et
428 al., 2008; Carles et al., 2016; El-Hossary et al., 2017; Green et al., 2019), these enzymes (or
429 their genes) could be used as biomarkers to predict the capacity of ecosystems to transform such
430 molecules.

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432

433 5. Conclusion

434

435 In this study, we showed, for the first time, that the NfrA2 nitroreductase of *Bacillus*
436 *megaterium* Mes11 played a key physiological role in the mesotrione transformation and that it
437 is still active in a realistic agricultural context of herbicide mixture exposure or even
438 overexpressed. The *Bacillus megaterium* Mes11 could thus be used for mesotrione
439 bioremediation and its active nitroreductase could have potential biotechnological applications
440 in the environmental domain.

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442

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448

449 Declaration of interests

450 The authors declare that they have no known competing financial interests or personal
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452

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632 **Figure captions:**

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634 **Fig. 1.** Circular genome map of *Bacillus megaterium* Mes11. From the innermost to the
635 outermost circle : the inner black circle shows the scale line in Mbps ; circles (1) and (2)
636 represent the G + C content and G + C skew; circles (3) and (4) represent the predicted coding
637 sequences on the forward and backward strands, colored by the COG functional categories.
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639 **Fig. 2.** Biotransformation kinetics of mesotrione, alone or in mixtures, by *B. megaterium* Mes11
640 and NfrA2 expression fold-change compared to a control culture without mesotrione. The
641 values are the mean \pm standard errors (n = 3) of experimental data. Statistical differences were
642 indicated between the four conditions at each time by lowercase letters (Tukey's test; a < b, P
643 < 0.05) and between each time and T0 (ANOVA, * P < 0.001). Meso: mesotrione; Nico:
644 nicosulfuron; S-meto: S-metolachlor.
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646 **Table 1:** General features of *Bacillus megaterium* Mes11 genome.
647

648 **Table 2:** Concentration of metabolites produced during the mesotrione (1 mM) degradation by
649 *B. megaterium* Mes11 and fold change in NrfA2 expression. Values are expressed as means (n
650 = 3) \pm standard error.
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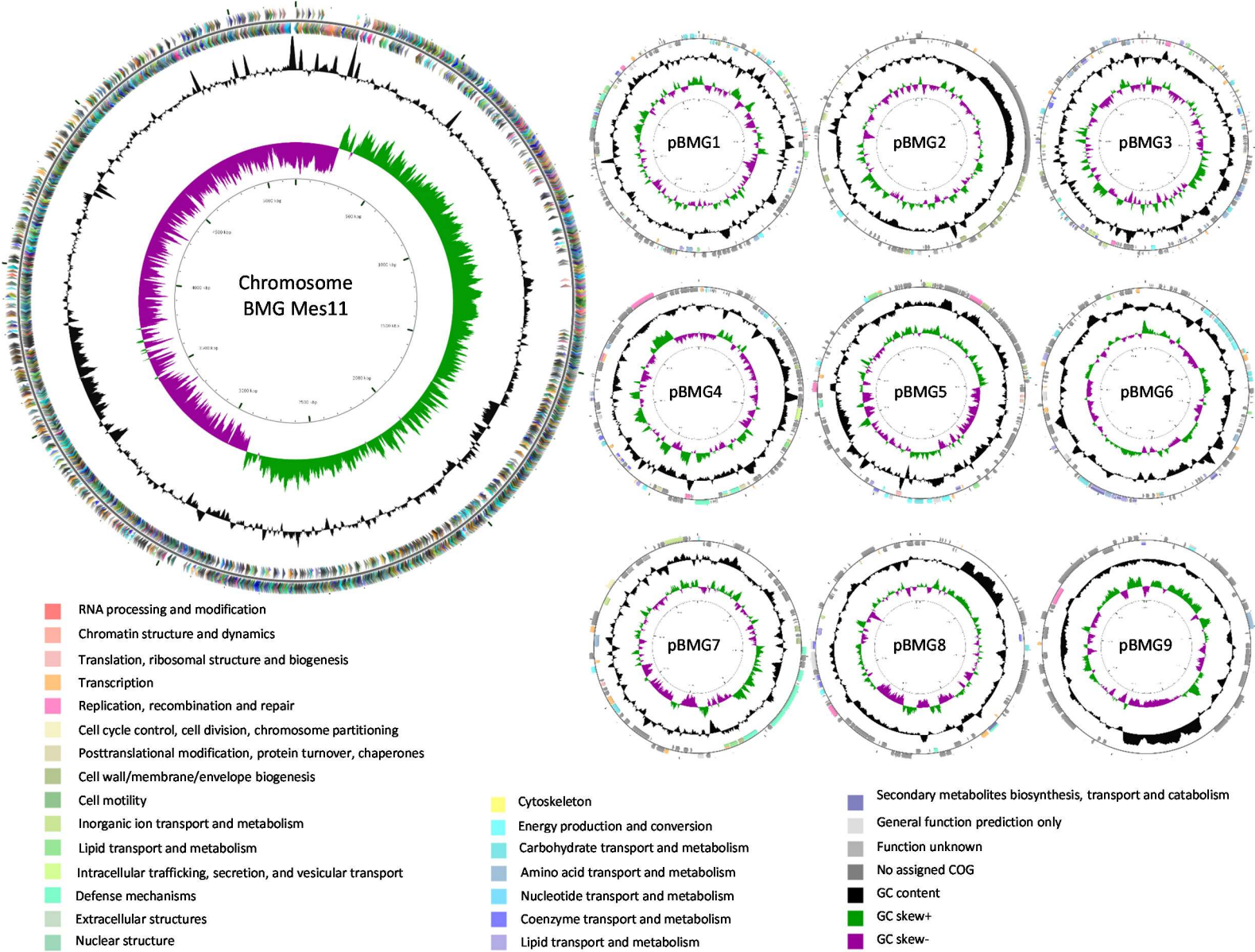
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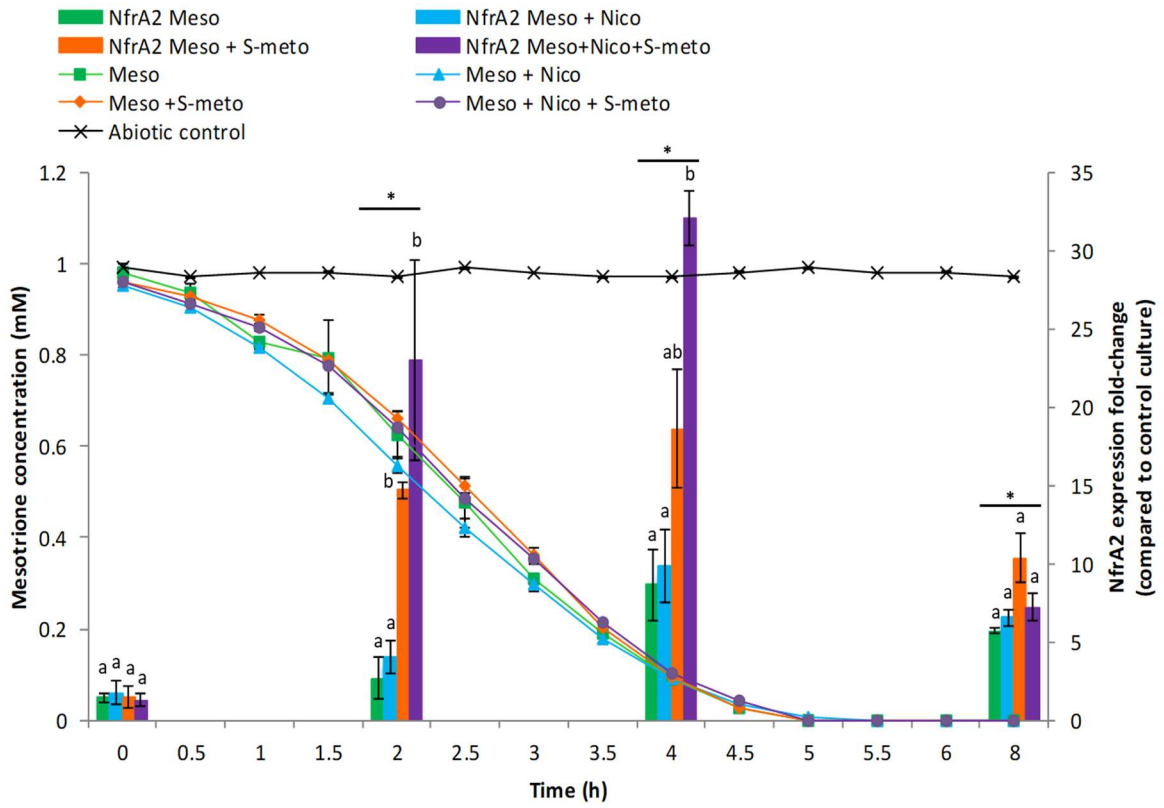
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666 Figure 2

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669 **Table 1:** General features of *Bacillus megaterium* Mes11 genome.

| Features | Genomic size (bp) | CG content (%) | Total genes | Genes with predicted functions | tRNA genes | rRNA operon |
|------------|-------------------|----------------|-------------|--------------------------------|------------|-------------|
| Chromosome | 5,191,612 | 38.2 | 5458 | 4055 | 121 | 11 |
| pBMG1 | 169,684 | 34.2 | 162 | 104 | 2 | - |
| pBMG2 | 122,563 | 33.7 | 83 | 32 | - | - |
| pBMG3 | 157,203 | 33.8 | 175 | 100 | 5 | - |
| pBMG4 | 139,476 | 33.6 | 128 | 64 | - | - |
| pBMG5 | 87,511 | 35.1 | 100 | 37 | - | - |
| pBMG6 | 105,079 | 33.7 | 109 | 63 | - | - |
| pBMG7 | 75,956 | 33.5 | 72 | 42 | - | - |
| pBMG8 | 70,948 | 35.1 | 91 | 47 | 17 | 1 |
| pBMG9 | 45,372 | 35.5 | 66 | 31 | 18 | 1 |

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Table 2: Concentration of metabolites produced during the mesotrione (1 mM) degradation by *B. megaterium* Mes11 and fold change in NrfA2 expression. Values are expressed as means (n = 3) ± standard error.

| Treatment | Mesotrione (mM) | Hydroxylamino derivative (mM) | Isoxazolol (mM) | AMBA (mM) | Mass balance (% initial dose) | NrfA2 fold change ^a (compared to control ^a) |
|----------------------|-----------------|-------------------------------|-----------------|-------------|-------------------------------|--|
| T0 | | | | | | |
| Meso | 0.98 ± 0.02 | 0 | 0 | 0 | 100% | 1.44 ± 0.34 |
| Meso + Nico | 0.95 ± 0.01 | 0 | 0 | 0 | 100% | 1.77 ± 0.78 |
| Meso + S-meto | 0.96 ± 0.01 | 0 | 0 | 0 | 100% | 1.46 ± 0.69 |
| Meso + Nico + S-meto | 0.96 ± 0.01 | 0 | 0 | 0 | 100% | 1.30 ± 0.38 |
| T2 | | | | | | |
| Meso | 0.63 ± 0.05 | 0.16 ± 0.02 | 0.14 ± 0.03 | 0 | 95% | 2.68 ± 1.35 |
| Meso + Nico | 0.56 ± 0.02 | 0.13 ± 0.01 | 0.11 ± 0.02 | 0.02 ± 0.02 | 86% | 4.02 ± 1.02 |
| Meso + S-meto | 0.66 ± 0.02 | 0.10 ± 0.01 | 0.09 ± 0.01 | 0 | 89% | 14.69 ± 0.49 |
| Meso + Nico + S-meto | 0.64 ± 0.01 | 0.10 ± 0.00 | 0.07 ± 0.01 | 0.02 ± 0.02 | 86% | 23 ± 6.35 |
| T4 | | | | | | |
| Meso | 0.09 ± 0.00 | 0.29 ± 0.01 | 0.41 ± 0.10 | 0.12 ± 0.02 | 93% | 8.64 ± 2.32 |
| Meso + Nico | 0.09 ± 0.01 | 0.24 ± 0.02 | 0.38 ± 0.02 | 0.17 ± 0.02 | 92% | 9.84 ± 2.32 |
| Meso + S-meto | 0.09 ± 0.01 | 0.22 ± 0.01 | 0.37 ± 0.02 | 0.20 ± 0.02 | 92% | 18.63 ± 3.78 |
| Meso + Nico + S-meto | 0.10 ± 0.00 | 0.19 ± 0.00 | 0.34 ± 0.01 | 0.22 ± 0.01 | 89% | 32 ± 1.75 |
| T8 | | | | | | |
| Meso | 0 | 0.33 ± 0.01 | 0.53 ± 0.01 | 0.17 ± 0.01 | 105% | 5.71 ± 0.19 |
| Meso + Nico | 0 | 0.32 ± 0.01 | 0.49 ± 0.00 | 0.19 ± 0.05 | 105% | 6.58 ± 0.53 |
| Meso + S-meto | 0 | 0.25 ± 0.00 | 0.54 ± 0.01 | 0.25 ± 0.01 | 108% | 10.36 ± 1.56 |
| Meso + Nico + S-meto | 0 | 0.25 ± 0.00 | 0.53 ± 0.00 | 0.26 ± 0.01 | 108% | 7.24 ± 0.86 |

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Meso: mesotrione; Nico: nicosulfuron; S-meto: S-metolachlor.

^a: the culture control was realized in GSM without herbicide.