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Engineering synthetic bacterial consortia for enhanced desulfurization and revalorization of oil sulfur compounds

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ABSTRACT

The 4S pathway is the most studied bioprocess for the removal of the recalcitrant sulfur of aromatic heterocycles present in fuels. It consists of three sequential functional units, encoded by the *dszABCD* genes, through which the model compound dibenzothiophene (DBT) is transformed into the sulfur-free 2-hydroxybiphenyl (2HBP) molecule. In this work, a set of synthetic *dsz* cassettes were implanted in *Pseudomonas putida* KT2440, a model bacterial "chassis" for metabolic engineering studies. The complete *dszB1A1C1-D1* cassette behaved as an attractive alternative — to the previously constructed recombinant *dsz* cassettes — for the conversion of DBT into 2HBP. Refactoring the 4S pathway by the use of synthetic *dsz* modules encoding individual 4S pathway reactions revealed unanticipated traits, e.g., the 4S intermediate 2HBP-sulfinate (HBPS) behaves as an inhibitor of the Dsz monooxygenases, and once secreted from the cells it cannot be further taken up. That issue should be addressed for the rational design of more efficient biocatalysts for DBT bioconversions. In this sense, the construction of synthetic bacterial consortia to compartmentalize the 4S pathway into different cell factories for individual optimization was shown to enhance the conversion of DBT into 2HBP, overcome the inhibition of the Dsz enzymes by the 4S intermediates, and enable efficient production of unattainable high added value intermediates, e.g., HBPS, that are difficult to obtain using the current monocultures.

1. Introduction

Crude oils contain undesirable contaminant molecules, such as thiophenic aromatics compounds, which have a negative impact on oil processing and pose serious environmental threats (Soleimani et al., 2007). A wide spectrum of desulfurization technologies have been developed to remove sulfur mainly from finished refinery products (Stanislaus et al., 2010). Hydrodesulfurization (HDS) treatment has proved to be the common technology of choice to reduce the level of sulfur in crude oil products. Significant environmental, technical and economic limitations have been reported in applying the HDS process (Babich and Moulijn, 2003).

During the past 30 years, research to develop alternative desulfurization technologies resulted in a biotechnological strategy to eliminate sulfur from thiophenic compounds (biodesulfurization (BDS)) via serial reactions known as the 4S pathway (Gray et al., 2003; Gupta et al., 2005; Kilbane, 2006; Monticello, 2000; Nuhu, 2013; Xu et al., 2009). This pathway was firstly reported in the gram-positive bacterium *Rhodococcus erythropolis* IGTS8 (Gallagher et al., 1993), but the 4S pathway has been also found in other bacteria (Duarte et al., 2001; Kilbane, 2006; Mohebali and Ball, 2008).

The 4S pathway provides a nondestructive oxidative process used by the cells to obtain the sulfur required for growth, which involves the transformation of dibenzothiophene (DBT), the model compound for sulfur heterocycles present in oil and refractory to HDS, into 2-hydroxybiphenyl (2HBP) and sulfite (Fig. 1A) (Gallagher et al., 1993). Sulfite is further assimilated via its oxidation into sulfate by a sulfite oxidoreductase (Aggarwal et al., 2012). In the 4S pathway, DBT undergoes three successive oxidation steps leading to the formation of DBT-sulfoxide (DBTO), DBT-sulfone ($DBTO_2$) and 2HBP-sulfinate (HBPS) mediated by two monooxygenases (DszC and DszA), followed by a hydrolytic step for sulfur removal as sulfite, and formation of 2HBP, which is mediated by a desulfinase (DszB). The three oxidation steps require reducing equivalents ($FMNH_2$) supplied by a flavin-reductase (DszD) (Fig. 1A) (Gray et al., 1996). The genetics of the 4S pathway has been elucidated in *R. erythropolis* IGTS8 being

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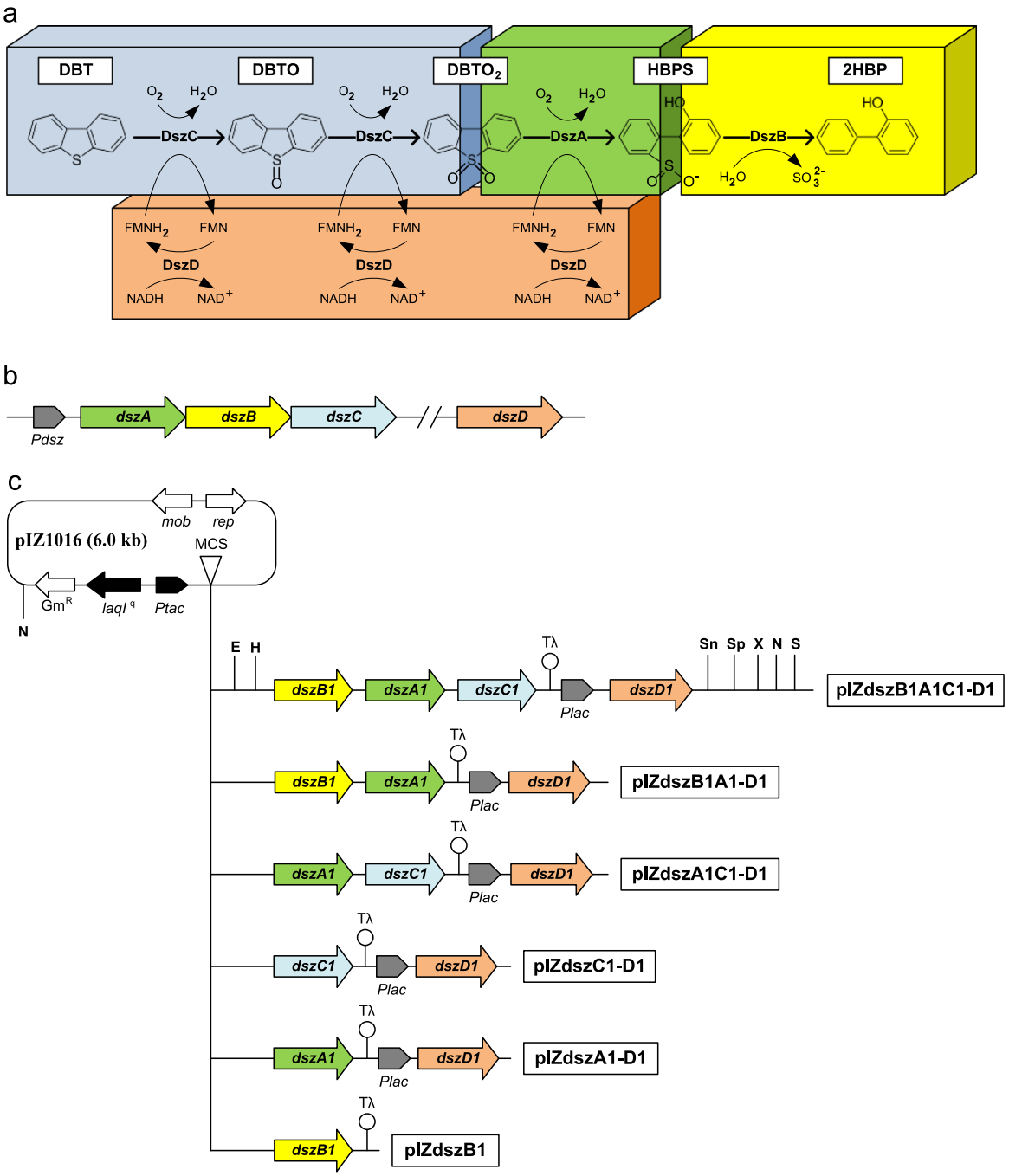

Fig. 1. The 4S pathway for DBT desulfurization, its genetic determinants, and design of *dsz* cassettes. (A) Scheme of the 4S pathway for DBT desulfurization. The three functional modules are based on the activity of the DszC monooxygenase, DszA monooxygenase and DszB desulfinase, and they are shown by blue, green and yellow boxes, respectively. Reducing power ($FMNH_2$) needed for the activity of the DszC and DszA monooxygenases is provided by the DszD flavin reductase (orange box). The metabolites are as follows: dibenzothiophene (DBT), dibenzothiophene sulfoxide (DBTO), dibenzothiophene sulfone (DBTO2), 2-(2-hydroxybiphenyl) sulfinate (HBPS), 2-hydroxybiphenyl (2HBP). (B) Organization of the *dsz* genes encoding the 4S pathway in a megaplasmid (*dszABC*) and in the chromosome (*dszD*) of *R. erythropolis* IGTS8. The color code of the *dsz* genes correspond to that of the enzymatic reactions in panel A. *Pdsz*, represents the promoter of the *dszABC* operon. (C) Scheme of the pIZ1016-derivative plasmids harboring the different *dsz* cassettes. The arrows indicate the direction of transcription of the genes. The color code of the synthetic *dsz* genes correspond to that of the enzymatic reactions in panel A. Only relevant restriction sites are shown (E, EcoRI; H, HindIII; N, NotI; S, SacI; Sn, SnaBI; Sp, SpeI; X, XbaI). The transcriptional regulatory elements, i.e., $lacI^q$/*Ptac* and *Plac* are shown by black and gray arrows, respectively. *Tλ*, indicates a transcriptional terminator of the lambda phage. The genes encoding gentamicin resistance (Gm^r), and the plasmid replication (*rep*) and mobilization (*mob*) functions are shown by white arrows.

coded by three megaplasmid-born genes (*dszABC*) and one chromosomal-born gene (*dszD*) (Fig. 1B) (Gray et al., 1996).

Attempts to remove sulfur from crude oil based on the 4S pathway have identified two major technical bottlenecks, which need to be overcome to develop a feasible bioprocess for sulfur removal in the oil industry. These major bottlenecks are bioprocess-related hurdles to develop a continuous bioprocess, and biocatalyst-related limitations to develop robust biocatalysts with a high catalytic desulfurization rate. Since low amounts of sulfur are required for bacterial growth, and the native regulation of *dsz* gene expression is sulfur-dependent (Li et al., 1996), the *dsz* genes are expressed at low level in almost all naturally occurring bacteria harboring the 4S pathway, which leads to low rates of DBT desulfurization from an industrial point of view (Kilbane, 2006).