IN SILICO STUDY OF ANTIVIRAL POTENTIAL OF BIOSURFACTANTS PRODUCED BY *Bacillus subtilis* UFPEDA 438 AGAINST SARS-CoV-2

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ABSTRACT

The present study aims to investigate the antiviral potential of surfactin and iturin produced by cultivation of *Bacillus subtilis* UFPEDA 438, using sugarcane molasses as a substrate, through *in silico* analysis, against the 4 main target proteins of SARS-CoV 2 being Papain-like protease (Plpro), Main protease C30 endopeptidase (Clpro), Spike protein and RNA-dependent RNA polymerase (RdRp). The results showed the greatest interaction of the studied biosurfactants was with the Spyke protein, which is crucial for the binding of the virus with the host human cell. The achieved results point to the extract produced as a promising constituent in the elaboration of products with antiviral activity against SARS-CoV-2.

Keywords: Biosurfactant. Iturin. Surfactin. Antiviral activity. Docking.

1 INTRODUCTION

Lipopeptides are biosurfactants, substances with a high surfactant potential where each family corresponds to a group of isoforms that differs in the peptide's composition and the length of the lipid chain. They are mainly produced by *Bacillus subtilis*. Among them, iturin and surfactin, lipopeptides with high surfactant and antibiotic potential, stand out[1]. As a lipopeptide, they are a set of isoforms, biosynthesized or engineered, where the relationship between their structures and properties helps choose and direct the product to the final application. The biosurfactants market, mainly divided into glycolipids and lipopeptides, was estimated to reach a turnover of 1.9 billion by 2027, with a CAGR (Compound Annual Growth Rate) of 11.2%. Such growth would be driven by the search for biodegradable surfactants, from renewable resources, and less toxic[2]. Sugar cane molasses, a by-product of sugar production, has high levels of fermentable sugars, around 30 to 40% (m/m) of sucrose, followed by smaller amounts of fructose, and glucose, and also pentosans and nitrogenous compounds, among others[3]. Therefore, sugarcane molasses has great potential to act as a substrate, serving as a source of carbon and energy for the production of biosurfactants, through submerged cultivation.

Surfactin has well-characterized antimicrobial activities, where its surfactant properties act, in most cases, disturbing or disrupting the integrity of the membrane of target cells[4] together with other biosurfactants produced by B. subtilis, namely: iturin, fengycin, lichenisin, mycosubtilisin and bacillomycin[5]. *In silico* studies through docking and molecular dynamics simulations have been a practical and valuable theoretical tool in several studies involving the interaction of surfactin with potential targets. However, 4 years after the pandemic caused by the SARS-CoV-2 virus, there are few studies in the literature that evaluate the use of surfactin and iturin as agents against the virus. Therefore, in the present study, the antiviral potential of a pre-purified extract, containing the lypopeptides (surfactin and iturin) produced by *Bacillus subtilis* UFPEDA 438, using molasses as substrate, against SARS-CoV-2, is evaluated based on an *in silico* investigation.

2 MATERIAL & METHODS

The biosurfactant production, recovery, and purification were carried out and it was possible to obtain an extract fraction containing up to 164.43 mg/L and 28.22 mg/L of surfactin and iturin, respectively[6]. Aiming to previously evaluate the interaction between lipopeptides extracted mainly from the cultivation of *B. subtilis* UFPEDA 438 and the main active proteins of the SARS-CoV-2 virus, and aiming to delimit likely targets to be attacked by them, thus resulting in antiviral activity, a total of four proteins were chosen based on the literature, namely the Papain-like protease (Plpro), the Main protease C30 endopeptidase (Clpro), the Spike protein and the RNA-dependent RNA polymerase (RdRp). The three-dimensional structures of the targets and the substance under study, surfactin, together with the second biosurfactant produced by *B. subtilis* strains, iturin, were collected in the PDB (Protein Data Bank) database[7] as indicated in Table 1. In the molecular dynamics study, it was observed that the 4OVZ and 6LU7 files present inhibitors in their structure, they were used to define the binding site and construction of the box grid centered on the coordinates of the PDB inhibitor, while the active site for Spike was demarcated based on the portion that is linked to angiotensin-converting enzyme 2 (ACE2). Finally, for RdRp, the active site was demarcated based on the catalytic domain of the target[14].

Table 1 Collection of structures by PDB for four SARS-CoV-2 proteins and biosurfactants whose in silico antiviral activity was evaluated in this study.

Target	Identifier	Author	Reference
Plpro	4OVZ	Báez-Santos et al. (2014)	(RCSB PDB, 2014)
3Clpro	6LU7	Jin et al. (2020)	(RCSB PDB, 2020)
Spike	6M0J	Lan et al. (2020)	(RCSB PDB, 2020)
RdRp	6NUS	Kirchdoerfer and Ward (2019)	(RCSB PDB, 2019)
Surfactin	2NPV	Tsan et al. (2006)	(RCSB PDB, 2006)
Iturin	2IH0	Volpon et al. (2006)	(RCSB PDB, 2006)

Such structures were then subjected to docking preparation by excluding solvents, adding hydrogen and electrical charges, and replacing the rotameter library with incomplete side chains. The molecular docking simulation between the ligand in the data set and the selected proteins was performed using Autodock Vina[15]. To analyze the fitting results in kJ/mol, the scoring function for empirical energy and ligand similarity was used to rank possible mechanisms of antiviral action. In this case, the UCSF Chimera program was used[16].

3 RESULTS & DISCUSSION

Through the molecular anchoring study with the main lipopeptides quantified in the purified sample, surfactin and iturin had their interactions with 4 proteins of the SARS-CoV-2 virus investigated, namely Papain-like protease (Plpro), C30 endopeptidase (Clpro), the Spike protein and the RNA-dependent RNA polymerase (RdRp), in order to evaluate the propensity for antiviral activity of the compounds. In the first stage, the RMSD (Root mean square deviation) value, an average measure of the distance between atoms of two or more macromolecules, was measured to validate the anchoring and interaction energy of existing inhibitors (EligPDB). For docking analysis, it was observed that all RMSD values were below 2.0 Å in redocking (Table 2), thus being considered valid for analysis with the study compounds

Table 2 RMSD value and energy values of the compounds and PDB inhibitors for each of the SARS-CoV-2 proteins studied.

Component	Plpror	3Clpro	RdRp	Spike
RMSD	0.83	0.91	---	---
PDN Inhibitor	-10.50[8]	-8.00[9]	---	---
Surfactin	-7.70	-6.00	-7.20	-8.60
Iturin	-6.70	-5.80	-5.80	-6.60

Table 2 provides information on the interaction energies of the compounds, the RMSD value for model validation based on the position of the active sites (Plpro and 3Clpro), and the region of action of PBD inhibitors 'Protein Data Base' for Spyke and RdRp and the interaction energy of these inhibitors (EligPDB). It is observed that the PDB inhibitors for the Plpro and 3Clpro proteins presented higher energy values than iturin and surfactin, compared to the studied lipopeptides, for Plpro conventional hydrogen bonds between iturin and its Alanine residues (Ala250) were observed.), Glutamine (Gln267), Tyrosine (Tyr289), and Proline (Pro249), in addition to a water bond, even so the energy value for surfactin was better, due to the large number of bonds with the hydrophobic aliphatic portion of the structure, providing alkyl-type interactions with the amino acid residues Leucine (Leu163), Tyrosine (Tyr265), Proline (Pro249 and Pro248) and pi-sigma bonds with the amino acid residue Tyrosine (Tyr269). Bonds with water molecules were also observed in their anchoring with surfactin. These same interactions were observed for the target's PDB ligand, which presents a large number of alkyl bonds, hydrogen bonds, and phi-phi bonds.

Among the targets studied, the lipopeptides produced here showed better interaction with the Spike protein of SARS-CoV-2, highlighting iturin as the most active candidate when compared to surfactin, with -8.6 kcal/mol and -6.6 kcal/mol, respectively. This difference in energy is observed due to the greater number of hydrogen bonds with the residues of alanine (Ala387), arginine (Arg403 and Arg408), asparagine (Asn33), lysine (Lys417), glycine (Gly416), glutamine (Gln409), and glutamic acid (Glu406) in the formation of Spike complex with iturin. On the other hand, surfactin has three hydrogen bonds with glycine (Gln409) and the same arginines (Arg403 and Arg408) in the carboxylic acid fragments. Alkyl-type bonds also occur in the most hydrophobic regions of the structure, highlighted by interactions with the amino acid residues lysine (Lys26), proline (Pro389), and alanine (Ala387) as illustrated in Figure 1. Previous studies simulated seven different lipopeptides, including surfactin, anchoring them individually against the Spyke (S)-glycoprotein of SARS-CoV-2, finding a free energy of -539.61 kcal between surfactin and the protein, characterizing it as a good agent for inhibiting this target[18].

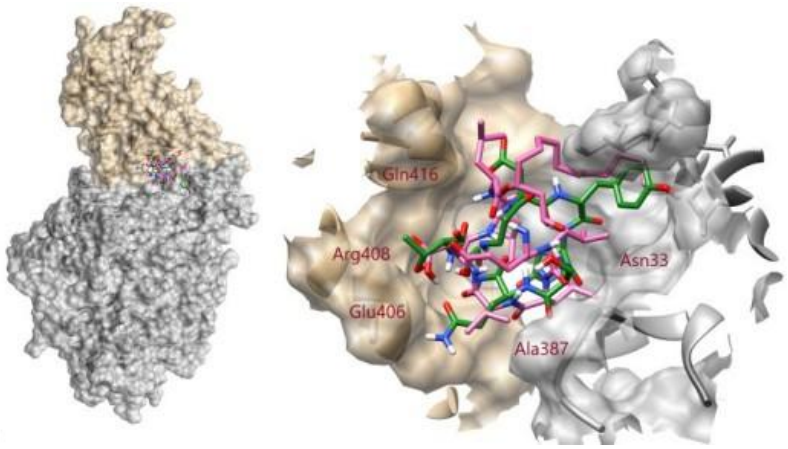

Figure 1 Anchorage and amino acid residues involved in the formation of the complex of compounds with Spyke proteins and ACE2. The figure highlights the target as the Spike protein (gold) and ACE2 (silver), iturin (green), and surfactin (pink).

4 CONCLUSION

The lipopeptides surfactin and iturin produced by *B. subtilis* UFPEDA 438 showed antiviral potential against SARS-CoV-2. The best interaction studied in silico of lipopeptides was with the Spike protein, with ituran showing a better interaction, free energy of -8.6 kcal/mol, than surfactin, which showed an energy of -6.6 kcal/mol.

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ACKNOWLEDGEMENTS

The authors thank CAPES (Finance Code 001) and CNPq for supporting this study.