Research Article

Mechanism of Inhibition of Growth of *E. coli* MTCC 1610 by Bisphenol S – An *in-silico* Study

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ABSTRACT

Present day's plastic industry is using Bisphenol S (BPS) as a safe replacement of Bisphenol A (BPA) due to its hazardous effects on mankind. Bisphenol S is considered as an Endocrine Disrupting Chemical (EDC), and its extensive use might exert adverse effects on human health due to its potential toxicity. Humans are often exposed to BPS through consumption of BPS-contaminated foods and drinking water; and the gastrointestinal system gets primarily exposed to BPS on consumption. The BPS on exposure might alter the gut microbial homeostasis and impair the proper functioning of the gut microbes. Further, the production of bioactive compounds like vitamins, amino acids, and lipids relies heavily on the gut microbiota. At present, there is no report available on the effect of BPS on *E. coli*, the most widely studied gut microorganism. In this study, the probable mechanism of action of BPS on the inhibition of growth of *E. coli* has been done through molecular docking analysis of BPS with FtsZ (Filamentous temperature sensitive mutant Z) protein having PDB ID of 6UNX. FtsZ protein is having crucial role in bacterial cell division.

Keywords: Bisphenol S, *E. coli*, Drug Likeliness, *In silico* Toxicity, FtsZ Protein, Molecular Docking.

INTRODUCTION

The scientific community has taken an interest in the endocrine-disrupting effects of bisphenols on human health due to their wide application in industries like plastic manufacturing, food packaging, pharmaceuticals, and personal care products. The plastic industry is currently using Bisphenol S (BPS) as a safe alternative to BPA in the production of clear plastic products[1].

Due to its greater thermal stability and decreased estrogenic activity compared to BPA, BPS has been considered as a substitute of BPA because it is less likely to get contaminated with the foods or substances by thermal leaching. However, BPS also seeps into food from storage containers, much like BPA does. Human exposure to BPS is confirmed by the presence of BPS in urine. The most likely route of exposure of BPS exposure into humans is from food consumption contaminated with the BPS [2-5].

According to toxicological studies, BPS exposure as low as 2 mg/kg in rats and mice results in impairment in the male reproductive system through decreased spermatozoa production and motility as well as changes to testicular histology[3,6]. According to other studies, BPS exposure as low as 1.5 µg/kg bw/day alters male thyroid hormone levels and induces obesity[7]. BPS has been observed to inhibit the contractile activity of the duodenal visceral smooth muscle (dVSM) and induces oxidative stress in dVSM[1].

There are various ways in which microbiota can impact biological processes. The microbiota is essential for the extraction of nutrients and energy from food because of its diverse metabolic genes, which produce distinct enzymes and biochemical pathways on their own[8]. Furthermore, the gut microbiota is crucial for the creation of bioactive substances like lipids, vitamins, and amino acids[9]. In terms of the immune system, the human microbiota plays a vital role in the development of the intestinal mucosa and immune system in addition to creating antimicrobial compounds that shield the host from foreign pathogens.

The FtsZ protein is essential for cell division in the majority of bacteria, including *E. coli*. FtsZ self-polymerizes in a GTP-dependent manner to produce proto-filaments and larger bundles thereof, which eventually congregate into a discontinuous ring-like structure at the internal side of the cytoplasmic membrane to check the planned location of cell division[10-11]. This structure serves as the scaffold for adhering proteins within the cytokinetic device.

Any exposure to environmental toxicants, might alter the gut microbial homeostasis and led to dysbiosis and can lead to onset of various diseases[12].The detrimental effects of Bisphenol S on intestinal microflora has not been studied till date so far. *Escherichia coli* is the most beneficial and frequently studied intestinal microflora. However, the effect on the growth of *E. coli* on BPS exposure has not been reported till date. Therefore, this study was carried on the *in silico* drug likeliness, toxicity prediction of BPS and the effect of BPS at the initial and mid-logarithmic phase of growth of *E. coli*. Further, to find possible mechanisms of BPS induced growth inhibition of *E.coli*, we performed molecular docking analysis of BPS with FtsZ (filamentous temperature-sensitive mutant Z) protein having PDB ID of 6UNX.

MATERIALS AND METHODS

In silico Drug likeliness and Toxicity

Drug likeliness of BPS was determined on the basis of Lipinsky's rule of five by using molinspiration and SwissADME server. For this purpose, canonical SMILES (Simplified Molecular Input Line-Entry System) formula of BPS was used. *In silico* toxicity of BPS was determined by using ToxiM server.

Determination of Effective Dose of BPS on *E. coli*

Effective dose of BPS on *E. coli* was determined by using spot test method with BPS concentrations of 50, 100, 200, 400 and 700 µg/ml. Sterile distilled water was used as control and DMSO was used as vehicle control. Experiments were carried out aseptically on sterile glass slides. The slides were then put inside the sterile Petri plates and were incubated at 37°C for overnight. All the experiments were done in triplicates.

Growth Curve of *E. coli*

Effect of BPS was studied by addition of BPS concentrations of 300, 500 and 700 µg/ml at the initial stage of the growth curve of *E. coli* and at the mid log phase in separate experiment. 50 ml of sterile nutrient broth was taken in 250 ml nephelometric flasks. 500 µl fresh nutrient broth culture of *E. coli* MTCC1610 was added in each flask. Then the flasks were placed at shaker incubator at 37°C with a rpm speed of 120. Untreated *E. coli* culture flask was considered as control. Reading was taken at ƛ = 610 nm with the help of a colorimeter at an interval of one hour. Reading was taken until stationary phase was reached.

In silico Pharmacokinetics and Toxicity Prediction of Bisphenol S (BPS)

The pharmacokinetic aspects, drug likeliness, and toxicity prediction of BPS were detected using four webservers: molinspiration, pkCSM, SwissADME, and ToxiM. These web resources suggest both predicted regression analysis and molecular similarity for the given molecule. The BPS median lethal dose (LD_{50}) was calculated with the Pro Tox-II server.

In silico BPS-FtsZ Protein Interaction through Molecular Docking

An online cavity-detection guided blind docking method was employed for 6UNX binding site detection with BPS, CB Dock2 [13]. The modified pdb file as receptor and BPS as ligand in SDF format, previously downloaded from PubChem database (CID: 6626) (https://pubchem.ncbi.nlm.nih.gov), were uploaded in CB Dock2 server (https://cadd.labshare.cn/cb-dock2/index.php), in order to study the probable mechanism of action of BPS on the inhibition of growth of *E. coli*. The natural ligand-bound to the protein 6UNX and water molecules were manually removed from the pdb file.

A molecular docking study was conducted using the free version of the Mcule.com (https://www.mcule.com) web platform. This program offers a well-curated chemical database and an excellent online drug discovery tool. Default settings were employed for the purpose of screening. The online servers ToxiM (http://metagenomics.iiserb.ac.in/ToxiM), SwissADME (https://www.swissadme.ch), and Molinspiration (https://www.molinspiration.com) were used for *in silico* toxicity analysis and drug likeliness analysis of the ligand using Lipinsky's rule of five with '0' violation. A ligand that has been virtually screened and filtered can expedite the virtual screening procedure and conserve online tool limit.

The binding capability of the filtered ligand against the *E. coli* cell division protein FtsZ was virtually tested. From the protein data bank (https://www.rcsb.org/), the crystal structure of the *E. coli* cell division protein FtsZ with PDB ID: 6UNX was obtained. *E. coli* cell division protein FtsZ was stripped of its attached ligand and water molecules by erasing it from a notepad file in pdb format belonging to 6UNX. Next, a pdb file containing the *E. coli* cell division protein FtsZ crystal was uploaded to the docking (vina) platform. The AutoDock Vina online program automatically processed the receptor by adding polar hydrogen atoms and Gasteiger charges after receiving the receptor protein upload. A 20Å × 20Å × 20Å binding site region was utilized, having coordinates of (X: 22, Y: - 3, Z: 19). The final compounds were sorted according to how little energy they needed to bond to the 6UNX crystal.

Docking Analysis

For the least binding energy pose, the produced protein-ligand complex was stored as a pdb file. After that, the protein-ligand complex was shown using the Biovia Discovery Studio Visualizer (https://www.3dsbiovia.com) so that the types of interactions and docking confirmation could be examined. Ramachandran plot analysis was performed on the docked protein-ligand complex (https://saves.mbi.ucla.edu). The PDBsum database (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html) was used to investigate the protein-ligand interaction, and LigPlot was created to display the 2D interaction for hydrogen bonds between amino acid residues and BPS.

Statistical Analysis

One-way ANOVA at 95% level of confidence was done for bacterial growth curve analysis by using GraphPad Prism version 8.4.3. P <0.05 was considered as significant.

RESULTS AND DISCUSSION

The *E. coli* MTCC 1610 growth curve analysis (Figure 1) demonstrates unequivocally how BPS inhibits growth. It lengthens the bacterium's generation period. This demonstrates that cell division is restricted significantly in a dose response manner. From the growth curve of *E. coli* MTCC 1610 with graded doses of BPS and one-way ANOVA analysis it is clearly seen that after two hours of addition of

BPS at the initial phase of the experiment, there is no significant difference in the growth. But from three hours of incubation, control culture enters into the log phase where as other treated cultures remained in the lag phase. From the fourth hour, culture with 300 µg/ml BPS was entered into the log phase but others did not. From eighth hours of incubation, bacterial culture with 500 µg/ml BPS was entered into the log phase. Bacterial culture with 700µg/ml BPS never entered into the log phase. There is significant difference in the growth curve pattern of bacterial culture with graded doses of BPS long with control culture. This study clearly indicates that BPS somehow interferes the cell cycle of *E.coli* by hampering the proteins regulating cell division.

For the addition of BPS at the mid log-phase of growth curve, there is significant difference in the growth with graded doses of BPS, but not so much significant as BPS was added at the initial phase of growth curve of *E. coli.*

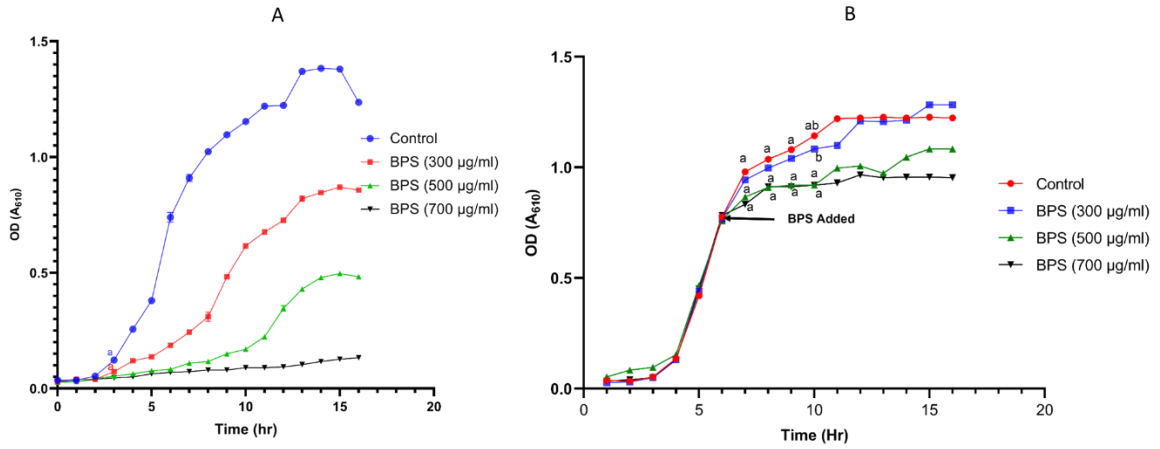

Figure 1: Effect of BPS on the growth of *E. coli* MTCC 1610 on application of graded doses of BPS. (A) represents the growth curve of *E. coli* MTCC 1610 where doses of BPS were added at the initial phase of growth curve and (B) represents the growth curve of *E. coli* MTCC 1610 where doses of BPS were added at the mid-log phase of growth curve.

Table 1: Chemical properties of Bisphenol S as obtained from PubChem

Molecular Formula	M.W. (g/mol)	LogP	PSA (Å^2)	H-bond acceptor	H-bond donor
$C_{12}H_{10}O_4S$	250.27	1.93	98.702	4	2

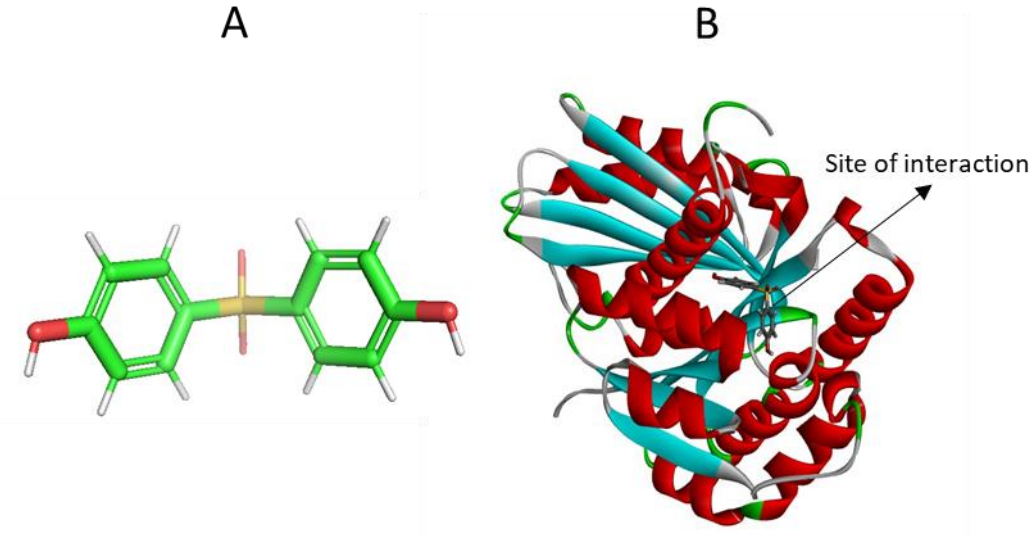

Figure 2: Structure of BPS (A) and Crystal structure of *E. coli* cell division protein FtsZ bound with BPS, visualized with Biovia Discovery Studio Visualizer (B)**.**

The characteristic features of BPS (Table 1) as virtually tested against the *E. coli* FtsZ protein are displayed in Figure 2. The calculated values for the minimal binding energy, pharmacokinetics, and toxicity characteristics were shown in Table 2 where it has been shown that -6.8 Kcal/mol was the lowest binding energy for BPS. These chemical exhibits lead likeliness, a high proportion of intestinal adsorption, and a relatively small volume of dispersion, all in accordance with Lipinsky's rule of five. This substance is not mutagenic and passes the simulated AMES test.

Table 2: Predicted minimum binding capacity, pharmacokinetics features and toxicity profile for Bisphenol S screened against *E. coli* FTsZ protein (PDB ID: 6UNX)

Property	Compound (BPS)
PubChem ID	6626
Canonical SMILE formula	C1=CC(=CC=C1O)S(=O)(=O) C2=CC=C(C=C2)O
Average binding energy (Kcal/mol)	-6.8
Rule of five violations (Lipinsky)*	0
Water solubility Numeric (log mol/L)	-2.817
Lead likeliness	Yes
Bioavailability score	0.55
Steady state volume of distribution (VD_{ss}) (log L/Kg)	0.154
Percentage of intestinal absorption (Human)	93.977
Total clearance (log ml/min/kg)	0.675
Ames Test (Mutagenicity)	No
Predicted lethal dose (LD_{50}) in rat (mg/kg)	1600
Predicted Toxicity through ToxiM#	0.95

Lipinski's Rule of Five* (Through MolInspiration: www.molinspiration.com):

a. Molecular Weight of the Compound: <500 Da

b. LogP Value: <5

c. No. of Hydrogen Bond Donors: <5

d. No. of Hydrogen Bond Acceptors: <10

#ToxiM Score: Classification score of any molecule with score greater than or equal 0.8 can be considered as Toxic. (http://metagenomics.iiserb.ac.in).

The median lethal dose (LD_{50}) of BPS is rather high. Figure 2 displays the docking image of FtsZ protein (6UNX) and BPS in order to examine the drug interaction with the protein to observe the BPS induced inhibition of the growth of *E. coli*. From the analysis, it is suggested that BPS might inhibit the bacterial growth by binding with bacterial cell divisional pacemaker protein- FtsZ.

The LigPlot picture (Figure 3) certainly demonstrates that BPS participates in both Pi hydrophobic interaction with arginine 142 and traditional hydrogen bonding with glycine 20, asparagine 24, and asparagine 186, respectively. The 307 residues in the docked protein-ligand complex, 255 are non-glycine and non-proline, according to Ramachandran plot analysis (Figure 4) and the 241 residues among non-glycine and non-proline fall in most favoured region (94.5%).

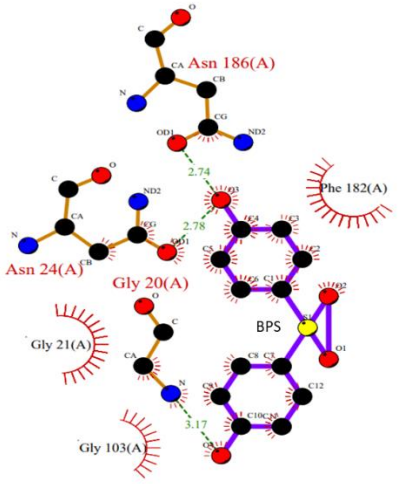

Figure 3: LigPlot of Protein-ligand to show hydrogen bonding between amino acid residues and BPS.

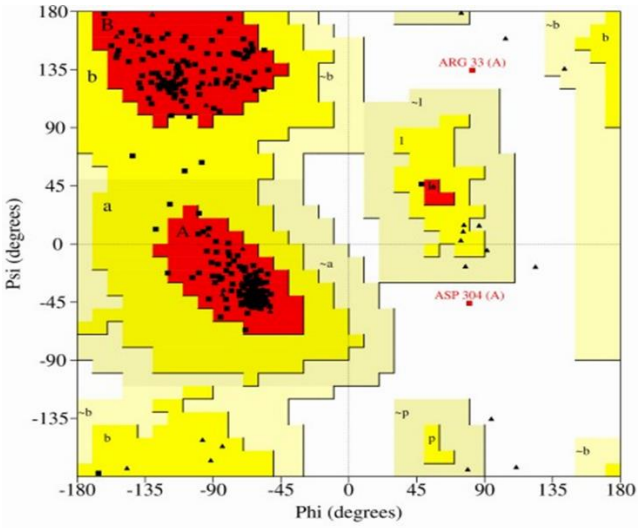

Figure 4: Ramachandran Plot analysis of *E. coli* FtsZ protein-BPS complex.

Table 3: Analysis of Ramachandran Plot of FtsZ-BPS docked complex.

Characteristics	No. of Residues	Percentage (%)
Most favoured regions [A,B,L]	241	94.5%
Additional allowed regions [a,b,l,p]	12	4.7%
Generously allowed regions [~a,~b,~l,~p]	0	0.0%
Disallowed regions [XX]	2	0.8%*
Non-glycine and non-proline residues	Total = 255	Total =100.0%
End-residues (excl. Gly and Pro)	4	
Glycine residues	41	
Proline residues	7	
Total Number of Residues	307	

CONCLUSION

This study indicates that BPS is interfering in the cell division of *E. coli* MTCC 1610 likely by interacting with cell divisional key protein FtsZ as observed from the *in-silico* study. Hence, from the study it is expected that BPS might inhibit the growth of *E. coli* and other gut microbes on exposure and may disrupt the gut microbial homeostasis inducing several health complications.

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REFERENCES

1. Roy D, Dutta M, Mondal M, Sarkar K, Paul G. Effect of Bisphenol S (BPS) on the contraction of duodenal visceral smooth muscle *ex vivo* in rat. Sci Arch. 2021; 2:99-108.
2. Yang Y, Guan J, Yin J, Shao B, Li H. Urinary levels of bisphenol analogues in residents living near a manufacturing plant in south China. Chemosphere. 2014 Oct 1;112:481-6.
3. Ullah H, Jahan S, Ain QU, Shaheen G, Ahsan N. Effect of bisphenol S exposure on male reproductive system of rats: A histological and biochemical study. Chemosphere. 2016 Jun 1;152:383-91.
4. Bousoumah R, Leso V, Iavicoli I, Huuskonen P, Viegas S, Porras SP, Santonen T, Frery N, Robert A, Ndaw S. Biomonitoring of occupational exposure to bisphenol A, bisphenol S and bisphenol F: A systematic review. Science of the Total Environment. 2021 Aug 20;783:146905.
5. Cao M, Wei J, Pan Y, Wang L, Li Z, Hu Y, Liang Y, Cao H. Antagonistic mechanisms of bisphenol analogues on the estrogen receptor α in zebrafish embryos: Experimental and computational studies. Science of the Total Environment. 2023 Jan 20;857:159259.
6. Lehmler HJ, Liu B, Gadogbe M, Bao W. Exposure to bisphenol A, bisphenol F, and bisphenol S in US adults and children: the national health and nutrition examination survey 2013–2014. ACS omega. 2018 Jun 18;3(6):6523-32.
7. Derakhshan A, Shu H, Peeters RP, Kortenkamp A, Lindh CH, Demeneix B, Bornehag CG, Korevaar TI. Association of urinary bisphenols and triclosan with thyroid function during early pregnancy. Environment international. 2019 Dec 1;133:105123.
8. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. nature. 2006 Dec;444(7122):1027-31.
9. Roberfroid MB, Bornet F, Bouley CE, Cummings JH. Colonic microflora: nutrition and health. Summary and conclusions of an International Life Sciences Institute (ILSI)[Europe] workshop held in Barcelona, Spain. Nutrition reviews. 1995 May 1;53(5):127-30.
10. Addinall SG, Bi E, Lutkenhaus J. FtsZ ring formation in fts mutants. Journal of bacteriology. 1996 Jul;178(13):3877-84.
11. Bi, E., &Lutkenhaus, J. FtsZ ring structure associated with division in Escherichia coli. *Nature, 1991;354*(6349):161–164.
12. Monahan LG, Robinson A, Harry EJ. Lateral FtsZ association and the assembly of the cytokinetic Z ring in bacteria. Molecular microbiology. 2009 Nov;74(4):1004-17.
13. Whiteside SA, Razvi H, Dave S, Reid G, Burton JP. The microbiome of the urinary tract—a role beyond infection. Nature Reviews Urology. 2015 Feb;12(2):81-90.
14. Liu Y, Yang X, Gan J, Chen S, Xiao ZX, Cao Y. CB-Dock2: Improved protein–ligand blind docking by integrating cavity detection, docking and homologous template fitting. Nucleic acids research. 2022 Jul 5;50(W1):W159-64.