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CIÊNCIAeNATURA

ISSN 2179-460X
Acesso aberto

Ci. e Nat., Santa Maria, v. 44, Ed. Esp. VI SSS, e17, 2022 • https://doi.org/10.5902/2179460X68835
Submitted: 8/12/2021 • Approved: 9/12/2021 • Published: 1/4/2022

Special Edition

The use of bacteria for bioremediation of environments contaminated with toluene: a molecular docking analysis

O uso de bactérias para biorremediação de ambientes contaminados com tolueno: uma análise molecular por *docking*

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ABSTRACT

Bacteria can be helpful organisms for environmental decontamination through bioremediation processes. Among the environmental contaminants, toluene is one of the compounds present in gasoline and can be toxic in the aquatic environment, causing significant damage to organisms in this ecosystem. The detailed understanding of the main proteins and processes involved in cellular bioremediation pathways is little explored, which encourages studies in the area to optimize these processes. The ABC transporter system is involved in the transport of a wide variety of inorganic compounds and complex organic molecules. Molecular docking (DM) is an *in silico* methodology in which two molecules are joined (receptor *versus* ligand), and the chemical characteristics of this bond are verified. possibilitando identificar espécies promissoras para a biorremediação do tolueno.Thus, through the molecular docking tool, this study aimed to evaluate the interaction between toluene and ABC transporters of bacterial species of *Staphylococcus aureus* and *Thermotoga maritima*, enabling the identification of promising species for toluene bioremediation. For the DM, UCFS Chimera software (for the elimination of heteroatoms and production of 3D images), AutoDock Tools (for preparation of proteins and toluene for docking), AutoDock Vina (for docking per se), and LigPlot + (for checking amino acids and types of binding between molecules) were used. The binding energy between ABC transporters and toluene was similar for both species, being -5.2 Kcal/mol for *S. aureus* and -5.5 Kcal/mol for *T. maritima*. The number of transporter amino acids bound to toluene differed for the two species: *S. aureus* bound through three amino acids, while *T. maritima* bound through seven amino acids. The results presented in this study demonstrate that both species evaluated are promising for *in vitro* and *in vivo* studies for toluene bioremediation.

Keywords: BTXs; Proteins; Fuels

RESUMO

As bactérias podem ser organismos úteis para a descontaminação ambiental através de processos de biorremediação. Dentre os contaminantes ambientais, o tolueno é um dos compostos presentes na gasolina e pode ser tóxico no ambiente aquático, causando danos significativos aos organismos deste ecossistema. A compreensão detalhada sobre as principais proteínas e processos envolvidos em rotas celulares de biorremediação é pouco explorada, o que encoraja os estudos na área para a otimização desses processos. Sabe-se que o sistema transportador ABC está envolvido no transporte de uma ampla variedade de compostos inorgânicos e de moléculas orgânicas complexas. O *docking* molecular (DM) é uma metodologia *in silico* na qual unem-se duas moléculas (receptor *versus* ligante) e verifica-se características químicas dessa ligação. Desse modo, o objetivo desse estudo foi avaliar, através da ferramenta de *docking* molecular, a interação entre o tolueno e transportadores ABC das espécies bacterianas de *Staphylococcus aureus* e *Thermotoga maritima*, possibilitando identificar espécies promissoras para a biorremediação do tolueno. Para o DM utilizou-se os *softwares* UCFS Chimera (para eliminação de heteroátomos e produção das imagens em 3D), *AutoDock Tools* (para preparação das proteínas e do tolueno para o *docking*), *AutoDock Vina* (para o *docking per se*) e *LigPlot* + (para verificação de aminoácidos e tipos de ligação entre moléculas). A energia de ligação entre os transportadores ABC e o tolueno foi semelhante para ambas as espécies, sendo para *S. aureus* -5.2 Kcal/mol e para *T. maritima* -5.5 Kcal/mol. O número de aminoácidos do transportador que se ligaram ao tolueno foi diferente para as duas espécies: *S. aureus* uniu-se através de três aminoácidos, enquanto *T. maritima* uniu-se através de sete aminoácidos. Os resultados apresentados nesse estudo demonstram que ambas as espécies avaliadas são promissoras para estudo *in vitro* e *in vivo* para biorremediação do tolueno.

Palavras-chave: BTXs; Proteínas; Combustíveis

1 INTRODUCTION

Environmental pollution and contamination have grown exponentially in recent years. Among the environmental contaminants, gasoline is one of the most representative pollutants found in the soil and has in its composition a mixture that includes a variety of BTXs compounds (benzene, toluene, and xylene) (AN; LEE, 2008). Sams *et al*. (2004) report that the BTXs can cause severe groundwater contamination, mainly due to spills involving the release of petroleum products such as gasoline, diesel fuel, and lubricating oil and from leaking oil tank heaters. Data indicate that about 5.74 million tons of oil and oil products were spilled into marine ecosystems between 1970 and 2013 (ITOPF, 2009). The presence of the BTXs in water can cause harm to the environment and public health, even at low concentrations (SAMS *et al*., 2004). Among BTXs, toluene - also known as methylbenzene, is a water-insoluble homolog of benzene that is toxic in the aquatic environment and can cause significant damage to organisms in this ecosystem, affecting growth, development, and reproduction (XU *et al*., 2020). Li *et al*. (2021) explored the ototoxicity of toluene in the early development of zebrafish embryos/larvae. They found that toluene can affect the development of the inner ear and lateral line systems in zebrafish, to the point that the lateral line system may be more sensitive to toluene than the inner ear.

An alternative for controlling this compound would be the bioremediation, a process in which living organisms-usually plants, microorganisms, or their enzymes, are used technologically to remove (remediate) or reduce pollutants in the environment. Bioremediation has received attention in the past two decades as an alternative method to remove or mitigate toluene contamination in the ecosystem (OYETIBO *et al*., 2017). Bacteria are key organisms for bioremediation due to their rapid growth rate, ability to adapt quickly to environmental conditions, and simple growth requirements (FIELD; SIERRA, 2008; PARSEK; MCFALL; CHAKRABARTY, 1995). The first bioassay for toxicity monitoring was developed using the luminescent bacterium *Vibrio fischeri* in the 1970s (CLÉMENT; CADIER, 1998). The biodegradation of toluene by a microbial consortium with *Pseudomonas sp.*, *Escherichia coli*, and *Bacillus sp.* has already been reported (RAJAMANICKAM *et al*., 2017). Some other examples of isolated bacteria that degrade toluene are: *Pseudomonas aeruginosa* AT18, *Acinetobacter genospecies* Tol 5, *Bacterium* Ex-DG74, *Magnetospirillum* sp. 15-1, *Bacillus cereus* THH39, *Burkholderia* sp. JS150, and *Thauera* sp. DNT-1 (AFROUZOSSADAT *et al*., 2013; HEYDARNEZHAD *et al*., 2018; JOHNSON; OLSEN 1997; KIM; JEON, 2009; MEYER *et al*., 2017; PEREZ *et al*., 2006; SHINODA *et al*., 2004).

Some of the mechanisms involved in bioremediation processes are well known, occurring through, for example, pathways such as alk (c5 to c12 n-alkanes), nah (naphthalene; PAHs) (WHYTE *et al*., 1997), and xyl (toluene; aromatic hydrocarbons) (NAKAI *et al*., 1983). Despite this, details about which proteins are involved in these pathways remain scarce. It is necessary to determine in more detail the identity of these proteins and the interaction between them, and the activation of their genes under polluting conditions (GILBERT, 2001). Regarding bioremediation, the ABC transporter system is known to be involved in the transport of a wide variety of molybdenum, nickel, arsenic, and more complex organic molecules (BEHERA *et al*., 2020). Behera *et al*. (2020) evaluated the presence of a microbial community with bioremediation potential in sediment samples from the Ganga and Yamuna Rivers (India). Through functional analysis, many bioremediation-related protein domains were found in the metagenome of the river samples, among them the ABC transporter (BEHERA *et al*., 2020). This mechanism was also reported in the degradation of alkaline lignin by *Bacillus ligniniphilus*. By comparing proteomic data and metabolomic analysis, positive regulation of the ABC transporter system was found (ZHU *et al*., 2017).

In silico methods are used before toxic xenobiotics biodegradation assays to find viable cultures for their application (KHAN; SAJID; CAMEOTRA, 2013). This can help decrease the time and money spent on the initial screening and identifying environmentally viable strains. Some studies have successfully applied *in silico* techniques involving the molecular docking to identify and study compounds/enzymes and amino acid residues involved in biodegradation (LIU *et al*., 2018; SRINIVASAN *et al*., 2019). Thus, through this tool, this work aimed to evaluate the interaction between toluene and ABC transporters of the bacterial species of *Staphylococcus aureus* and *Thermotoga maritima*, enabling the identification of promising species for toluene bioremediation.

2 MATERIAL AND METHODS

The proteins were selected in the Protein Data Bank (PDB) by searching the term "ABC transporter." The unmutated structure of this transporter was found for two organisms: Staphylococcus aureus (PDB ID: 2HYD) and Thermotoga maritima

(PDB ID: 3QF4) (BERMAN *et al*., 2000). The chemical structure of toluene was obtained from the PubChem database (PubChem ID: 1140) (BOLTON *et al*., 2008). The heteroatoms were removed from the proteins by using UCSF ChimeraX software (GODDARD *et al*., 2018), and with AutoDock Tools, the proteins (removing the information from water molecules, adding polar hydrogens and Kollman charges) and the toluene ligand (updating Gasteiger and TORSDOF charges) were prepared. Molecular docking analyses were performed by AutoDock Vina (TROTT; OLSON, 2010). A "blind docking" was performed, performing the complete protein's coverage and increasing the exhaustiveness parameter. The configurations present in Table 1 were used to perform the docking simulations. The PyMol software (SCHRÖDINGER, 2015) was used for conversion into PDB format and LigPlot + (WALLACE; LASKOWSKI; THORNTON, 1995) for binding analysis on specific residues.

Table 1 – Summary of the parameters used in AutoDock Vina software to molecular docking analysis

PDB ID	**Grid box size**	**Coordinates of the center of the grid box**	***Exhaustiveness***
2HYD	x: 98; y: 60; z: 112	x: 116.899; y: 67.679; z: 142.384	500
3QF4	x: 118; y: 62; z: 82	x: -53.901; y: 5.998; z: 35.316	500

Source: Authors, 2021

3 RESULTS AND DISCUSSION

Bioinformatic tools offer a fast and robust way to screen targets for bioremediation. The molecular docking tool can help to screen the degradation susceptibility of the pollutant by already characterized enzymes. In addition, the existence of several methodologies and software packages available for molecular docking is another critical factor, as it provides predictions coupled with good performance and speed with low computational cost (GUEDES *et al*., 2014).

Although molecular docking studies are widely used for drug discovery, their use in predicting targets for bioremediation is still developing (SRIDHAR *et al*., 2013).

In this study, molecular docking analysis between bacterial ABC transporters and toluene was performed considering active site interactions. The results were examined based on binding energies, binding sites, and amino acid residues. The bacterial species showed similar binding free energy (BFE) between protein and ligand (Table 2) and < - 5.2 Kcal/mol, which may indicate a similar ability to bioremediate toluene through pathways involving the ABC protein. A BFE affinity < 0 means that a ligand and its receptor can bind spontaneously, and the lower the BFE value, the stronger the binding capacity (DU *et al*., 2016).

Table 2 – Binding free energy values (BFE) and amino acids (AA) involved in the interaction of the ABC transporters with the toluene

Specie	**PDB ID**	**BFE**	**AA**
Staphylococcus aureus	2HYD	-5.2 Kcal/mol	Lys 477 (B chain); Tyr 349 e 391 (A chain)
Thermotoga maritima	3QF4	-5.5 Kcal/mol	Phe 218 (chain A); Ile 141 (B chain); Phe 117 (B chain); Leu 116 e 120 (B chain); Val 195 (A chain) e 140 (B chain);

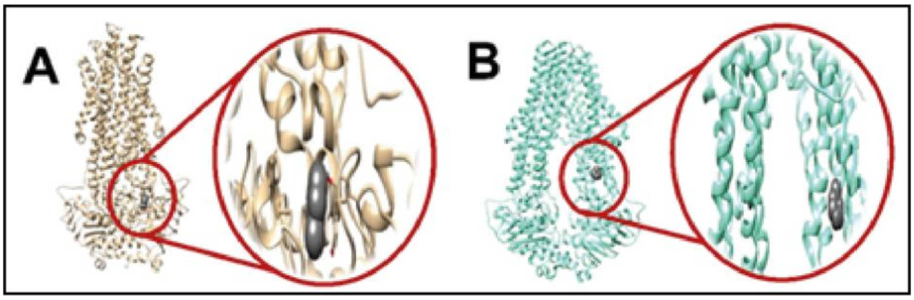
Source: Authors, 2021

The ABC protein can be an important target, as it is a transporter that may be a determinant for the entry of toluene into bacterial cells to initiate bioremediation (ORELLE *et al*., 2019). The ABC protein of the *T. maritima* showed a FEB slightly better than of the *S. aureus*. In addition, the ABC protein of the *T. maritima* was had the highest number of amino acid residues involved in binding (Table 1). For both bacteria, the toluene was ligated near the transmembrane region (Figure 1).

The ABC transporters of the plasma membranes of Gram-negative and Gram-positive bacteria are widely different in their organization and composition

(GOFFEAU; HERTOGH, 2013). However, these structural differences were minimal for the binding stability between the ABC transporter and the toluene since *S. aureus* (Gram-positive bacteria) and *T. maritima* (Gram-negative) showed little difference between the FEBs (Table 2).

Figure 1 – Toluene chemical structure binding with ABC transporter protein (generated using UCSF Chimera software)



A: ABC transporter of *S. aureus;* B: ABC transporter of *T. maritima*. In both, the toluene chemical structure is in dark gray.
Source: Authors, 2021

Staphylococcus is Gram and catalase-positive cocci, which strains grow on standard media, broth, or plain agar, with a pH = 7 and at an optimum temperature of 37°C. The distribution of S. aureus is extensive since this bacterium can significantly resist desiccation and cold (SANTOS *et al*., 2007). Despite being a human bacterial pathogen that causes a wide variety of clinical manifestations (TONG *et al*., 2015), the use of *S. aureus* (strain K1) in bioremediation has already been reported. Zou *et al*. (2014) analyzed metal-contaminated locals and reported the ability of *S. aureus* to resist and reduce chromium (VI) to chromium (III) and adsorb uranium (VI). Tariq *et al*. (2019) confirmed through biochemical and phylogenetic analyses that the *S. aureus* K1 strain is promising and can remove chromium from metal-polluted environments. Furthermore, biofilms produced by *S. aureus* - one of the structures responsible for the bacterium's pathogenicity, have been tested for uranium bioremediation ability (SHUKLA *et al*., 2020). Biosorption and enzymatic removal of the *S. aureus* biofilm played a role in remediating up to 47% of 10 ppm uranium (VI) (SHUKLA *et al*., 2020).

Thermotoga maritima is a hyperthermophilic Gram-negative bacterium that utilizes a vast network of ABC transporters to efficiently metabolize various carbon sources to produce hydrogen (LATIF *et al*., 2015). *T. maritima* grows anaerobically and has an optimum growth temperature of around 80 °C (HUBER *et al*., 1986). Being a source of thermostable enzymes and an efficient hydrogen producer, the *T. maritima* has great biotechnological applications (CONNERS *et al*., 2006). Despite this, no studies were found relating the bioremediation capacity of this bacterium. Thus, this study may be indicating a new candidate for further studies.

4 FINAL CONSIDERATIONS

Environmental contaminants are a concern and have gained attention in recent years due to the consequences for the environment and human and animal health. Remediation means, methods, and technologies are needed to reverse this scenario. The use of bacteria to remediate polluted and/or contaminated sites is a great strategy. The molecular docking may represent a fast and low-cost way to aid in research for the bioremediation of hydrocarbon-contaminated locals, assisting in choosing the ideal bacterium to carry out the depollution and/or decontamination process. In this study, *S. aureus* and *T. maritime* bacteria, concerning binding on ABC transporters, demonstrated similar interaction abilities with toluene, indicating a similar capacity for bioremediation. The results presented in this study show that both species evaluated are promising for *in vitro* and *in vivo* studies for toluene bioremediation.

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How to quote this article

RANGEL, E. M.; SILVA, E. F.; MACAGNAN, K. L.; RIBEIRO, L. V.; CARDOSO, T. F.; GARCEZ, D. K. The use of bacteria for bioremediation of environments contaminated with toluene: a molecular docking analysis. **Ciência e Natura**, Santa Maria, v. 44, Ed. Esp. VI SSS, e17, 2022. DOI: 10.5902/2179460X68835.