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REVIEW ARTICLE

Deinococcus as new chassis for industrial biotechnology: biology, physiology and tools

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Summary

Deinococcus spp are among the most radiation-resistant micro-organisms that have been discovered. They show remarkable resistance to a range of damage caused by ionizing radiation, desiccation, UV radiation and oxidizing agents. Traditionally, *Escherichia coli* and *Saccharomyces cerevisiae* have been the two platforms of choice for engineering micro-organisms for biotechnological applications, because they are well understood and easy to work with. However, in recent years, researchers have begun using *Deinococcus* spp in biotechnologies and bioremediation due to their specific ability to grow and express novel engineered functions. More recently, the sequencing of several *Deinococcus* spp and comparative genomic analysis have provided new insight into the potential of this genus. Features such as the accumulation of genes encoding cell cleaning systems that eliminate organic and inorganic cell toxic components are widespread among *Deinococcus* spp. Other features such as the ability to degrade and metabolize sugars and polymeric sugars make *Deinococcus* spp. an attractive alternative for use in industrial biotechnology.

Deinococcus, originally identified as *Micrococcus*, are coccoid or rod-shaped nonsporulating bacteria known for their resistance to multiple stresses and their capacity to repair DNA damage with unparalleled efficiency compared to other known bacterial species. *Deinococcus radiodurans* strain R1 was the first discovered deinobacteria and was isolated in 1956 (Anderson *et al.* 1956) from X ray-irradiated canned meat. More than 60 different species have been isolated from very diverse environments, such as air dust (Weon *et al.* 2007; Yang *et al.* 2009, 2010) and air sample (Yoo *et al.* 2010), activated sludge (Im *et al.* 2008), desert soils (De Groot *et al.* 2005; Rainey *et al.* 2005), arsenic polluted water (Suresh *et al.* 2004), cold environments in Antarctica (Hirsch *et al.* 2004), hot springs or biofilms at the surface of paper machines (Ferreira *et al.* 1997; Kolari *et al.* 2002), radioactive sites (Siebert and Hirsch 1988) faeces (Brooks and Murray 1981), gut of a wood-feeding termite (Chen *et al.* 2012) and Phoenix spacecraft surface (Stepanov *et al.* 2014). These species grow at temperatures ranging from 4 to 55°C and have been regrouped in a distinct (Rainey *et al.* 2005) eubacterial phylogenetic lineage related to the *Thermus* genus. The closest *Deinococcus* relatives are Trueperaceae, Thermales (*Marinithermus*, *Thermus*, *Oceanithermus* and *Vulcanithermus*); no members of this genus have been implicated as pathogens.

Robust cells that are able to assimilate all of the most common sugars and synthesize the biological macromolecules necessary for growth from cheap carbon sources without auxotrophy are of considerable interest for biotechnological applications. Indeed, several *Deinococcus* species meet this requirement because they are capable of growing on minimal media and metabolizing multiple sugars. Furthermore, the well-known robustness of this bacterium and its physiological specificities makes it an attractive chassis for future biotechnological applications. However, many bacteria with attractive properties are recalcitrant to genetic manipulation because of the lack of plasmids and shuttle vectors, the scarcity of selection markers, and low transformability or narrow host range. Approximately, 900 publications referenced in Medline describe the ability of *D. radiodurans* R1 to resist radiation

and to overcome oxidative stress. Most of the genetic tools available for *Deinococcus* originate or were developed for this strain, and it can be easily engineered. The second most well-studied species is *Deinococcus geothermalis*, with approx. 50 publications referenced in Medline, but there is only one report describing the transformation of this strain with an autonomous plasmid originally constructed for *D. radiodurans* (Brim *et al.* 2003). In comparison, 300 000 publications mention *Escherichia coli*, and 100 000 publications discuss *Saccharomyces cerevisiae*. The focus on the resistance of *Deinococcus* to radiation has somewhat masked the potential interest in the industrial applications of this genus. In this review, we summarize the physiological and genomic properties of *Deinococcus*, the tools available to engineer the different species and the most recent applications.

Genomic organization

The sequencing of the *Deinococcus* genome has provided useful information regarding the biology, physiology, biological diversity and biotechnological potential of these bacteria. However, many of these areas have not yet been studied in detail.

Deinococcus radiodurans R1 contains 3195 genes and has a high GC content (67%; similar to *Thermus thermophilus*) and a 3·25 Mb genome (two chromosomes of 2·6 and 0·4 Mb, a megaplasmid of 0·17 Mb, and a small plasmid of 45 kb) (White *et al.* 1999). *Deinococcus geothermalis* (Makarova *et al.* 2007) and *Deinococcus grandis* have approximately the same genome size, while the genome of *Deinococcus gobiensis* (Yuan *et al.* 2012) is 4·4 Mb and that of *Deinococcus hopiensis* is 6·69 Mb. *Deinococcus proteolyticus* (Copeland *et al.* 2012) has the smallest published genome (2·84 Mb). This suggests a strong interspecies genome diversity (Fig. 1) and genomic plasticity compared to *Escherichia* (genome size 4·5–5·8 Mb). This observation was confirmed experimentally via the duplication of a selection marker flanked by a direct repeat of host DNA sequences in *D. radiodurans* (Smith *et al.* 1988). After amplification by antibiotic selection, approx. 50 copies per cell had accumulated (10% of the genome). This experiment demonstrated the plasticity of *D. radiodurans* and its ability to replicate and express extremely large foreign DNA pieces. Makarova estimated that 10–15% of the *D. radiodurans* genome originated from horizontal transfer, and most of these genes are located on the megaplasmid (Makarova *et al.* 2001).

Deinococcus is polyploid and contains four copies of the genome in stationary phase and 10 copies during the exponential phase (Hansen 1978). This can contribute to the well-known genome stability of *Deinococcus*. However, *Thermus*, one of its closest phylogenic relatives, is also polyploid and is not resistant to UV or radiation, suggesting that genome stability is most likely multifacto-

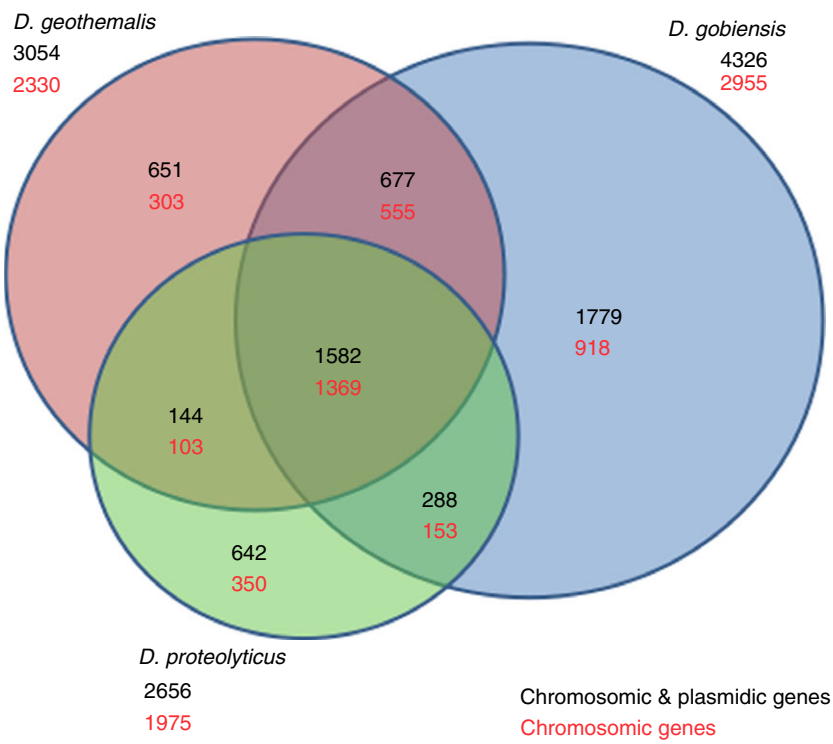

Figure 1 A genomic comparison of *Deinococcus proteolitycus* (2·84 Mb), *Deinococcus geothermalis (*3·2472 Mb) and *Deinococcus gobiensis* genomes (4·41 Mb). Putative orthologous genes were determined by reciprocal best-hits *BLAST* with an e-value cut-off of 10^{e-3}. Most of the core genome is conserved and located on the chromosome, but the plasmids are expectedly more divergent.

rial (Ohtani *et al.* 2010). The polyploidy affects genetic engineering strategies because homogenous chromosomal modification must be achieved.

Computational analysis revealed that the *D. radiodurans* genome possesses mobile genetic elements, including a large number of insertion sequences (ISs) elements (Makarova *et al.* 2001). The first IS in *D. radiodurans* may be related to the IS4 family of *E. coli* and was discovered within the *uvrA* gene of the *mtcB* mutant 2621 (Narumi *et al.* 1997). The IS8301 element (now named IS*Dra*2) was later found in the *pprI* gene (Hua *et al.* 2003). Pasternak *et al.* 2010 showed that the frequency of IS*Dra*2 transposition is increased 50 to 60-fold following UV or γ-radiation because both processes generate large quantities of single-stranded DNA. IS transposition appears to be a major event in spontaneous and induced mutagenesis in *D. radiodurans* (Mennecier *et al.* 2006).

Physiology and biology

Deinococcus are resistant to harsh environments

Deinococcus radiodurans exhibits unparalleled resistance to oxidative stress (Slade and Radman 2011; Misra *et al.* 2013). Oxidative stress can be caused by reactive oxygen species produced by respiration, exposure to irradiation or chemical agents. *Deinococcus radiodurans* can survive high doses of ionizing radiation (up to 2000 double-stranded breaks per bacteria) without considerable irreversible DNA or protein damage, and can reassemble its chromosome with a high fidelity. The resistance of this bacterium to these stresses is due to multiple converging mechanisms.

Two different pathways for the assimilation of glucose, the pentose phosphate and glycolysis, are present and active in *Deinococcus*. Liedert *et al.* (2012) proposed that *D. geothermalis* grows in aerobic conditions by preferentially channelling glucose to the pentose phosphate pathway generally involved in the regeneration of NADPH rather than NADH, in agreement with our observations on exponentially growing *D. geothermalis* (Leonetti, J.-P., unpublished results). The pentose phosphate pathway and the generated NADPH are directly involved in repair mechanisms and resistance to oxidative stress (Juhnke *et al.* 1996; Rui *et al.* 2010). The under-utilization of glycolysis can be a mean to limit NADH formation and to avoid the generation of reactive oxygen species during its recycling by the respiratory chain. This trait is a corolar of the resistance of *Deinococcus* to irradiation and is of significant importance for biotechnology as NADPH is used by several anabolic pathways, such as amino acids synthesis (e.g. arginine, proline, isoleucine, methionine and lysine), vitamin synthesis (e.g. pantothenate, phylloquinone, and tocopherol), polyol synthesis (e.g. xylitol), isoprenoid synthesis and fatty acid synthesis. In addition, NADPH is the source of reducing equivalents for cytochrome P450 hydroxylation of aromatic compounds, steroids, alcohols and drugs. The most commonly used micro-organisms in bioproduction, such as *E. coli* and *S. cerevisiae*, produce primarily NADH and *Deinococcus* could be used as a preferential host to build NADPH-consuming pathways. However, reliable metabolic models of *Deinococcus* are still missing in the literature, which makes metabolic engineering more time consuming and labour intensive like for most of the new alternative chassis. Furthermore *Deinococcus* has never been fermented yet at a scale compatible with an industrial application.

Although *Deinococcus* is a nonsporulating bacteria, it is extremely resistant to desiccation. Mattimore and Battista (Mattimore and Battista 1996) have shown that *D. radiodurans* R1 can survive 6 weeks of desiccation, while the viability of *E. coli* was reduced by six logs after only 7 days. It is believed that resistance to ionizing radiation and desiccation are closely linked evolutionary processes because desiccation resistance appears to require extensive DNA repair (Mattimore and Battista 1996). *Deinococcus* possesses a pathway for the synthesis of trehalose from malto-oligosaccharides (Timmins *et al.* 2005) and can produce trehalose (unpublished results), a common osmolyte. The protective role of this osmolyte in osmotic stress, heat shock and dehydration has been well-characterized in other species (Purvis *et al.* 2005; Yu *et al.* 2012). Surviving desiccation is a major issue for the production of starter cultures for industrial applications (Hofman and Thonart 2010).

The ability of cells to tolerate stress is often limiting in industrial environments and is multifactorial. Wang & *et al.* (Wang *et al.* 2012, 2013) found that the transcription regulator *irrE* from *D. radiodurans*, when transformed into *E. coli*, enhanced tolerance to ethanol, butanol and acetate (10–100-fold), and conferred significant cross-tolerance to two other common inhibitors found in lignocellulosic hydrolysates, 5-hydroxymethyl-2-furaldehyde and vanillin. The *irrE* gene encodes a general switch transcription factor that is responsible for the extreme radioresistance of *D. radiodurans* (Earl *et al.* 2002).

Deinococcus has an unusual thick cell envelope

Deinococcus often forms tetrads (Fig. 2a,c). Most bacteria can be easily classified as Gram-positive or Gram-negative due to the presence or absence, respectively, of a thick cell wall (Fig. 2d) of peptidoglycan that sequesters the stain after treatment. The cell envelope of *Deinococcus* differs depending on the species and exhibits an unusual structure and composition. *Deinococcus indicus*, *D. gran-*

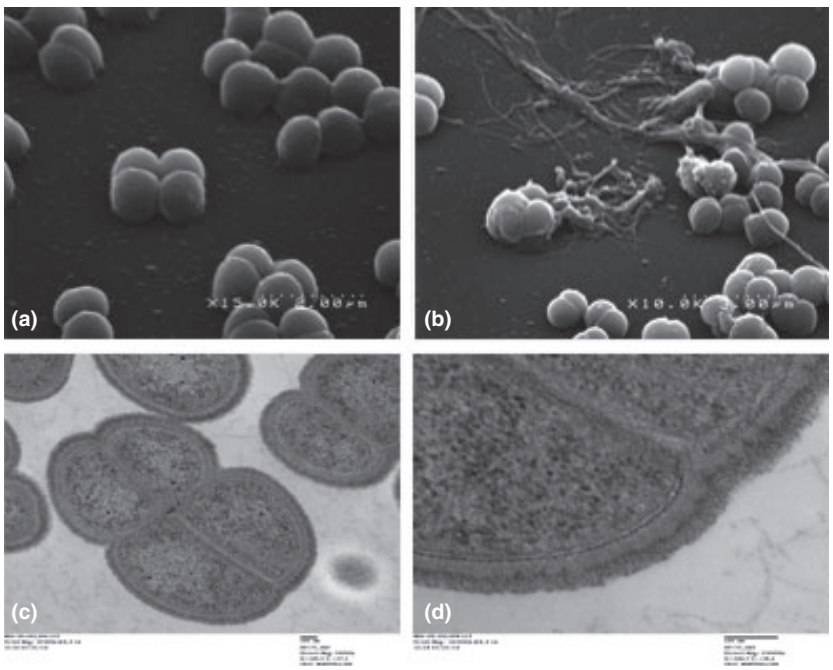

Figure 2 *Deinococcus geothermalis* often forms tetrades (a, c) and presents an unusual thick cell envelope (c, d). Scanning electron microscopy (SEM) images of *D. geothermalis* planktonic cells (a) and cells in contact with Whatman paper (b) Transmission Electron Microscopy (TEM) images of *D. geothermalis* (c, d).

dis and *D. deserti* strain YIM 007^T stain Gram-negative, whereas a majority of the other *Deinococcus* species stain Gram-positive. The Gram-positive coloration of many *Deinococcus* is reminiscent of the thick peptidoglycan layers of Gram-negative bacteria that are difficult to decolorize.

As reviewed by others (Makarova *et al.* 2001), the following six layers of the cell envelope have been identified by electron microscopy: (i) the most internal layer is the plasma membrane common to all cells and is composed of unusual lipids including alkylamine chains. (ii) it is followed by a perforated peptidoglycan cell wall. (iii) the third layer is unique and may be composed of a matrix of tiny uncharacterized compartments. (iv) the fourth layer is another plasma membrane and is the outer membrane. (v) the fifth layer is a distinct electrolucent zone. (vi) the sixth layer consists of regularly packed hexagonal protein subunits (the S-layer, or hexagonally packed intermediate layer), typical of other bacterial S-layers. Several strains of *Deinococcus* are surrounded by a dense carbohydrate coat.

Whether this thick unusual structure is directly involved in resistance to xenobiotics is unknown, but *D. geothermalis* strain T27 has been reported to survive in the presence of high concentrations of ethyl acetate, toluene and diethylphtalate provided as a nonaqueous layer to a cell suspension (Kongpol *et al.* 2008).

Deinococcus forms biofilms

Deinococcus geothermalis forms biofilms (Fig. 2b). This species is frequently found on paper machines (Väisänen *et al.* 1998; Kolari *et al.* 2001), and it is very difficult to remove from steel surfaces. *Deinococcus geothermalis* E50051 was reported to persist after 1 h of washing with 0·2% NaOH or 0·5% sodium dodecyl sulphate, in contrast to other biofilm formers, such as *Burkholderia cepacia* F28L1, *Staphylococcus epidermidis* O-47 and *D. radiodurans* strain DSM 20539. The attachment of *D. geothermalis* involves thread-like structures that interact with the machinery surface (Saarimaa *et al.* 2006). Pili-mediated adhesion is common to multiple Gram-negative and Gram-positive pathogenic bacteria. Saarimaa *et al.* characterized the adhesion threads of *D. geothermalis* as glycosylated type IV pili that are closely related to the type II protein secretion system (Sandkvist 2001; Peabody *et al.* 2003). Type IV pili genes homologous to putative *pil* genes in *D. radiodurans* have also been implicated in the natural competence of *T. thermophilus* HB27, a close relative of *Deinococcus*, and in the secretion of diverse extracellular enzymes (Friedrich *et al.* 2002).

Bacterial biofilms can impair or enhance industrial processes. Very often biofilms are considered a defect because they can clog tubing and impair fermentation processes. However, biofilm formation can be advantageous as it can be used to increase the biomass density in a reactor because it is not dependent on cell recycling, there is no need for re-inoculation in repeated-batch fermentation, and it can prevent 'wash out' when using continuous processes at a high dilution rate. Furthermore, biofilms are often more resistant to extreme conditions. Biofilm formation is not inherent to the *Deinococcus* family or even to *D. geothermalis*.

Genetic engineering and tools

An extensive genetic toolbox exists for some but not all members of the *Deinococcus* clade. This toolbox provides a solid technological basis for the rapid construction of genetic variants, underlying future biotechnological applications.

Transformability

Genetic engineering of bacteria requires the transfer of circular or linear DNA into the intended host. The first evidence that *Deinococcus* was able to take up exogenous DNA was obtained in 1968 by Moseley and Setlow (Moseley and Setlow 1968). The authors co-incubated DNA conferring resistance to an antibiotic with *D. radiodurans* cells sensitive to the antibiotic and obtained antibiotic-resistant clones. In addition to *D. radiodurans,* it was later shown that *D. geothermalis* strain DSM11300 (Brim *et al.* 2003) can be transformed by a $CaCl_2$-dependent technique at a frequency of 5×10^2 transformant per μg of DNA, and *D. grandis* strain ATCC43672 (Satoh *et al.* 2009) can be transformed by electroporation with heterologous DNA at a frequency of $1{\cdot}4 \times 10^4$ transformants per μg of DNA. In our hands, chemical transformation is the most efficient procedure for transforming *D. radiodurans* R1 and *D. geothermalis* DSM11300 (unpublished results). The mechanism underlying DNA uptake remains poorly understood. The following four basic mechanisms can be used in DNA translocation: (i) natural transformation, (ii) transformation using chemical competent cells, (iii) electroporation and (iv) conjugation.

Plasmid and vectors

Although more than 60 *Deinococcus* species are presently known, most of the genetic engineering tools available are devoted to *D. radiodurans* (Table 1). Two large (>40 kb) shuttle vectors, pS28 and pS19 (Smith *et al.* 1989), built from *E. coli* vector pS27 and the full-length *D. radiodurans* stain SARK cryptic plasmids pUE10 (37 kb) and pUE11 (45 kb) have been shown to replicate in *E. coli* and *D. radiodurans* (Smith *et al.* 1989). Three smaller derivatives of the historical vector pUE10 have been constructed, including the 16-kb plasmid pI3 (Masters and Minton 1992), the 27-kb plasmid pMD66 (Daly *et al.* 1994), and pRAD1, a 6·3-kb plasmid. These vectors have been extensively used to engineer *D. radiodurans* (Meima and Lidstrom 2000).

The plasmid pMD66 is also able to replicate into *D. geothermalis* (Daly *et al.* 1994). It was used to engineer this bacterium for use in the bioremediation of high-temperature radioactive waste environments. *Deinococcus geothermalis* transformation with this plasmid is 1000-fold less efficient than the *D. radiodurans* one (8×10^5 and 5×10^2 transformants per μg of DNA, respectively).

Versatile shuttle vectors for *D. grandis* have also been constructed (Satoh *et al.* 2009). The plasmid pZT23 can transform *D. grandis* at an efficiency of approx. $6{\cdot}6 \times 10^3$ transformants per μg of DNA, and pZT27 and pZT29 can transform this species at efficiencies of approx. $1{\cdot}1 \times 10^4$–$1{\cdot}4 \times 10^4$ transformants per μg of DNA respectively.

The available vectors are, however, quite large and rather species-specific. The development of new, more versatile vectors with a broader host range would be useful tools for the genetic manipulation of other *Deinococcus* spp.

Selection and counter-selection markers

Selection markers can confer resistance to antibiotics, antimicrobials, or heavy metals or can be metabolic markers. They are used for the selection of plasmid- or insert-containing clones from a large bacterial population. In practice, antibiotic and metabolic markers are frequently used by molecular biologists. Antibiotic resistance cassettes are often derived from broad-spectrum resistance elements and are generally active in many bacterial species. Kanamycin cassettes derived from Tn9, chloramphenicol cassettes derived from Tn9O3, and tetracycline,

Table 1 An overview of the most frequently used plasmids for the transformation of *Deinococcus*

Plasmids	Host(s)	Size (kb)	References
pS28	*Deinococcus radiodurans-Escherichia coli*	>40	Smith *et al.* (1989) and Brim *et al.* (2003)
pS19	*D. radiodurans-E. coli*	>40	Smith *et al.* (1989)
pUE10	*D. radiodurans-E. coli*	37	Smith *et al.* (1989)
pUE11	*D. radiodurans-E. coli*	45	Smith *et al.* (1989)
pI3	*D. radiodurans-E. coli*	6·3	Masters and Minton (1992)
pRAD1	*D. radiodurans-E. coli*	6·3	Meima and Lidstrom (2000)
pMD66	*Deinococcus geothermalis* or *D. radiodurans-E. coli*	28	Daly *et al.* (1994)
pZT23	*Deinococcus grandis-E. coli*	2·4	Satoh *et al.* (2009)

hygromycin (Harris *et al.* 2004) and spectinomycin (Bouthier de la Tour *et al.* 2009) resistance genes can be used to select transformants or recombinant *D. radiodurans* or *D. geothermalis* (Smith *et al.* 1988; Pasternak *et al.* 2010). The following antibiotic selection markers can also be used on *D. geothermalis* in our laboratory: hygromycin, oxytetracycline (but not tetracycline), bleocine, kanamycin, cloramphenicol and erythromycin (Gerber, E., unpublished results). More recently, an a-amylase-containing vector was transformed into a *D. geothermalis* strain lacking its endogenous α-amylase, and the transformant were selected on agar plates containing starch (unpublished results).

In contrast to selection markers, counter-selection markers serve to eliminate unwanted genetic elements such as DNA segments encoding selection markers. Counter-selection can be achieved by exploiting unique metabolic properties present in one bacterium but not another. For example, *Bacillus subtilis* can express the gene *sacB*, a levansucrase that catalyses the synthesis of high molecular weight fructose polymers in the presence of sucrose; however, the expression of the same gene in *E. coli* (Gay *et al.* 1985) is toxic and favours the elimination of *sacB* from the strain. Taking advantage of this property, *sacB* has been used in many different Gram-negative bacteria as a counter-selection marker, thus bypassing the need for an antibiotic resistance marker. This type of selection was used successfully in *D. radiodurans* (Pasternak *et al.* 2010) but produces partial phenotypes that are difficult to select in *D. geothermalis* (unpublished results).

Generation of chromosomal mutants

Genetic engineering often necessitates the generation of chromosomal modifications. Chromosomal mutants can either be generated randomly or targeted at specific site and can involve insertions, deletions or the modification of the native DNA sequence. Chromosomal mutations make it possible to stably express genes without maintaining a plasmid in the cell. However, insertions or deletions at specific loci require detailed knowledge of the targeted genome and of the contribution of each locus to strain fitness.

The first *Deinococcus* chromosomal mutant was constructed by homologous recombination (Smith *et al.* 1988) in *D. radiodurans*. Homologous recombination occurs when nonreplicative plasmids encoding an antibiotic resistance marker flanked by homology regions are integrated into the bacterial chromosome. The first report demonstrating that duplication insertion can be used to create insertional mutations in *D. radiodurans* was published in 1999 (Markillie *et al.* 1999). The same technique was used to engineer *D. radiodurans* for organopolluant degradation by cloning the *todC1C2BA* operon from *Pseudomonas putida* F1 (Lange *et al.* 1998) and for metal remediation by inserting the *merA* gene from *E. coli* strain BL308 into the *D. radiodurans* chromosome (Brim *et al.* 2000). Chromosomal replacement was first used in 2000 (Meima and Lidstrom 2000) by replacing the α-amylase gene with a gene encoding 1,4-α-D-glucan glucanohydrolase. The same study showed that the use of linearized DNA greatly enhanced the occurrence of replacement recombinants.

Significant progress has been made in engineering *D. radiodurans*. A current goal is to engineer *Deinococcus* strains as resistant to higher temperatures. *Deinococcus geothermalis* has been identified as the best candidate strain for this purpose. *Deinococcus geothermalis* can express genes of interest cloned into pMD66 and its derivative at a high temperature (50°C) (Brim *et al.* 2003). Suicide plasmids and linear DNA fragments can be used to obtain double-crossover recombinants in *D. geothermalis* with an excellent efficiency (unpublished results). In our experience, despite that *Deinococcus* forms tetrads and harbours multiple copies of its genome, 50% of the modifications made on the chromosome of *D. geothermalis* are readily homogeneous after a single plating under antibiotic selection pressure. Twenty-five to thirty per cent more homogeneous clones can be obtained by serial (2–3) plating at higher antibiotic concentrations. Finally, 15–20% of the constructions cannot be homogenized, possibly because the gene is essential and cannot be completely deleted, or because the gene is toxic when expressed by all the copies. This limits the use of several technologies such as marker-free multiplexed genome editing (CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats).

Deinococcus as host bacteria for biotechnological applications

Several studies have demonstrated the robustness of this poly-extremophilic genus for use in the metabolism or bioremediation of toxic compounds such as organic molecules or heavy metals in radioactive environments.

A *Deinococcus* strain expressing the toluene dioxygenase from *Ps. putida* was able to bioremediate toluene, chlorobenzene, 3-4 dichlorobutene and indole under irradiation (60 Gy/h for 24 h) and was resistant to 1 g l^{-1} toluene (Lange *et al.* 1998). Brim *et al.* (2000) have also generated several *D. radiodurans* strains expressing the Hg (II) resistance gene (*merA*), a mercuric reductase gene from *E. coli* strain BL308. This strain resisted the bactericidal effect of ionic Hg (II) at concentrations up to 50 mmol l^{-1}, and it was able to reduce toxic Hg (II) to

the much less toxic and volatile elemental Hg (0). The authors also noted the remarkable genome plasticity of *D. radiodurans,* bacteria able to maintain, replicate and express extremely large segments of foreign DNA integrated in multicopy in the chromosome.

In addition to these interesting perspectives, bacteria belonging to the *Deinococcus-Thermus* group possess other specific characteristics. Since the first report of the genus *Deinococcus*, more than 50 species within this genus have been discovered from various environments. Several species can grow on and/or digest various biomasses because they encode biomass digestion enzymes and are able to assimilate a variety of sugars present in lignocellulosic biomass. Numerous reports have described the ability of *D. geothermalis* to form biofilms and the problems that this ability poses during the papermaking process (Väisänen *et al.* 1998; Kolari *et al.* 2003). One of the most distinctive features of *D. geothermalis* in comparison with *D. radiodurans* is its great abundance of genes encoding sugar metabolism enzymes. D-xylose, in the form of xylan polymers, is a complex polysaccharide found in lignocellulosic biomass. *Deinococcus geothermalis* contains genes encoding xylanases, enzymes involved in xylane and xylose metabolism most of which form a cluster in a megaplasmid. In contrast to the proteolytic lifestyle of *D. radiodurans*, *D. geothermalis* is a proficient hydrolytic organism (Väisänen *et al.* 1998; Kolari *et al.* 2003), and most of these functionalities were acquired by horizontal gene transfer (Omelchenko *et al.* 2005). Several of these enzymes have been patented (Claverie *et al.* 2012).

The production of bio-energy and biomolecules in a sustainable fashion is becoming increasingly important. In recent years, the mechanism by which new industrial strains are generated has changed dramatically in response to massive sequencing projects that have identified new genes, the development of new bioinformatic tools, and increasing knowledge of bacterial metabolism. These advances have made it possible to construct synthetic pathways for the production of a variety of chemical compounds from sugars. *Escherichia coli* and *S. cerevisiae* remain the most commonly used chassis in industry. However, there now is an increasing demand for micro-organisms that are able to use cheaper carbon sources, such as organic wastes or lignocellulosic biomass. Because it is able to digest cellulose and xylan and assimilate all of the sugars present in lignocellulosic biomass, *D. geothermalis* meets this requirement and has been patented for this purpose (Leonetti and Matic 2009). Interestingly, *D. geothermalis* T27 (Kongpol *et al.* 2008) is also able to tolerate high concentrations of solvent (decane, ethyl-acetate, etc.), an unusual property that is of major interest in industrial processes. Substantial progress has been made regarding our knowledge of the *Deinococcus* genome, physiology and fermentation capabilities. This knowledge coupled with the ability to genetically modify different *Deinococcus* species makes it possible to build industrial strains with the ability use organic wastes and lignocellulosic biomass more efficiently and with improved tolerance to various abiotic and biotic stresses. However, one key aspect to engineer rapidly cost efficient strains is the understanding of the physiology of the chassis and the availability of reliable metabolic models. With only 900 publications on *Deinococcus* ranks far beyond *E. coli* and *S. cerevisiae* in term of scientific knowledge, which makes the strain optimization process more time consuming and labour intensive.

Conflict of Interest

All the authors are employees of Deinove, a company exploiting *Deinococcus* in synthetic biology.

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