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REVIEW ARTICLE

Mechanistic Understanding of *Gordonia* sp. in Biodesulfurization of Organosulfur Compounds

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Abstract
Although conventional oil refining process like hydrodesulfurization (HDS) is capable of removing sulfur compounds present in crude oil, it cannot desulfurize recalcitrant organosulfur compounds such as dibenzothiophenes (DBTs), benzothiophenes (BTs), etc. Biodesulfurization (BDS) is a process of selective removal of sulfur moieties from DBT or BT by desulfurizing microbes. Therefore, BDS can be used as a complementary and economically feasible technology to achieve deep desulfurization of crude oil without affecting the calorific value. In the recent past, members of biodesulfurizing actinomycete genus *Gordonia*, isolated from versatile environments like soil, activated sludge, human beings etc. have been greatly exploited in the field of petroleum refining technology. The bacterium *Gordonia* sp. is slightly acid-fast and has been used for unconventional but potential oil refining processes like BDS in petroleum refineries. *Gordonia* sp. is unique in a way, that it can desulfurize both aliphatic and aromatic organosulfurs without affecting the calorific value of hydrocarbon molecules. Till date, approximately six different species and nineteen strains of the genus *Gordonia* have been recognized for BDS activity. Various factors such as enzyme specificity, availability of essential cofactors, feedback inhibition, toxicity of organic pollutants and the oil–water separations limit the desulfurization rate of microbial biocatalyst and influence its commercial applications. The current review selectively highlights the role of this versatile genus in removing sulfur from fossil fuels, mechanisms and future prospects on sustainable environment friendly technologies for crude oil refining.

Introduction

The third most abundant heteroatom in crude oil is sulfur which contributes approximately 10% of the total crude oil chemical composition. It is estimated that 50–95% of the sulfur content in petroleum and its derivative fractions is in the form of thiophenic sulfur where alkylated derivatives of dibenzothiophenes (DBTs) comprise the predominant form of polyaromatic sulfur hydrocarbons [1, 2].

Biodesulfurization of organosulfur compounds by microbes has attracted a lot of attention in the recent past due to its economical, ecological and environmentally benign bioprocess. However, further investigations are required to enhance and remodel the process to make it more suitable for use in industry [3].

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Research in biotechnology and petroleum microbiology suggests that strains of *Gordonia* sp. have the potential to break down refractory crude oil pollutants like polycyclic aromatic hydrocarbons (PAHs) and organosulfur compounds due to long chain hydrophobic mycolic acids located in their cell wall [4]. *Gordonia*, is a gram positive, non-motile, catalase-positive, aerobic bacterium. This versatile genus has been retrieved from different land and water ecosystems for its deterioration capability of oil pollutants [5, 6]. Several examples illustrate the potentiality of this genus in degrading wide variety of organic pollutants. Two strains of *G. rubripertincta*; strain SBUG 1971 and SUBG 1972 have been reported to degrade twenty four n-alkanes, twenty two n-alkylcyclohexanes, twenty branched chain alkanes, thirteen alkylbenzenes, four n-alkyl-substituted naphthalenes, three n-alkyl-substituted biphenyls and many other oil components in traces [7]. Further, long chain hydrocarbon decomposing *G. amicalis* strain LH3, showed 17.8% of paraffin degradation and 44.7% of oil viscosity reduction [8]. *Gordonia amicalis* IEGM^T was identified as a reliable and homogeneous strain on par with the well-studied *Rhodococci* model having a 4S metabolic pathway for DBT desulfurization [9]. There are about six species and

nineteen strains of *Gordonia* sp. known to show biodesulfurization (BDS) activity (Table 1) [10–37].

Few researchers have reviewed the potential biotechnological prospects of the *Gordonia* sp. [4, 6, 38, 39]. Arenskötter et al. [38] reviewed the biological significance of the genus with emphasis on its role in various biotechnological fields in regards to catabolic and metabolic abilities of genus *Gordonia*. Drzyzga [4] highlighted the strengths and weaknesses of the genus in regards to various industrial applications as well as its pathogenic role in causing disease in humans. A review of *Gordonia* shows the clinical significance of this versatile genus, both in healthy and immune compromised patients [39]. The ecological significance, diversity and adaptability of *Gordonia* sp. in relevance to different metabolites were highlighted by Sowani et al. [6]. Several species of the genus have been reported to have catabolic adaptations, for bioremediation purposes such as bioaugmentation [40]. Many factors including enzyme specificity, cofactor ($FMNH_2$) availability, feedback inhibition, oil–water separation and toxicity of organic pollutants affect the desulfurization rate of microbial biocatalyst and influence its commercial applications. Therefore, strategies to optimize industrial scale BDS processes are needed for oil refineries. The present review will provide insights into the role of *Gordonia* sp. in a novel biorefining process known as BDS. In this paper, mechanisms and pathways involved, recent technological developments, as well as genetic engineering opportunities for enhancing the BDS process are explained.

Gordonia sp. as Potential Biodesulfurizing Bacteria

Hydrodesulfurization vs Biodesulfurization

Emissions of SO_x from vehicular exhaust pose a dire threat to the environment. Therefore, to limit the sulfur content in the emissions, governing authorities of many developing countries have recommended petroleum refineries to adopt deep desulfurization of crude oil [41]. The recommended

Table 1 Overview of *Gordonia* species or strains showing biodesulfurization activity (BDS)

Species	Strain	Source	BDS compound	References
G. alkanivorans	RIP190A	Soil, Iran	Dibenzothiophene Biodesulfurization	[10, 11]
		Recombinant *Gordonia* species	Dibenzothiophene Biodesulfurization	[12, 13]
	1B	Soil, Portugal	Dibenzothiophene-desulfurizing	[14–16]
	CGMCC6845	Soil, China	Dibenzothiophene, benzothiophene other thiophene analogs desulfurizing	[17]
G. amicalis	IEGM[T]	Soil, Russia	Dibenzothiophene-desulfurizing	[9]
	F.5.25.8	PETROBRAS, Brazilian oil company	Dibenzothiophene-desulfurizing and carbazole-metabolizing	[18]
		Federal University of Rio de Janeiro, Brazil	–	[19]
	1D	Soil, Russia	Oil (dibenzothiophene) desulfurizing	[20]
G. desulfuricans	–	Soil, UK	Benzothiophene-desulfurizing	[21]
G. nitida	CYKSI	–	Oil (dibenzothiophene)-desulfurizing	[22, 23]
G. rubripertincta	T08	–	Benzothiophene desulfurization	[24]
	ICP172	–	Dibenzothiophene desulfurization	[25]
G. terrae	C-6	Soil, China	Benzothiophene desulfurization	[26]
Gordonia strains without species assignment				
Gordonia sp.	213E	Soil, Scotland	Benzothiophene desulfurization	[27]
	C-6	GuDao Oil Field, China	Benzothiophene desulfurization	[28]
	CYKS1	wastewater discharge, Korea	Diesel oil- and dibenzothiophene-desulfurizing	[29]
	TM414	–	Dibenzothiophene-desulfurizing	[30]
	ZD-7	Hangzhou Refinery sludge, China	Dibenzothiophene-desulfurizing	[31]
	IITR100	Soil, India	Thianthrene	[32]
			Dibenzyl sulfide	[33]
			Benzo[b]naphtho[2,1-d]thiophene	[34]
			Heavy crude oil and hydrodesulfurized diesel	[35]
	JDZX13	Soil, China	Dibenzothiophene-desulfurizing	[36]
	AHV-01	Soil, Iran	Dibenzothiophene-desulfurizing	[37]

limit of sulfur content set by the Environmental Protection Agency (EPA) of USA for gasoline is 15 ppm and diesel is 30 ppm [42]. Desulfurization is a key step in the preprocessing of fossil fuels to achieve compliance with these regulations [43]. Hydrodesulfurization (HDS) is the standard, traditional process routinely accepted in oil industries for cutting back the sulfur components present in crude oil and petroleum refining purposes. However, HDS suffer from major setbacks as it is dependent on severe operating conditions like that of extremely high pressure (1–18 MPa), and high temperature (200–240 °C) to fulfil the stringent EU sulfur regulations (500 to < 10 ppm) [44, 45]. Sulfides, mercaptans, thiophenes and their derivatives such as DBTs are the forms of sulfur found in liquid fuels [46]. The removal of these refractory compounds by conventional but sophisticated HDS has made the process both expensive and energy- exhaustive. Moreover, expensive catalysts used in the process constitute a greater problem of disposal as they are hazardous in nature. In addition to this, HDS is not efficient in attaining the reduced contents of sulfur limited by the Environmental Protection Agency, EPA [47, 48].

The major obstacle in petroleum desulfurization has been overcome through an alternative, emerging microbial process of BDS that exhibit a lot of potential for refining crude oils. Apart from being environmentally friendly, the major advantage of the technology is that it can work at ambient conditions [49, 50]. The following are some general conclusions drawn about BDS from previous studies (a) various factors such as the amount and accumulation of reduced cofactors, enzyme activity, movement of substrate and intermediates across cell membranes do not affect the rate of biodesulfurization; (b) Competitive enzyme inhibition is the best model for explaining the mechanism of biodesulfurization and its transition states; (c) The number of substituent's of DBT determines the biodesulfurization rate which is specified by depletion in inhibition constant and the increase in Michaelis–Menten constant [46].

Metabolic Pathways of Biodesulfurization

Microorganisms have the tendency to extract sulfur from different sources to fulfill their nutrient needs through their metabolic pathways. Thiophenic compounds like DBT and benzothiophenes (BT) can be consumed by certain microorganisms, leading to a decrease in sulfur content in fossil fuels. While HDS results in increased amount of H_2S due to reduction of sulfur moieties, BDS is based on oxidative pathways, making it a promising and less-demanding technology under ambient conditions [51]. There are two principle pathways that have been reported for DBT utilization such as ring-destructive (degradation) i.e. Kodama pathway and sulfur specific (desulfurization) pathway i.e. 4S metabolic pathway [52].

The Kodama pathway of DBT degradation is comparable to the naphthalene degradation, which is among the most common path for oxidative cleavage of C–C bond [52, 53]. This destructive oxidative C–C bond scission mechanism is rather undesired and is not commercially exploited due to fragmentation of phenyl ring by sequential degradation/oxidation of C–C bonds, and the reduced calorific value of the fuels (Fig. 1) [47, 54].

The key features in the pathway are addition of hydroxyl group (hydroxylation) followed by breakage of C–C bonds which lead to the disruption of the aromatic ring and finally hydrolysis [42]. Diverse groups of microorganisms are known to follow this destructive pathway. The microbial cleavage of DBT following the Kodama pathway was tested by growing *Pseudomonas jianii* DDC279 in a DBT rich minimal salt medium where five water-soluble products were obtained among which 3-hydroxy-2-formyl-benzothiophene was accounted for the maximum quantity [55]. The Kodama pathway prefers to catalyze unsubstituted homocyclic rings of DBTs over their methylated derivatives [53]. The reactive steps in the pathway begins with addition of oxygen to the DBT compound resulting in cleavage of the aromatic ring and accumulation of an unstable, water-soluble end product, 3-hydroxy-2-formyl -benzothiophene (HFBT). The number of carbon atoms in HFBT is lower than that of DBT, thereby halting the desulfurizing process altogether [52].

Contrary to Kodama pathway, the 4S metabolic pathway does not disrupt the DBT aromatic ring. It specifically attacks the C–S bond, removing the sulfur entity from the ring through successive oxidation steps. As the DBT is oxidized, the following products are formed: dibenzothiophene sulfoxide, dibenzothiophene sulfone, 2-hydroxybiphenl sulfinate and finally 2-hydroxybiphenyl (2-HBP) and other water-soluble sulfates (Fig. 2). The 4S metabolic pathway does not affect the oil's combustion value which is recognized as the most valuable potential application pathway [36, 56]. There are several reports that suggest an expanded account of the 4S pathway where 2-methoxybiphenyl (2-MBP), 2,2-dihydroxybipheny or biphenyl are the end product instead of 2-HBP. However, their level of toxicity and inhibition of desulfulrizing capacity of the bacteria is yet to be explored [57]. The 4S metabolic pathway is divided into three major stages: (a) cleavage of the sulfur component by oxidation which results in activation of the thiophene ring; (b) cleavage of thiophenic ring which results in sulphinate, an aromatic compound and (c) eradication of sulfinate group.

There are 22 identified bacterial genera and several other unidentified bacteria are reported to utilize the 4S metabolic pathway, but most of the research is focused on *Rhodococcus* sp. [58]. Application of muti-enzymatic

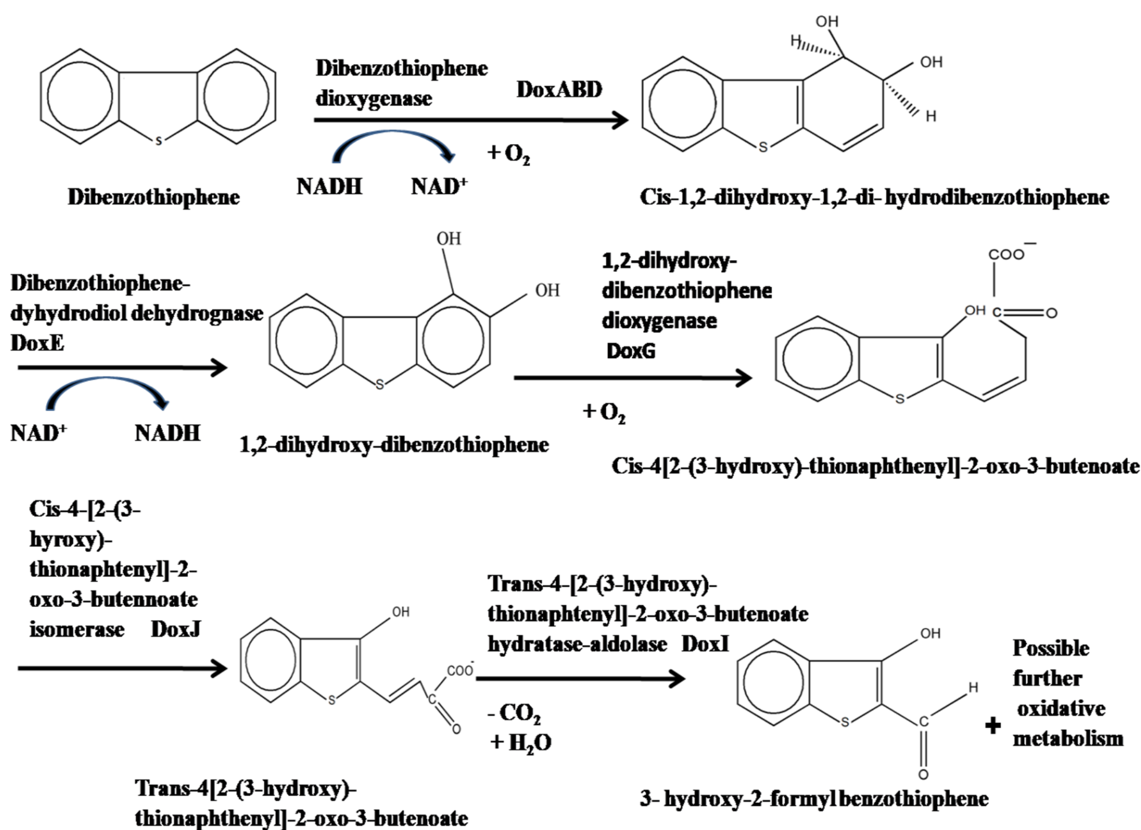

Fig. 1 Biodesulfurization of dibenzothiophene by Kodama pathway

4S metabolic pathway has suggested to be adopted as an alternative technology in oil production fields and refineries. It is therefore necessary to promote the commercialization of BDS in large scale industrial applications by deepening our understanding of the mechanism, genes involved and inhibitory nature of the end products [3, 36]. The US Company, Energy BioSystems Corporation undertook one such industrial BDS project and used three bioreactors to lower sulphur concentration from 5 barrels of oil per day [59].

Genetics of Biodesulfurization

DBT metabolism involves several catabolic genes known as *dsz, bds, mds, sox* and *tds.* Among these genes, "*sox*" designation for DBT desulfurization has been now dismissed as numerous independent unlinked genes have also designated as *sox* (Mohebali and Ball 2016) [58]. BDS of organosulfur compounds like DBT is carried out by activity of three enzymes encoded by *dszA*, *dszB* and *dsz*C genes. *Rhodococcus erythropolis* IGTS8 has a linear megaplasmid with size of 120–150 kb which bears these three genes arranged and expressed in a 4 kb fragment of the megaplasmid in the same orientation. All the three *dsz* genes are regulated by a *dsz* operon [34, 59–61]. *Rhodococcus erythropolis* IGTS8 is a model bacterium and is among the first strains reported to be DBT-BDS where DBT is successfully transformed into 2-HBP following the 4S pathway. 4-S metabolic pathway of *R. erythropolis* IGTS8 involves DszC (45-kDa protein) and DszA (50-kDa protein) monooxygenases enzymes and a desulfinase enzyme, DszB (40-kDa protein) [58, 62].

DBT is degraded into 2-HBP through successive oxidative step wherein the first catalyzing step is regulated by *dszC* gene and the enzyme encodes for this is a monooxygenase, DBT-monooxygenase. This enzyme oxidizes DBT to DBT-sulfoxide and DBT- sulfoxide to DBT- sulfone. Second gene, *dszA* encodes a monooxygenase, called DBT sulfone monooxygenase, which acts on DBT-sulfone converting it further into 2-hydroxybiphenyl-2-sulfinic acid. Further oxidative step is catalyzed by a desulfinase enzyme, 2-hydroxybiphenyl sulfinate desulfinase encoded by *dszB* gene. During this step, 2-hydroxybiphenyl-2-sulfinic acid is converted to 2-hydroxybiphenyl and sulfite [63]. DszC and DszA are flavin-dependent monooxygenases that require reductive flavin nucleotides ($FMNH_2$) as cofactors for their catalytic activities. Studies indicates an additional presence

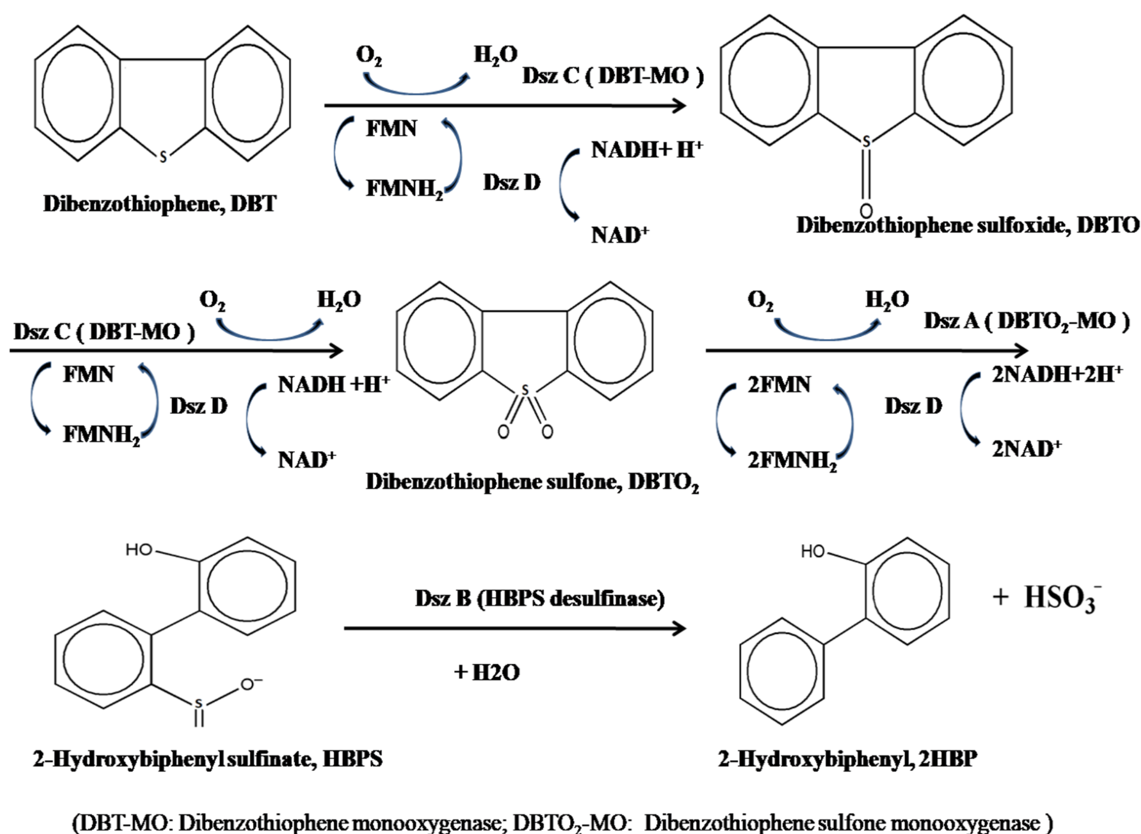

Fig. 2 4S Metabolic pathway of biodesulfurization of dibenzothiophene

of chromosomal gene, *dszD* encodes protein DszD, reduced nicotinamide adenine dinucleotide (NADH) or flavin mononucleotide (FMN) or simply flavin reductase. However, various factors such as enzyme specificity, limited availability of enzyme and essential cofactor ($FMNH_2$), feedback inhibition, toxicity of organic pollutants and the oil–water separations limits the desulfurization rate of microbial biocatalyst and influence its commercial applications [59, 64].

Diverse bacterial genera isolated from various environments possess *dsz* genes which support the assumption that these genes are horizontally transferred and have only a few differences in their sequences. Six different types of organization in *dszABC* genes nucleotide sequences were reported. Type 2 (*dszABC-type-2*) has been reported from *Gordonia alkanivorans* 1B whereas type -3 (*dszABC-type-3*) was detected from *G. amicalis* strain F.5.25.8. The homology of *dszABC*-type-2 and *dszABC*-type-3 with that of *dszABC*-type-1 found in *Rhodococcus* sp. IGTS8 is 90% and 84% respectively. Similarly, *dszABC*-type-3 and type-4 have been found from thermophilic *Bacillus subtilis* and *Paenibacillus* sp. respectively. Type-5 and type-6 organizations were described from *Mycobacterium* sp. [65]. The size of *dszA, dszB* and *dszC* range from 1362 to 1425 bp, 1062–1098 bp and 1245–1254 bp respectively [58, 65]. The expression patterns of *dszA*, *dszB,* and *dszC* genes in *Gordonia* species were found to be in the same orientation under the control of a single promoter [58]. Sequencing studies indicated a 10 bp gap between *dszC* and *dszB* genes along with a 67 bp overlap between initiation codon of *dszB* and the termination codon of *dszA* [65]. Removal of the overlap between *dszA* and *dszB* gens in *dsz* operon can substantially improve the biodesulfurization activity of *Gordonia* species [58]. Shavandi et al. [13] discussed that rearrangement *of dszABC* operon into *dszBAC* would facilitate a higher biodesulfurization activity. Further, results of Southern blot and pulsed field gel electrophoresis specify that, *dsz* operon of *G. alkanivorans* RIPI90A is located on bacterial chromosome where as *dsz* operon of *Rhodococcus* strain IGTS8 is located on 120 kb megaplasmid [13]. Although there is a higher homology of *dsz* operons between strain RIPI90A and strain IGTS8, the homology between promoter sequences of both strains is minimal (> 52.5%) [13].

Taxonomy, Diversity and Cultural Characteristics of *Gordonia* sp.

The bacterial genus, *Gordonia* is very versatile for its ability to convert and decompose a wide range of compounds,

including polyaromatic sulfur heterocycles (PASHs) that are present in fossil fuels. There are several strains in this diverse catabolic genus which are capable of desulfurizing not only DBT, but also benzothiophene (BT) and other thiophenic compounds, which most common *Rhodococci* strains cannot do [43]. A comparative characteristic of the efficiency of *Gordonia* species in fuel desulfurization showed that strains CYKS1, SYKS1 were capable of desulfurizing middle distillate unit feed (1500 ppm), light gas oil (3000 ppm) and diesel fuel (250 ppm) of oil fractions with 50–70% and 35–76% of degree of desulfurization respectively [51]. Table 2 summarizes the desulfurization efficiency of various strains of *Gordonia* sp. [10–37].

Tsukamura, in1971 proposed *Gordona* as a new genus of coryneform bacteria isolated from sputa of patients with pulmonary disease and from soil [66]. In order to pay tribute to an eminent American bacterial taxonomist, Ruth E. Gordon, the name *Gordona* for this genus was proposed. The genus was later renamed as *Gordonia* based on biochemical, molecular and 16S ribosomal RNA sequence evaluation. The cell wall composition of *Gordonia* sp. consists of high molecular weight, long-chained fatty acids like mycolic-acid with an average carbon atom number of 50–60 and a range of 0–5 double bonds, as well as MK-9-(H) menaquinone [67, 68]. Phylogenetic analysis places this genus in the family Gordoniacea, suborder Corynebacterineae, and order Actinomycetales [6, 69].

Phylogenetic relationships based on 16S rRNA gene analyses of 39 *Gordonia* species isolated from soil, rhizosphere, waste water, marine animals, activated sludge and human beings has been published [6]. Seven strains of *Gordonia* sp. isolated from soil and sludge samples have shown siderophore production, lignin, xenobiotic, thermo-halotolerant alkane degradation and metabolism of organo sulfur compounds. *G. desulfuricans, G. terrae*, *G. amicalis,* and *G. alkanivorans* are thoroughly studied *Gordonia* species for their role in crude oil component degradation. Twenty four *Gordonia* species isolated from diverse environments have shown a potential role in biodegradation of hydrocarbons and pollutants. Among these, ten species, whose roles are

Table 2 Comparisons of biodesulfurizing efficiency among different strains of *Gordonia* sp. reported in the literature

Species	Strain	Substrate	Reaction time/rate	Concentration of the end product	References
G. alkanivorans	RIP190A	0.5 mM DBT	60 h	0.053 mM 2-HBP	[10]
	RIP190A Recombinant strain	0.5 mM DBT	30 h	≈65 µM 2-HBP	[12]
	1B	0.2 mM DBT	168 h	≈120 µM 2-HBP	[14]
		0.25 mM DBT (RPS hydrolyzate)	100 h	≈110 µM 2-HBP	[15]
		0.5 mM	240 h	≈260 µM 2-HBP	[16]
G. amicalis	F.5.25.8	1 mM DBT	120 h	≈0.19 mM 2-HBP	[18]
G. nitida	CYKSI	0.3 mM DBT	12 h	≈0.15 mM 2-HBP	[22]
		0.3 mM DBT	72 h	-	[23]
G. rubripertincta	T08	0.19 mM BT	48 h	0.11 mM Phenolic compounds (Coumaranone)	[24]
	ICP172	≈0.6 mM DBT	2.5 h	≈0.15 mM 2-HBP	[25]
G. terrae	C-6	0.3 mM BT	48 h	0.15 mM Phenolic compound	[26]
Gordonia strains without species assignment					
Gordonia sp.	213E	0.2 mM BT	60 h	0.2 mM HPEal	[27]
	C-6	0.5 mM BT	24 h	o-hydroxystyrene or o-hydroxyhyacinthin	[28]
	CYKS1	0.1 mM DBT	120 h	≈0.28 mM 2-HBP	[29]
	ZD-7	0.2 mM DBT	48 h	≈0.12 mM 2-HBP	[31]
	IITR100	0.25 mM TA	144 h	o-hydroxyphenyl phenylsulfone	[32]
		0.3 mM DBS	96 h	benzoic acid	[33]
		0.3 mM BNT	192 h	2-Phenyl naphthalene-1-ol	[34]
		0.9 mM DMDBT, 0.8 mM BNT	72 h	2-hydroxy-3,3' dimethyl biphenyl, α- hydroxy β phenyl naphthalene	[35]
	JDZX13	0.3 mM DBT	47 h	2-HBP (100% DBT consumed)	[36]
	AHV-01	1 mM DBT	96 h	70.29 µM 2-HBP	[37]

HPEal 2-(2'-hydroxypheny1) ethan 1-al, *2HBP* 2-hydroxybiphenyl, *DBT* Dibenzothiophene, *BT* Benzothiophene, *TA* Thianthrene, *DMDBT* dimethyldibenzothiophene, *DBS* dibenzyl sulfide, *BNT* benzonaphthothiophene

unclear, have been found to be associated with marine fauna, and three species, *G. aichiensis*, *G. sputa*, and *G. otitidis*, have been linked to human pathologies [6, 40].

Various phenotypic, biochemical, chemotaxonomic and molecular results distinguish *Gordonia* from other members of the Actinobacteria group. Members of this genus are partially acid-fast, arylsulfatase-negative, bacilli or cocci in shape, sensitive to lysozyme, hydrolyses urea, and shows positive nitrate reduction. Their gram character is positive to variable. Few members of *Gordonia* are known to produce pigmented colonies with a broad range of colors including orange to orange-red, pale grey, white, peach, yellow, tannish to deep pink and also bear shiny, slimy, smooth to rough and uneven surfaces. One of the best methods for identifying this genus is to observe its growth in medium containing carbohydrates, amino acids and different concentrations of sodium chloride at different temperatures [4, 6, 39, 40, 69–71]. Depending on the type of medium used for growth, the colony characteristics may vary within one species [72].

16S rRNA gene sequence analysis helps to identify *Gordonia* species, however, for species level identification a reference laboratory is required [73, 74]. The homology of 16S rRNA gene sequences among different *Gordonia* species is found to be very high. It ranges 99.9% between *G. alkanivorans* and *G. nitida*; and 94.8% in between *G. amarae* and *Gordonia sihwensis*. The intrageneric DNA–DNA closeness varies from 3.7% (shared by *G. amarae* and *Gordonia nitida*) to 52% (shared by *G. rubripertincta* and *G. alkanivorans*) and the G + C% ratio of *Gordonia* genome extends from 63 to 69 mol% [38].

Biodesulfurization Potential of *Gordonia* sp.

Gordonia species are recognized as an effective biocatalyst, capable of degrading as well as transforming organosulfur pollutants from oil contaminated soil and water. In the recent past, *Gordonia* sp has been recognized as a potential biodesulfurizing bacteria as it can degrade both aromatic and aliphatic hydrocarbons [75]. Some of the sulfur compounds that are degraded by different *Gordonia* strains are mentioned in the following.

Thiols, sulphides, thiophenes and its substituted derivatives constitute the major portion among the 200 types of organosulfur compounds found in crude oils. According to a study of the thiophenic compounds found in crude oil from Texas, 70 percent of the sulfur is in the form of DBT and its alkylated form [76]. Many other reports in the last few decades also share a similar background. Therefore, DBT has been exploited as a model compound in biodesulfurizing research.

The genus *Gordonia* comprises of many species which are capable of using DBT as a sole source of sulfur, e.g. *G. amicalis* strain IEGM^{T} [9], *G. alkanivorans* RIPI90A [10–13], *G. amicalis* F.5.25.8 [18, 19], *G. alkanivorans* 1B [14–16], *G. nitida* CYKSI [22, 23], *G. rubripertincta* ICP172 [25], and *Gordonia* sp. strain TM414 [30].

Gordonia sp. IITR100 is a robust, aerobic bacterium that can desulfurize a broad range of organosulfur compounds such as thiophenic (DBT, benzonaphthothiophene) and non-thiophenic (thiathrene). The presence of a nucleotide sequence of 11.5 kb region (GenBank accession number KC693733.1) containing the *dszABC* operon in *Gordonia* sp IITR100 shown > 99% sequence similarity with that of *Gordonia* strains 1B, RIPI90A and CYKS2. The size of the *dsz* genes are 1425, 1098 and 1251 for *dszA, dszB* and *dszC* respectively [32, 65]. Draft-genome sequence of *Gordonia* sp. strain IITR100 reported the presence of five genes involved in the BDS process, which includes 2-hydroxybiphenyl-2-sulfinate desulfinase, dibenzothiophene (DBT) desulfurization enzyme, DBT monooxygenase, sulfite reductase, and flavin reductase [77]. Another study reports strain IITR100 as one of the pioneer strains among the members of *Gordonia* genus showing remarkable biodesulfurizing capacity of heavy and hydrodesulfurized oil by reducing its sulfur quantity by 76% and 70–98% (70– < 2 ppm) respectively. Since the bacterium can also degrade aliphatic C–S bonds, 31% of viscosity reduction was observed until 6 days of incubation after which hydrocarbon framework of C–C bonds were disturbed [35].

Gordonia alkanivorans RIPI90A is widely used organism in BDS experiments conducted till date as it has the advantage of desulfurizing alkylated derivatives of DBT such as 4-dimethyldibenzothiophene (4-MDBT), 4, 6-dimethyldibenzothiophene (4, 6-DMDBT) etc. when used as sulfur sources and not as carbon sources. However, the strain prefers 4-MDBT over DBT and 4, 6-DMDBT, DBT sulfone over DBT and cannot desulfurize BT. In another study, DBT biodesulfurizing potential of *G. alkanivorans* RIPI90A was reported to be repressed by amino acids methionine, cysteine and sulfate. This was however controlled by adopting *G. alkanivorans* RIPI90A transformants with a stable plasmid pRSG43 harbouring a sulfate non-repressible, inducible *lac* promoter where *dszABC* genes were cloned and expressed for efficient BDS process with higher specific activity. Analytical studies for alternative promoters in effective expression of *dszABC* operon in *Gordonia* have also been done [10, 12, 13].

Repression effect of desulfurization by sulfate has been described to be characteristic of the 4S metabolic pathway [14]. A major hurdle in mass production of desulfurizing resting cells is repression of BDS by presence of sulfate. The problem can be tackled by replacement of sulfate by DBT as the main substrate for the 4S pathway. However, in practical purpose it is not possible. Therefore, dimethyl sulfoxide

(DMSO) was used as the sulfur source for mass production of desulfurizing bacterium *G. alkanivorans* RIPI90A, [11].

The first report on the utilization of alternative raw materials as substitutes for refined substrates (namely glucose) in biodesulfurization studies was done by using *G. alkanivorans* strain 1B [15]. Since the 4S pathway is a multienzymatic metabolic pathway, it depends on both reducing cofactors and the nutrient composition. The effect of various metal ions was observed on the growth and BDS activity of *G. alkanivorans* strain 1B, a potential BDS bacterium. Among all the nutrients, zinc showed the most significant effect, probably acting on DszA (DBT sulfone monooxygenase) and improved the production of 2HBP by 26% when its concentration was increased from 1 to 10 mg L^{-1} [16]. Although the pathways for DBT desulfurization and BT desulfurization varies, *G. alkanivorans* strain 1B has been reported to desulfurize both DBT and BT simultaneously [14]. A study on the effect of different carbon sources on BDS activity of the strain revealed the affinity of the strain towards fructose, producing 9.29 mM/hour of 2HBP from DBT [78].

DBT-desulfurizing bacterium, *G. amicalis* F.5.25.8 is highly stable, resistant to high temperature (42 °C) and mutagens. However, the remarkable and unique property of this strain lies on its location of the *dsz* genes. Unlike *Rhodococcal* BDS strains which have *dszABC* genes arranged on plasmid, *G. amicalis* F.5.25.8 and *G. alkanivorans* RIPI90A are reported to have these genes on the chromosome. PCR amplification based on *Rhodococcus* designed primers was only able to yield *dszA* and *dszB* genes. *G. amicalis* F.5.25.8 showed a high degree of BDS at a rate of 73% [18]. Characterization of entire DNA sequences, promoter region along with the *dszABC* genes as well as highly conserved regions of the strain F.5.25.8 showed 85% homology with *dsz* genes of *G. alkinovorans* 1B [19].

G. nitida CYKS1, another oil-desulfurizing biocatalyst adopted a unique strategy for its production and DBT-BDS rate. Two separate sulfur sources were used for the purpose. In a two-stage fermentation model, magnesium sulfate was used as the first sulfur source during the cell growth stage which was replaced by DBT in the desulfurization activity induction stage. The result generated a highly capable deep desulfurizing biocatalyst which can give rise to exceedingly low-sulfur petroleum oils. The level of sulfur in diesel oil was reported to have decreased to 61 mg-sulfur L-oil-1 in 20 h from 250 mg-sulfur L-oil^{-1} [22]. CYKS1 was found to take sulfur molecules from organic sulfur compounds for its growth [23]. Desulfurization activity of nocardioform bacteria, *Gordonia* sp.TM414 was increased by introducing *dsz* expression vector into the strain TM414 for practical application [30].

Apart from the studies on the metabolic pathways and varying desulfurizing rate of different *Gordonia* strains, reports on the studies of the genome sequences are also present which will give a platform of possibilities for exploitation of this genus ability in biotechnological applications. Draft genome sequence analysis of *Gordonia sp.* 1D showed that apart from genes involved in gentisate–naphthalene, salicylate, and alkane degradation, it also contain genes for DBT metabolism. The thermotolerant strain 1D is unique its ability to oxidize hydrocarbons found in crude oil at temperature of 45–50 °C. The strain is a kind of prototypical of *Gordonia amicalis* and effective in exploiting long chains of alkane group (upto C_{36}). At 45 °C, magnitude of crude oil degeneration in liquid medium and soil is 38% and 40% respectively [20].

G. alkanivorans strain CGMCC6845 (GenBank accession number AYXO00000000) is a DBT desulfurizing halotolerant bacterium which can also degrade other thiophenes like BTs. From the draft genome sequence analysis, it was found that the strain has 9 hydrocarbon degrading genes, and only 1 dibenzothiophene desulfurizing gene [17]. The biodesulfurization pathway involved in BT desulfurization is not yet fully understood but is proposed to be having of four intermediates of sulfur containing compounds. The *bds*ABC operon in the pathway shares a low degree of similarity with that of DBT desulfuring enzymes of 4S metabolic pathway [79].

There are number of BT desulfurizing genes which play an important role in BT biodesulfurization pathways. The first three steps in the proposed biodesulfurizing pathway of BT involve five genes that encode for $FMNH_2$-dependent monooxygenase and alkanesulfonate monooxygenase. A desulfinase gene along with transport proteins were also assumed to play catalyzing role in the last step of hydrolyzation of benzene sulphonate or benzene sulfinate to sulphonate or sulfinate [6]. There are several reports on desulfurization of benzothiophene by *Gordonia* species. The genetic analysis of *G. terrae* C-6 revealed presence of 135 genes up-regulated with BT desulfurization. Out of these, three genes arranged in a chromosomal operon, *bds*ABC, was reported to have a high percentage of similarity with that of DBT *dszABC* operon [26].

G. desulfuricans, 213E was established as the first BT-desulfurizing bacterium which uses BT and fructose as sulfur and carbon sources respectively. The strain was able to desulfurize BT in absence of sulfates forming four intermediates, where 2-(2'-Hydroxyphenyl) ethan-l-al (HPEal) got accumulated as the final product. Coincidently, the first three steps in the BT metabolizing pathway were homologous to 4S metabolic pathway of DBT biodesulfurization suggesting presence of enzyme similar to that of DszC [27].

However, BT-desulfurizing pathway of *G. rubropertinctus* strain T08 differed from previously reported pathway by Gilbert et al. [27]. The metabolites produced by the strain

were identified and based on them, a new BT-desulfurizing pathway was designed. The proposed catabolic sequence is BT → BTO (Benzothiophene S-oxide) → BTO2 (Benzothiophene S, S-dioxide) → BT-sultine → BT-sultone → *o*-hydroxystyrene → (coumaran) → coumaranone → (coumaranone derivative). Coumaran and coumaranone derivative were not detected in the study (Fig. 3) [24].

Earlier studies showed that the type of sulfur compounds present in crude oil can be used to predict the desulfurization technology needed for its degradation. Nevertheless, recent evidence points to other factors affecting the technology, such as the degree of substitution of groups in the sulfur ring and the amount of aromatic compounds of three rings in the crude oil. DBT and its methylated derivatives progressively inhibits desulfurization of other thiophenic compounds like that of benzo[b]naphtho[2,1-d]thiophene (BNT) [80].

Gordonia sp. IITR100 is one of the striking examples of BNT-desulfurizing strains, which degrades BNT using enzymes analogous to the 4S pathway of DBT biodesulfurization and forms intermediate metabolites such as BNT-sulfone, BNT-sulfinate, and BNT-hydroxide (Fig. 4). The strain prefers to degrade DBT over BNT during the first four days of incubation, and will only desulfurize BNT if it is the sole surface source [34].

Studies on the desulfurization of sulfide compounds are limited and one of such examples is the desulfurization of dibenzyl sulfide (DBS) by a newly identified strain, *Gordonia* sp. IITR100. The pathway proceeds via formation of metabolites such as DBS sulfoxide, DBS sulfone and benzoic acid. The strain shows immense potentiality to degrade DBS and can be used in petroleum biodesulfurization as it is capable of desulfurizing both thiophenic and sulfidic compounds [33].

Metabolic pathway for the degradation of non-thiophenic sulfur compound, thianthrene, by *Gordonia* sp. IITR100 has found to be similar to that of 4S metabolic pathway. The strain exhibited exceptional thianthrene (TA) desulfurization in comparison to DBT indicating a substantial acceptance of TA by the biodesulfuring enzymes [32].

Genetic Engineering Approaches in *Gordonia* sp. for Enhanced Biodesulfurization

Biotechnological potential of *Gordonia* sp. have not attained a huge success as exploitation in genetic tools such as obtaining a new plasmids or electroporation protocols developed so far have been limited. The availability of *E.coli/*

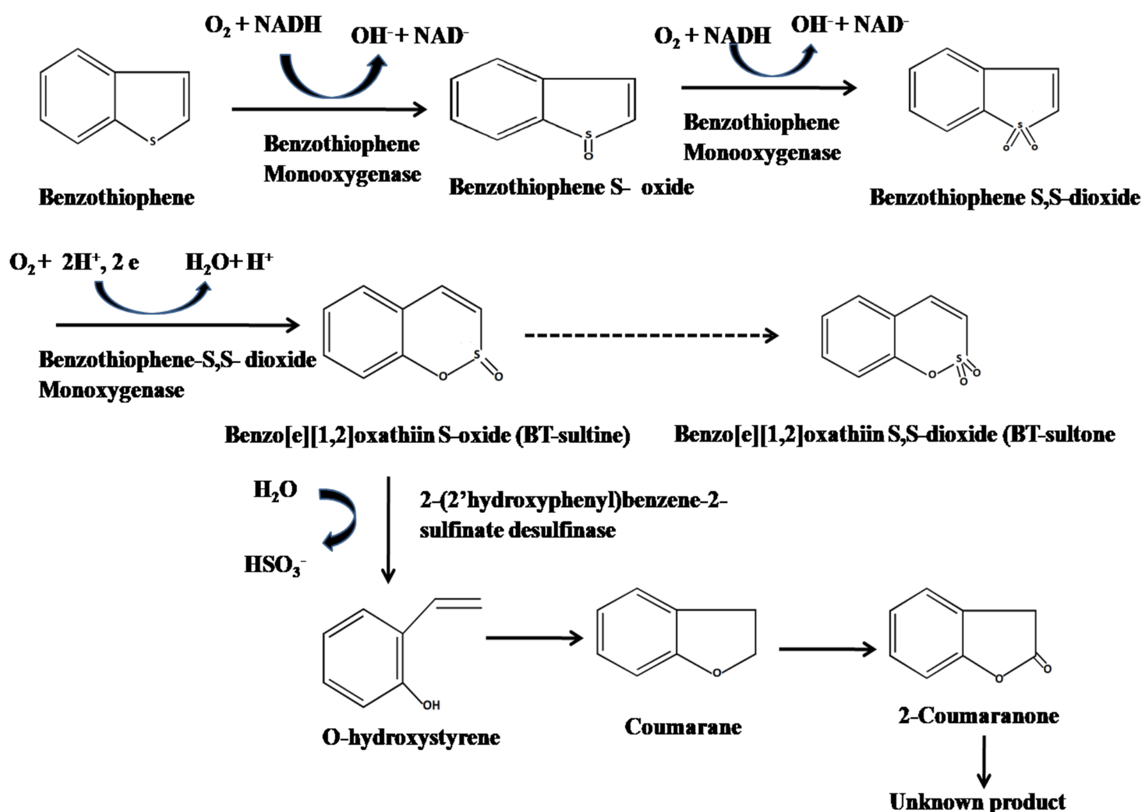

Fig. 3 Biodesulfurization pathway of benzothiophene

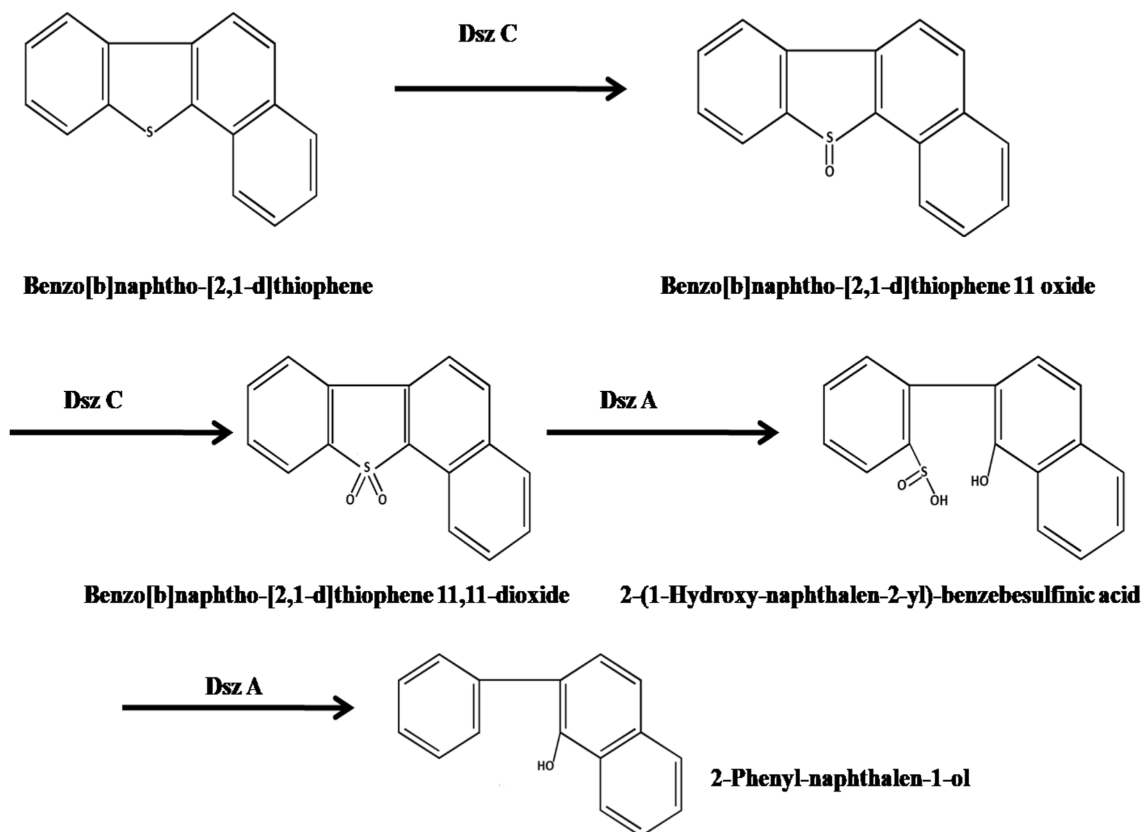

Fig. 4 Biodesulfurization pathway of Benzo[b]naphtho-[2,1-d]thiophene (BNT). *Figures were prepared using Chemoffice2004

Gordonia shuttle vectors for transformation works in the genus are very limited and are all based on origin of replication of plasmids belonging to *Rhodococcus* genus. Few examples include *E.coli/Rhodococcus* (*Gordonia*) shuttle vectors such as pNC9501, pNC9503; pBBRKmNC903 and pOpaCOS contain a common pNC903 ori, and kanamycin resistance gene [4, 81].

Another example, pRSG43 have been recently exploited for carrying out gene transfer and expression studies of *dszABC* genes in the *Gordonia alkanivorans* RIPI90A by adopting electroporation protocols. The recombinant strain was capable of increasing the desulfurizing activity by 2.67-fold under the regulation of *lac* promoter. However, the same shuttle vector did not produce any transformants in the case of *Gordonia* sp. IITR 100 (Genbank accession number GU084407), and therefore electroporation protocol for the genus was revised. A new, optimized protocol was suggested which required presence of glycine and a stronger permeabilizing reagent like Tween80. This protocol can be exploited for genetic manipulation studies in different species of the genera for enhancement of biodesulfurization [12, 82].

E. coli (SG13009) cells have been utilized as an expression host for *dsz*AB genes of *G. alkanivorans* strain 1B. The recombinant strain could desulfurize DBTs in LB medium containing other sulfur compounds such as sulfates, showing no sulfate repression of the *dsz*AB genes expression [83]. DBT biodesulfurization activity of *G. alkanivorans* RIPI90A was studied by conducting an intensive analysis of the promoter involved in the *dsz* operon. The study showed that regardless of high similarity in *dsz* upstream sequences with that of *R. erythropolis* IGST8, *dsz* promoter of the strain RIPI90A varied greatly in its sequences and location. A PCR-walking method was validated for cloning and sequencing of the *dsz* regulatory genes. Using *Gordonia*-specific primers, *dszABC* genes were amplified and it was reported that alterations in the orientation of *dszABC* operon by deletion of overlapping sequences may bring about remarkable increase in desulfurizing activity [13]. In a previous study, heterologus expression of *hpaC* oxidoreductase from *Escherichia coli* W was able to supply required $FMNH_2$ to the Dsz monooxygenases [60] and significantly improved the DBT desulfurization.

With a demand in studies of degradation, synthesis and transformation of numerous compounds by *Gordonia* sp., a new strong, stationary phase and auto inducible promoter was characterized from a biodesulfurizing bacterium, *Gordonia* sp. IITR100, which could also perform well in *E. coli* cells. The aim of the study was to tackle the screening

process more efficiently, so that positive constructs can be easily electroporated in IITR100 cells. One of the merits of this promoter is overexpression of heterologous proteins that do not lead to changes in the physiology of the bacterial cells which can prove beneficial in expression studies considering the rising demand in biotechnological field [84].

Even now in the field of biodesulfurization, vectors availability for knock-out mutants or transposon mutagenes for *Gordonia* sp. is are very limited. However, the increasing number of projects on whole genome sequencing will pave a way in the upcoming years for the genus to succeed in establishment of their metabolic as well as biotechnological prospects [4] (Table 3) [17, 20, 77, 85, 86].

Conclusions and Future Perspectives

It has been decades since attempts have been made to implement BDS in oil refining industries, but difficulties in achieving satisfactory results led researchers to seek new knowledge about this novel bioprocess. It is evidenced that successful commercialization of biodesulfurization process depends on various factors such as enzymatic activity of microorganisms, toxicity of the end product (2-HBP), and separation of microbial biomass/oil during BDS process [59]. However, this could be addressed with the rapid advent of computational analysis and modern genetic engineering technologies.

The commercialization of the BDS process will require genetically engineered microorganisms and novel biocatalysts as the desulfurizing rate of known BDS microorganisms is very low (55 fold lower than required) [87]. Molecular regulation of metabolic genes involved BDS pathways is still poorly understood. Therefore, further studies are required to understand metabolic gene expression and their regulation in the host cell during BDS pathways [58]. One possible way to achieve this is to regulate the BDS metabolic mechanisms from the available genome sequences of desulfurizing biocatalysts [88]. The available draft genome sequences of five different *Gordonia* spp. with desulfurizing activity (*G. alkanivorans* CGMCC6845 [17], *G. amicalis* 1D [20], *G. amicalis* CCMA-559 [85], *G. terrae* C-6 [86], *Gordonia* sp. IITR100 [77]) provide insight on the genes which are responsible for regulation of the BDS pathway.

The low solubility and adsorption of high molecular weight DBT and its hydrocarbons derivatives limits their availability to microorganisms and affects the biocatalysts ability. The difference in the interfacial tension between water and oil volume ratio is among the prominent drawback in the development of petroleum biotechnological processes. To control and cutback the operational costs linked with water dissociation and disposal, the interfacial tension should be preferably reduced [89]. This problem could be solved by application of suitable biosurfactants without effecting the biocatalyst cellular growth.

It has been observed that the presence of high number of disulfide bonds in proteins make them tolerant to various factors such as pH, solvents and temperature. Therefore, modifications in desulfurizing enzymes by incorporation of cysteine in its framework can have potential implications in industrial mass production of BDS biocatalysts. Further, the highly stable cysteine-rich desulfurizing enzymes will make the bacterial cells to have higher demand for sulfur and may even provide better tolerance against end products like 2-HBP. This will selectively enhance the desulfurization efficiency of the organism [57]. Investigations on efficient bioreactor systems for dealing with multiphase liquid mixture like air-lift reactor operation with immobilized cells can provide important information on the future development of BDS processes for deep desulfurization of fossil fuels [23]. Studies on purification and characterization of desulfurizing

Table 3 Summary of the published draft genome sequences of different biodesulfurizing *Gordonia* species

Gordonia species	GenBank accession no	Source	Size (MB)	GC%	Protein coding sequence	tRNA species	rRNA operons	References
G. alkanivorans CGMCC6845	AYXO00000000	Soil, China	5.0	67.43	4476	49	3	[17]
G. amicalis 1D	–	Soil, Russia	5.15	67.3	4772	47	3	[20]
G. amicalis CCMA-559	AWTB00000000	Oil contaminated mangrove swamp, Brazil	5.1	65.09	4736	–	–	[85]
G. terrae C-6	CP016594.1	Crude oil contaminated soil, China	5.17	67.9	4645	46	–	[86]
Gordonia sp. IITR100	MVKV00000000	Soil, India	5.28	–	4766	–	–	[77]
G. amicalis BDS-I	PRJNA641974	Hydrocarbon contaminated soil, India	5.18	67.4	4464	46	4	Unpublished

enzymes in *Gordonia* sp. can provide new avenues in petroleum biotechnology [16].

The BDS process alone should not be viewed as a complete replacement technology for oil refining processes, but rather as an interdependent complementary technology to conventional HDS. Integration of BDS systems along with HDS technology to eliminate recalcitrant components existing in fossil fuels shows a greater possibility of success in the nearest future for oil industries [58].

The present review summarizes relevant details of desulfurizing potentialities of *Gordonia* species that can be utilized and exploited in the field of petroleum biotechnology for the improvement of biorefining of crude oils. Draft genome sequencing data of the *Gordonia* species further enhanced our understanding of genetics behind biodesulfurization of organosulfur aliphatic and aromatic compounds in crude oils. Comprehensive understanding of different gene clusters involved in BDS pathways would enable us to go forward in strain improvement programmes and genetic manipulation studies for further enhancement of the process [6]. This genus could possibly play a significant role in petroleum biorefining process in the near future.

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Data Availability The original data compiled for the article will be maintained by the corresponding author.

Declarations

Conflict of interest All the authors declare that they have no conflict of interest.

Ethical Approval The work involves no plant, animal and human experiments. All prevailing local, national and international regulations and conventions and normal scientific ethical practices have also been respected.

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