Gene 490 (2011) 18–25

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

In vitro evolution of an atrazine-degrading population under cyanuric acid selection pressure: Evidence for the selective loss of a 47 kb region on the plasmid ADP1 containing the *atzA*, *B* and *C* genes

F. Changey, M. Devers-Lamrani, N. Rouard, F. Martin-Laurent *

INRA, Université de Bourgogne, Microbiologie du Sol et de l'Environnement, 17 rue Sully 21065 Dijon Cedex, France

ARTICLE INFO

Article history:
Accepted 9 September 2011
Available online 18 September 2011

Received by Jean-Marc Deragon

Keywords:
Experimental evolution
Adaptation
Molecular evolution
Cyanuric acid
Genetic plasticity
Pseudomonas sp. ADP

ABSTRACT

The adaptation of microorganisms to pesticide biodegradation relies on the recruitment of catabolic genes by horizontal gene transfer and homologous recombination mediated by insertion sequences (IS). This environment-friendly function is maintained in the degrading population but it has a cost which could diminish its fitness. The loss of genes in the course of evolution being a major mechanism of ecological specialization, we mimicked evolution *in vitro* by sub-culturing the atrazine-degrading *Pseudomonas* sp. ADP in a liquid medium containing cyanuric acid as the sole source of nitrogen. After 120 generations, a new population evolved, which replaced the original one. This new population grew faster on cyanuric acid but showed a similar cyanuric acid degrading ability. Plasmid profiles and Southern blot analyses revealed the deletion of a 47 kb region from pADP1 containing the *atzABC* genes coding for the enzymes that turn atrazine into cyanuric acid. Long PCR and sequencing analyses revealed that this deletion resulted from a homologous recombination between two direct repeats of a 110-bp, identical to IS*Pps1* of *Pseudomonas huttiensis*, flanking the deleted 47 kb region. The loss of a region containing three functional genes constitutively expressed thereby constituting a genetic burden under cyanuric acid selection pressure was responsible for the gain in fitness of the new population. It highlights the IS-mediated plasticity of the pesticide-degrading potential and shows that IS not only favours the expansion of the degrading genetic potential thanks to dispersion and duplication events but also contribute to its reduction thanks to deletion events.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

In the course of their evolution mainly driven by environmental stresses, bacteria have elaborated a wide range of physiological responses to survive in different ecosystems. They also use a large set of genotypic variations to face environmental changes. Microbial genomes are indeed characterized by a tremendous genetic plasticity (Arber, 1993) which allows them to acquire new genes by horizontal gene transfer (HGT) through different processes on the one hand, and, to lose preexisting genes by different processes such as mutation or deletion on the other hand. Horizontally acquired genetic material ranges from single genes to complete genomic islets that give additional functionality to their host populations (Lawrence, 1999). Combined with short generation times and large numbers, genetic plasticity offers bacterial populations a tremendous adaptation potential which compensates for the absence of sexual reproduction. Genomic changes, which underlie the evolutionary adaptation of bacterial populations to their environment, are not necessarily beneficial; indeed, several of them may be deleterious and cause cell death. On the contrary, some are favorable, giving a higher fitness to the new population, leading to its selection over the original population, thereby affecting the ecological distribution of bacterial populations (Parnell et al., 2010). However, a specific adaptation leading to the colonization of an ecological niche might be deleterious if environmental conditions happen to change.

The study of bacterial adaptation often deals with new microbial functions which are still in the process of environmental evolution. So far most studies have been carried out about resistance to antibiotics, whose main evolution-driving mechanism is the survival of resistant populations (Mazel and Davies, 1999). Recently we suggested that the adaptation of bacterial populations to pesticide degradation

Abbreviations: %, percentage; ADPE, newly evolved *Pseudomonas* sp. ADP population; ADPO, original *Pseudomonas* sp. ADP population; ARDRA, amplified rRNA restriction analysis; AU, arbitrary unit; bp, base pair; C, carbon; °C, celcius degree; Cm, centimeter; Dig, digoxigenine; DNA, deoxyribonucleic acid; dNTP, deoxyribonucleoside triphosphate; g, gram; h, hour; HGT, horizontal gene transfer; HPLC, high performance liquid chromatography; IS, insertion sequence(s); kb, kilobases; LB, luria broth medium; λ, lag time; mL, milliliter; mm, millitmeter; MSA, mineral salt atrazine medium; MSCA, mineral salt cyanuric acid medium; nm, nanometer; U, unit; μg, microgram; μl, microliter; μm, micrometer; OD600, optical density at 600 nm; pADP1, plasmid of *Pseudomonas* sp. ADP; PCR, polymerase chain reaction; rpm, rotation per minute; sec, second; TY, tryptone-yeast extract broth medium; Taq, thermophilus aquaticus; v/v, volume per volume.

* Corresponding author. Fax: +33 3 8069 3224.
E-mail address: fabrice.martin@dijon.inra.fr (F. Martin-Laurent).

0378-1119/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2011.09.005

could also provide an interesting model (Devers et al., 2008). In this case, the major factor driving bacterial evolution is the formation of a fully functional catabolic pathway enabling pesticide use as a source of C and energy. In this context, we showed that the herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) used for 50 years on crops such as corn represents an interesting model (Devers et al., 2008). It is not easily degraded and is rather mobile (Belluck et al., 1991) thereby contaminating water resources. As a result of regular exposure to this herbicide, bacterial populations able to degrade and use atrazine as a nitrogen and energy source for their growth have been selected and several of them have been isolated and characterized (Devers et al., 2007a, 2007b; Mandelbaum et al., 1995; Piutti et al., 2003; Rousseaux et al., 2001; Topp et al., 2000). *Pseudomonas* sp. ADP (Mandelbaum et al., 1995) which harbors the full catabolic pathway made of the *atz A, B, C, D, E* and *F* genes located on pADP1, a large self-transmissible plasmid (Martinez et al., 2001), is often used as a model. The early steps of the pathway, made of the *atzA*, *atzB* and *atzC* genes, transform atrazine into cyanuric acid (1,3,5-triazine-2,4,6-triol). These three genes surrounded by IS sequences are detected on different replicons, so they are not yet fixed at a permanent location (Rousseaux et al., 2001). On the contrary, the *atzDEF* genes coding for the enzymes that open the *s*-triazine ring of cyanuric acid are organized in an operon placed under the regulation of the *atzR* transcription factor (Garcia-Gonzalez et al., 2005). Recently, we showed in an evolutionary experiment conducted on *Pseudomonas* sp. ADP maintained under a selection pressure exerted by atrazine that a newly evolved population showing an increased fitness as compared to the initial population was selected after 320 generations (Devers et al., 2008). Further analyses showed that the gain in fitness of the newly evolved population resulted from a gene dosage effect due to the duplication of the *atzB* gene by homologous recombination mediated by IS sequences. Although the study highlights the plasticity of the atrazine-degrading genetic potential, indicating that the atrazine-catabolic genome may expand as a result of selection pressure, it also questions the maintenance cost of this adaptation in changing environmental conditions. Indeed, several authors suggest that genetic adaptation selected in a given environment might result in a lower fitness toward other environmental conditions (Zinser et al., 2003). Evidence for expansion and reduction of genomes has recently been discovered (Barrick et al., 2009) and adaptive losses of gene activities, leading to an increased fitness under selective conditions have been observed (Furuta et al., 2011; Roth, 2011). In addition, several authors suggest a rapid loss of *atz* genes when atrazine-degrading isolates are cultivated on rich media without any atrazine selection pressure, thereby suggesting a propensity of these strains to eliminate the genes that constitute a genetic burden under unfavorable conditions.

In order to assess the dynamics of the atrazine-degrading genetic plasticity under unfavorable conditions, we investigated the evolution processes of *Pseudomonas* sp. ADP populations placed under cyanuric acid selection pressure. We designed an evolutionary experiment in which *Pseudomonas* sp. ADP harboring the plasmid ADP1 was subcultured each week in a liquid mineral salt medium containing cyanuric acid as the sole source of nitrogen. The fitness of the initial and of the final population was assayed and the organization of the atrazine-degrading potential characterized.

2. Materials and methods

2.1. Strain and media

The strain used in our experiments was *Pseudomonas* sp. ADP (Mandelbaum et al., 1995). As previously described by Devers et al. (2005), it was maintained on MSA medium containing atrazine (30 mg.l^{-1}) as the sole source of nitrogen and citrate (1 g.l^{-1}) as a carbon source. *In vitro* evolution was performed with mineral liquid medium containing cyanuric acid (0.5 g.l^{-1}) as the sole source of nitrogen (MSCA) and citrate (1 g.l^{-1}) as a carbon source. A rich medium (TY) containing 5 g.l^{-1} tryptone, 3 g.l^{-1} yeast extract and 10 mM $CaCl_2$, was used to determine the growth kinetics of the strains. Knapp buffer (KH_2PO_4 1 g.l^{-1}; K_2HPO_4 1 g.l^{-1}; $MgSO_4$, $7H_2O$ 0,04 g.l^{-1}; $FeCl_3$ 0,004 g.l^{-1}) was used for batch experiments aimed at estimating the cyanuric acid degradation ability of the strains. LB medium (10 g.l^{-1} tryptone; yeast extract 5 g.l^{-1}; and NaCl 10 g.l^{-1} containing 100 µg.ml $^{-1}$ampicillin) was used for cloning PCR amplicons.

2.2. In vitro evolution experiment

Pseudomonas sp. strain ADP was grown in MSCA liquid medium at 28 °C on a shaker at 150 rpm. Each week, the optical density at 600 nm (OD_{600}) of the culture was estimated by spectrophotometry (Biophotometer, Eppendorf, Germany). An aliquot was collected to perform molecular analyses based on nucleic acid extraction and another one was collected to store the cells at −80 °C in 25% glycerol. An aliquot equivalent to an OD_{600} of 0.2 was then inoculated to 200 mL of fresh MSCA medium and incubated under the conditions described above. This procedure was repeated for approximately 120 generations. All along the *in vitro* evolution experiment, the purity of the culture was checked by plating the bacterial cells on solid MSCA medium and performing amplified rRNA restriction analysis (ARDRA) as described previously (Devers et al., 2008).

2.3. Growth kinetics

The growth kinetics of the original *Pseudomonas* sp. ADP (ADPO) and newly evolved (ADPE) strains were determined by inoculating an aliquot of the cell culture equivalent to an OD_{600} of 0.02 in 100 ml of MSCA or TY medium, in triplicate for each strain and each medium. Cell cultures were incubated at 28 C on a shaker at 150 rpm. Throughout the 15-hour incubation period, 1 ml aliquots were regularly collected to measure the OD_{600} by spectrophotometry. In addition, 1 ml aliquots were regularly collected from cultures grown on MSCA to estimate the amount of cyanuric acid remaining in the culture medium by high performance liquid chromatography (HPLC). Briefly, samples were filtered on 0.2 µm filters, transferred into HPLC vials (32 × 11.6 mm-Cleanpack) and analyzed by an HPLC system (LC Star System®, Varian) equipped with a Microsorb-MV C18 column (length 25 cm, internal diameter 4.6 mm, Varian). The solvent was composed of methanol/ultra pure water (75/25; v/v) delivered at a flow rate of 1 ml.min^{-1}. Under these conditions cyanuric acid detected at 225 nm showed a retention time of about 3 min.

To determine the growth parameters of ADPO and ADPE on MSCA medium, the bacterial growth curves were fitted to the modified Gompertz model using SigmaPlot® 6.10 software (Zwietering et al., 1990). The model equation is: $y = A.\exp\{-\exp[1 + \mu m.\exp(1).(\lambda\text{-}t)/A]\}$ where y is the $OD600_{nm}$ (AU); t is the time (h) ; µm is the maximum specific growth rate (h^{-1}) ; A the maximum $OD600_{nm}$ value obtained (AU) and λ lag time (h). The parameters were validated by a Student *t* test ($p < 0.005$).

As ADPO and ADPE yielded a biphasic growth curve on TY medium, the data could not be fitted into the Gompertz model. As a consequence, their growth rate was estimated by fitting linear regressions on the two phases observed.

To compare the growth parameters of ADPO and ADPE, the kinetic parameters were subjected to a single factor analysis of normality and a Kruskal-Wallis test followed by a Fisher procedure ($p < 0.01$)

2.4. Cyanuric acid degradation ability of crude cell extracts

The microbial biomass was produced on MSCA and MSA liquid media for strains ADPE and ADPO, respectively. The cells were harvested by centrifugation at 3,300 g for 5 min. Bacterial pellets were washed twice in Knapp buffer. The cell membrane was then disrupted

Download English Version:

https://daneshyari.com/en/article/2818237

Download Persian Version:

https://daneshyari.com/article/2818237

Daneshyari.com