FDA-Approved Drugs that can Prevent Cytokinesis of the *Caulobacter crescentus* Bacteria

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ABSTRACT

The overuse of antibiotics in recent years has resulted in a development of antibiotic-resistant bacteria. There are two potential solutions to overcome this problem. New drugs can be developed, but this takes considerable time and money. Old drugs can also be remade and reused, and bacteria may have a harder time becoming resistant. The energy of interaction between protein FtsZ (found in *Caulobacter crescentus* bacteria) and 7,409 Food and Drug Administration (FDA) approved drugs was calculated using a Python code, where negative values represented high energy of interaction and positive values, low energy of interaction. The drugs that had the highest energy of interaction are recommended for further experimentation.

KEYWORDS: Antimicrobial resistance, Superbugs, Antibiotics, *Caulobacter crescentus*, FtsZ Protein, Energy of Interaction

INTRODUCTION

Since the post-antibiotic era, "superbugs" have become a rising threat to treating diseases and infections. Superbugs is a term used to describe strains of bacteria or fungi that are resistant to most antibiotics used today. Resistant bacteria that cause pneumonia, skin infections, etc. are just some of the dangers we now face (Dugassa and Shukuri, 2017). Antibiotic resistance is a natural process of evolution that happens over time when bacteria slowly adapt or mutate to ensure their survival against drugs that are meant to kill them. Kapoor et al. (2017) reviewed the resistance mechanisms.

Developing new drugs and replacing existing antibiotics is not a good solution, because new drugs require lengthy clinical trials and toxicity tests, and approval is a complex procedure—the FDA only approved 1-3 new medications per year in the last 50 years (Figure 1). Also, it may not take very long for bacteria to become resistant to these new drugs, and the range of medications we can still use will get smaller and smaller.

An important characteristic of an antimicrobial drug is selective toxicity; it discerns the microbial target from host cells and only kills or prevents growth in the microbes while causing little to no harm to the host. Most of the drugs going through clinical trials are antibacterial because bacteria provide a better target variety for selective toxicity, compared to fungi or viruses. Each category of antibacterial drugs has a unique way to affect the microbes (Kirmusaoglu et al., 2019). Antibiotics can slow down or stop the growth of bacteria by targeting the cell wall or membrane. They also target protein and nucleic acid synthesis. Protein synthesis is performed by ribosomes which are nucleoprotein (nucleic acid bonded to

protein) complexes that are made up of a small and large subunit. Antibiotics can also work as antimetabolites by blocking the folate metabolism (therefore DNA synthesis) in a pathway that involves para-aminobenzoic acid (PABA) and two acids that help make folic acid: dihydrofolic acid (DHF) and tetrahydrofolic acid (THF). Antibiotics can block DNA gyrase, which

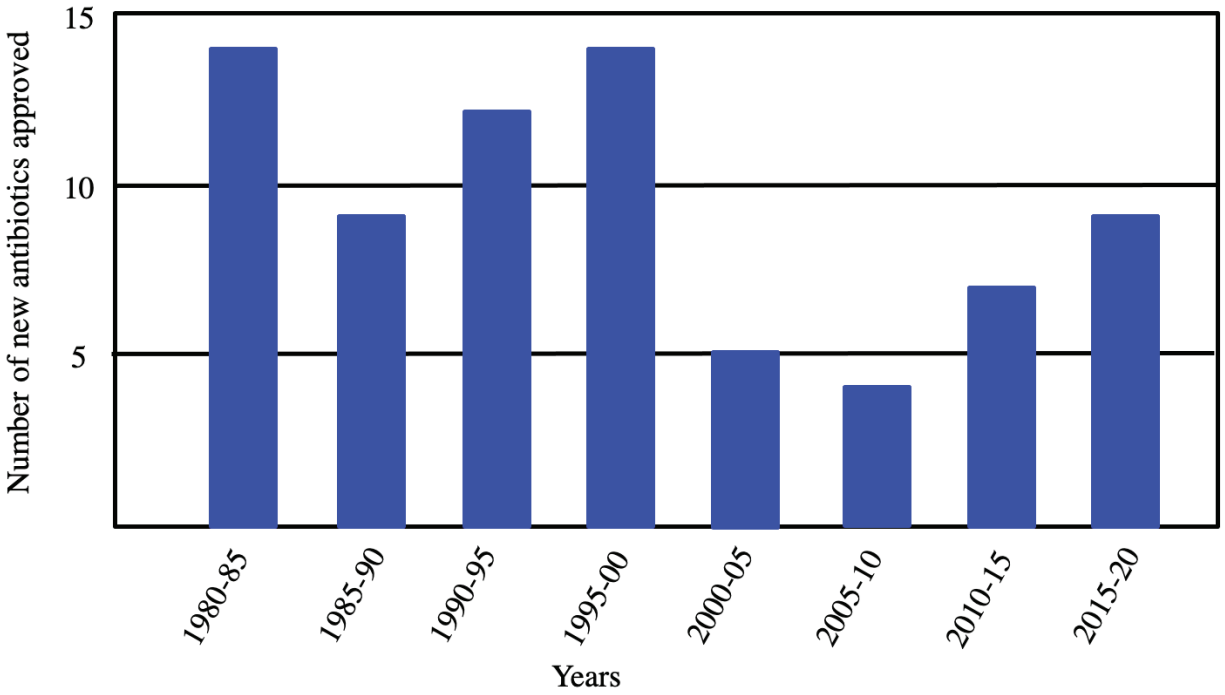

Figure 1. Number of new antibiotics approved by FDA (using data from the Wall Street Journal).

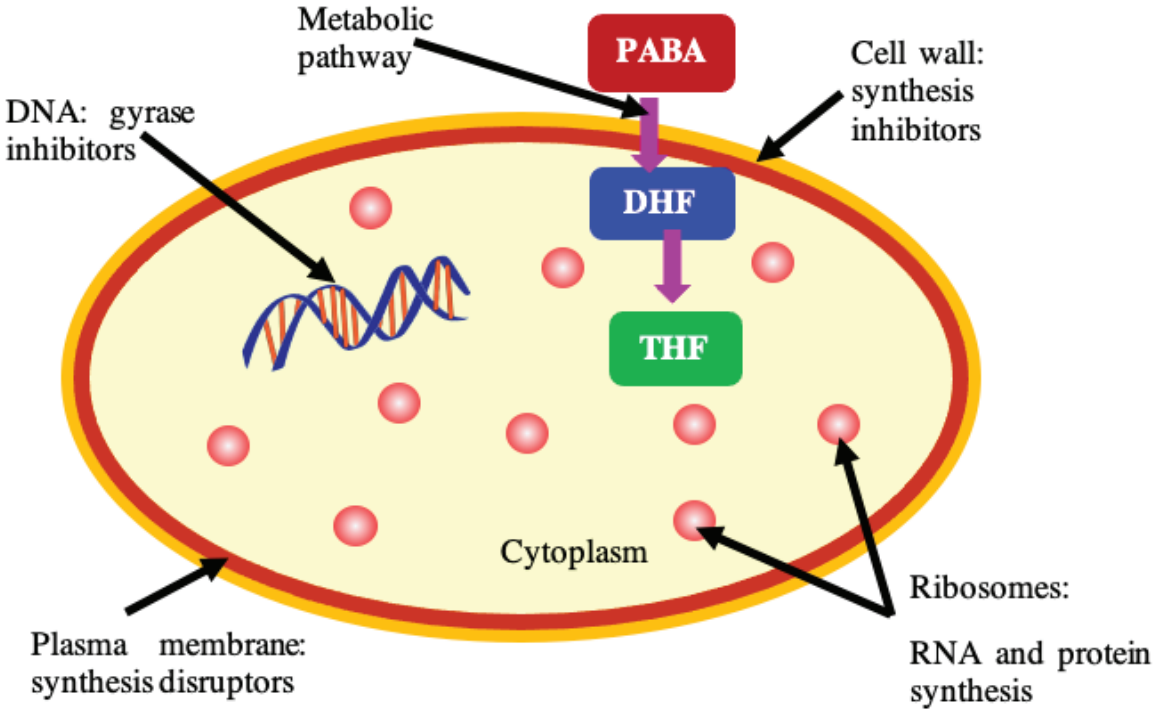

Figure 2. Mechanisms of work of antibacterial drugs.

is an enzyme that modifies the molecular arrangement of DNA, playing a role in replication and transcription. Figure 2 illustrates these mechanisms.

Antimicrobial resistance (AMR) occurs when a strain of bacteria resists antibiotics that usually prevent or slow their growth, which allows them to resist drugs. Since the 1990s, bacteria have been growing more harmful because of resistance genes (resistomes), which allow them to become resistant to various antibiotics. Today, many bacterial pathogens are resistant to antibiotics because they get or create antimicrobial-resistant genes, found mainly on plasmids and chromosomes. There are various methods like conjugation, transformation, and transduction when vulnerable strains can get resistance genes with transposons that support different resistance genes to combine with host chromosomes or plasmids. There are currently four basic mechanisms of bacterial drug resistance (Kapoor et al., 2017): target alteration, change to membrane permeability, efflux pump, and antibiotic degradation via enzymes (Figure 3).

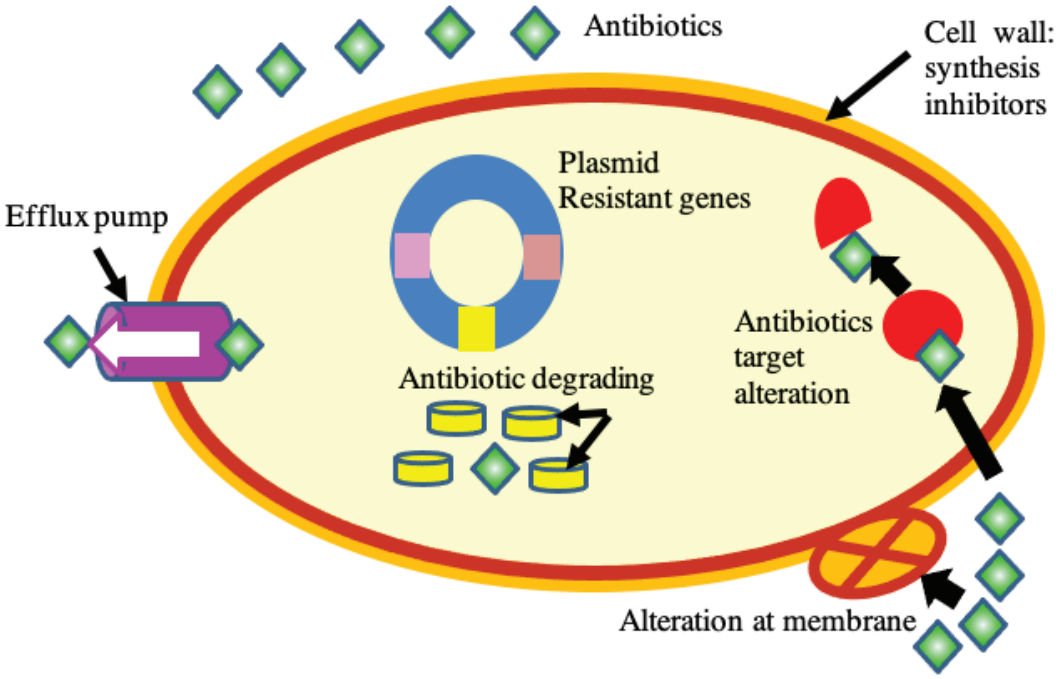

Figure 3. Four mechanisms of drug resistance: target alteration, change to membrane permeability, efflux pump, and antibiotic degradation via enzymes.

As superbugs become a bigger threat, the problem of treatment and developing new drugs is arising. New drugs that can effectively fight bacterial infections and diseases are needed. Developing drugs that can kill the bacteria is not the best approach. Instead, we need to slow down or even stop bacterial growth, while discontinuing the use of antibiotics, because they have little to no effect on the bacteria anymore.

This paper proposes an alternative approach that is based on the observation of the role of the protein FtsZ (Figure 4) during cytokinesis of the bacteria *C. crescentus* (de Boer et al., 1992). This protein moves to the middle of the cell during division and assists in cytokinesis. For this project, the ability of various inhibitors to block the active site of FtsZ was investigated. If the active site is blocked, the protein cannot participate in the bacterial division.

The principal work here is to calculate the energy of interaction between FtsZ and several inhibitors. The inhibitors were 7409 FDA-approved drugs that are not used as antibiotics- the bacteria could have resistance against it. Information about these drugs is taken from Kyoto Encyclopedia of Genes and Genomes (KEGG) Database (LigandBox) (Kawabata et al., 2013) because this database has the widest range of publicly available information about the drugs. It is convenient to use *C. crescentus* as a model bacterium because it is easy to track its cell division: each stage of the division is unique and contrasting. Using this kind of approach for various other bacteria could be a key for finding or developing drugs that bacteria aren't resistant to and will take a long time to develop resistance.

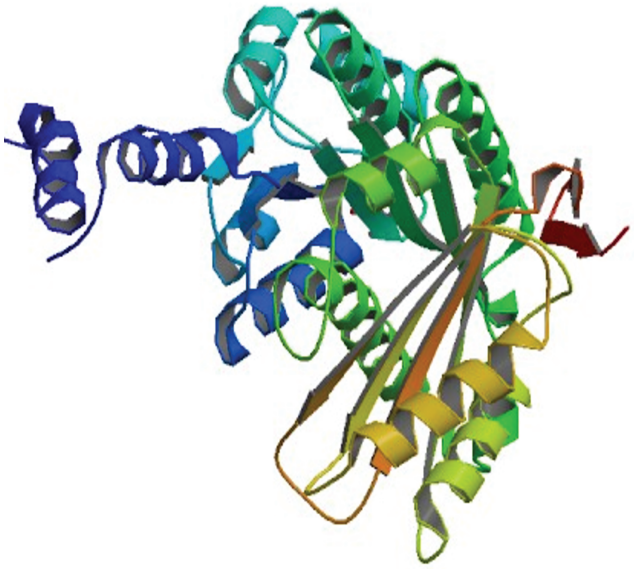

Figure 4. Crystalline structure of filamenting temperature-sensitive mutant Z (FtsZ) protein (PDB code: 1W59; image generated by PyMOL).

MATERIALS AND METHODS

To calculate the energy of interaction, data from FDA-approved drugs provided by KEGG, which is a collection of databases dealing with genomes, biological pathways, diseases, drugs, and chemical substances, was used. The KEGG DRUG database contains information about the active ingredients of approved drugs in Japan, the USA, and Europe. Part of this database is used in LigandBox (Kawabata et al., 2013), a 'ready-to-dock' database of small chemical compounds for virtual drug screening on computer docking studies. The database is downloadable in the form of a MOL2 file and, in December 2019, contained 7,409 records. The records were transformed to a PDBQT file using PubChem, an open-access database of chemical molecules and their activities against biological assays. The system is maintained by the National Center for Biotechnology Information which is part of the United States National Institute of Health (NIH). PubChem contains substance descriptions and small molecules with fewer than 1000 atoms and 1000 bonds. The database is growing continually. The energy of interaction between 7,409 ligands available in Ligand Box and FtsZ protein was calculated using the AutoDock Vina program which is a suite of automated docking tools designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of a known 3D structure. It is an open-access program developed by the Molecular Graphics Lab at The Scripps Research Institute, La Jolla, CA. Each molecule available in the Lig and Box was placed at the active site of the FtsZ protein in the Autodock Vina code. After the energy of interaction was calculated, the result was recorded in the output file. Finally, all the records in the output file were ranked by energy value. For this procedure, a Python utility program was written.

Python Code

The algorithm of the Python utility code is given in Figure 5.

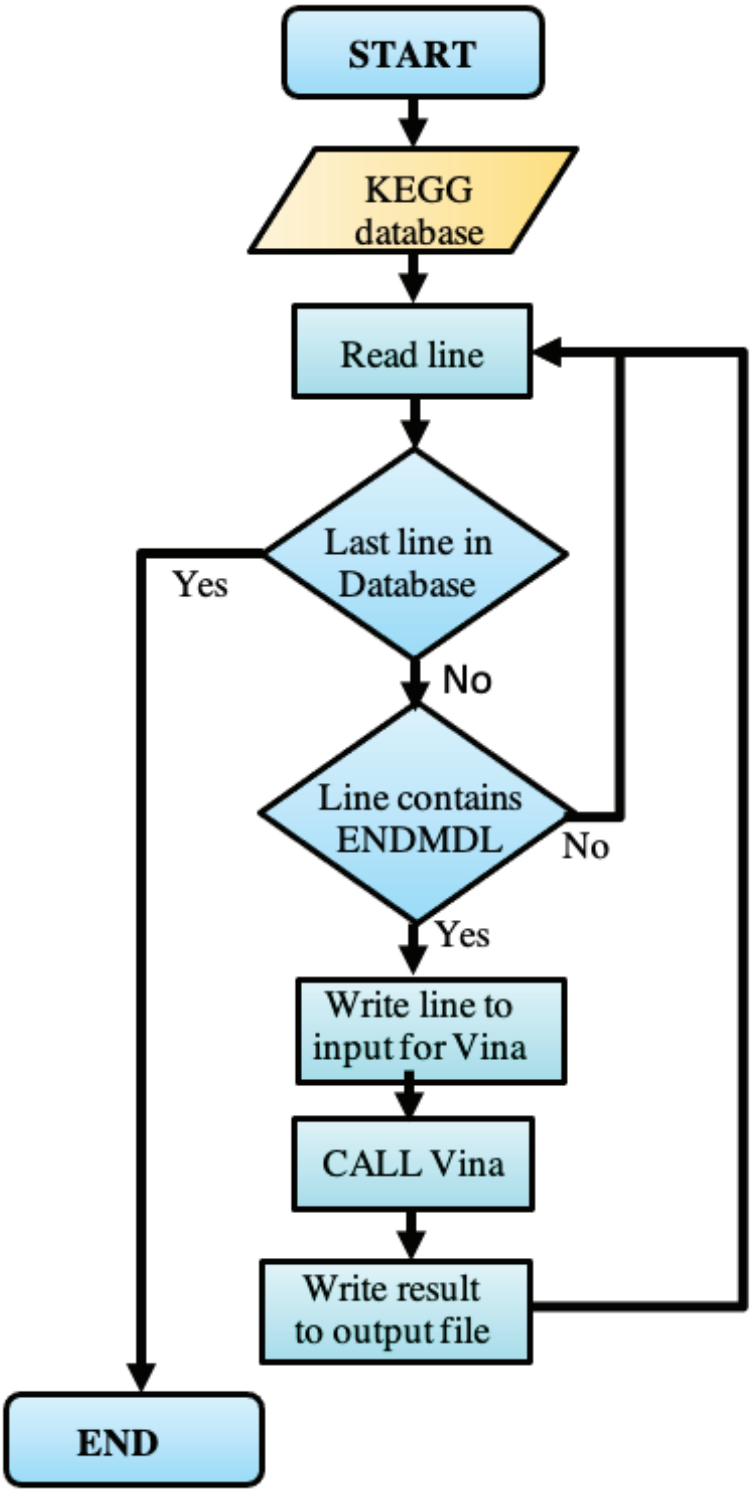

Figure 5. Each line in the KEGG database was analyzed. If the line contained ENDML, that line became the input for the AutoDock Vina program. This program was then called, and the result was written for the output file.

Docking the Inhibitors

The energy of interaction between FtsZ and the inhibitors was calculated with Autodock Vina. Negative energy corresponds to the attraction between the inhibitor and protein. Autodock Vina considers Coulombic forces between the atoms as well as Van der Waals's forces. The input data includes pdb files with the crystalline structure of the FtsZ protein taken from the PubChem database, the configuration file with the approximate coordinates of the active site, and the size of the optimization box that binds the space for the search of the optimal position of the ligand, and the pdb file with the molecule of the ligand read from the KEGG database. The resulting energy change due to the presence of the inhibitor is given as the function of the ligand orientation angles. Those with maximal absolute value were used. Autodock Vina gives the calculated changes in free energy in kcal/mol-1 (Table 1).

Table 1 shows results of the calculations for the twenty drugs which showed the highest energy of the bound state for the FtsZ–ligand complexes. (-11 kcal mol^{-1} or higher). For reader convenience, the KEGG number of each drug (taken from the database), the chemical formula, international ID, and the name used in the pharmaceutical industry are provided.

KEGG number	Energy (kcal/mol^{-1})	Chemical formula	International ID	Name
00006725-01	-12.1	C35H34N6O3F	D08981	Quarfloxin
00004026-01	-12	C35H30N4O4	D05029	Midostaurin
00005945-01	-11.6	C20H10N3O3F5Cl2	D07964	Fluazuron
00006874-01	-11.6	C22H24N6O2	D09610	Emicerfont
00004241-01	-11.4	C21H16N2	D05359	Paranyline hydrochloride
00007306-01	-11.3	C26H17N3O3F9Cl	D10361	Afoxolaner
00007374-01	-11.3	C33H39N7O4F2	D10465	Golvatinib tartrate
00006706-01	-11.3	C28H22N7OF3	D08953	Nilotinib
00007202-01	-11.2	C24H17N2O5F2	D10134	Lumacaftor
00007108-01	-11.2	C30H40N4O4F	D09981	Ulimorelin
00003390-01	-11.2	C25H21N4O4	D03978	Eltrombopag olamine
00006583-01	-11.1	C20H15N4O3F3	D08654	Trovafloxacin
00004025-01	-11.1	C18H13N3FCl	D05028	Midazolam maleate
00001979-01	-11.1	C20H15N4O3F3	D02123	Trovafloxacin mesylate
00000511-01	-11.1	C18H13N3FCl	D00550	Midazolam
00002557-01	-11.1	C30H22N4O4	D02773	Adozelesin
00000653-01	-11.1	C18H13N3FCl	D00696	Midazolam hydrochloride
00007109-01	-11	C30H40N4O4F	D09982	Ulimorelin hydrochloride
00004251-01	-11	C25H23N4O4Cl	D05378	Pazinaclone
00002865-01	-11	C41H50N6O2	D03249	Bisoctrizole

Table 1. The results of the calculations for the twenty drugs that showed the highest energy of the bound state for the FtsZ–ligand complexes. (-11 kcal mol^{-1} or higher).

RESULTS

The docking results show that the FtsZ molecule forms very stable complexes with the drug molecules listed in Table 1. This means that the drug molecules can only be in the free stage if all of the FtsZ molecules are already occupied. FtsZ binds to ligands unless it finds other molecules that might form more stable complexes, i.e. a molecule that has a higher energy of interaction with FtsZ (attraction is represented by a negative sign, while repulsion is positive). This means that these drugs can block FtsZ which assists in the cytokinesis of *C. crescentus* (Figure 6) and, therefore, can be used as potential antibiotics against this bacteria.

To verify this hypothesis, a follow-up experimental study is required. It will be necessary to break apart and examine the DNA of the *C. crescentus* bacteria that contains the protein FtsZ, insert the drug into the DNA, and examine its effects on the division of the bacteria. This experiment was not done because the COVID-19 pandemic prevented the author from working in the lab. Using this kind of approach for various other bacteria could be the key to finding or developing drugs that bacteria aren't resistant to and will take a long time to develop resistance against.

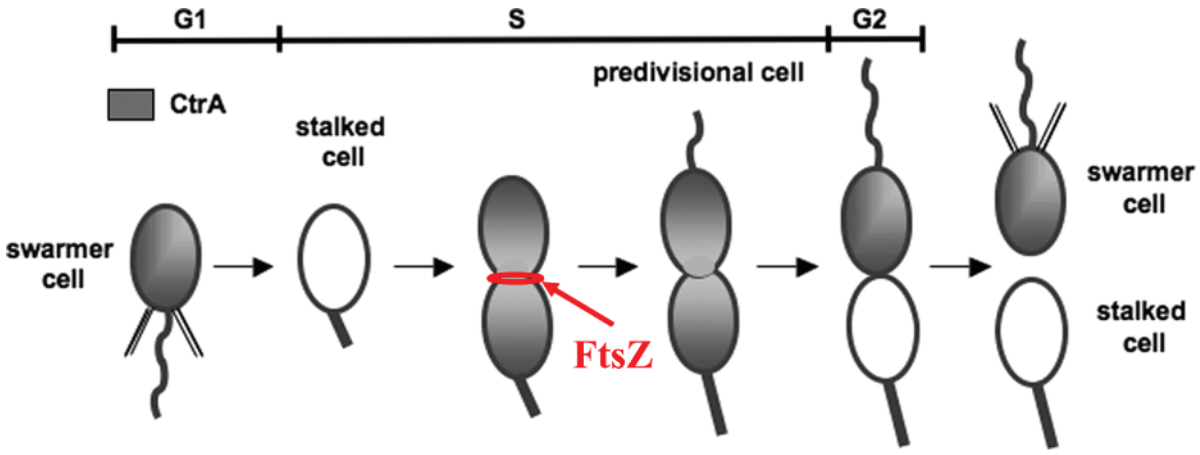

Figure 6. A schematic diagram of the *C. crescentus* cell cycle. (England et al., 2010).

CONCLUSIONS

The paper identified FDA-approved drugs that form stable complexes with FtsZ, a protein that assists in the cytokinesis of the bacteria *C. crescentus*. While this bacterium is mostly used, for example, as an important model organism for studying the regulation of the cell cycle, the proposed approach can be applied to other bacteria as well and could be a key for finding or developing drugs that bacteria aren't resistant to.

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