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In Silico Based Bioinformatics Project During the COVID-19 Lockdown Period: An Alternative to Wet Lab Study

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Abstract: In the weeks and months following the COVID outbreak, millions of students around the world are forced to switch over from traditional classroom learning to online learning. This unprecedented move had tremendous impact on the mode of learning project based courses. In particular, the field of biological science project based courses which require the students and faculty to work in the laboratory was impacted. 5 students and a faculty from Kalasalingam Academy of Research and Education used modern in silico tools, databases and software to study a biological problem related to antibiotic resistance in a bacterium called Enterobacter hormaechei subsp. hoffmannii OIPH-N069. The students used freely available modern bioinformatics tools, databases and software such as NCBI, PROTPARAM, SOPMA, BLAST, CLUSTAL OMEGA, CDD, SWISS-MODEL server, PATCHDOCK, PYMOL and CARD to carry out the research work. This method of doing the project has resulted in the phase I completion of the project course successfully. This methodology of usage of freely available tools, databases and software will help students worldwide to pursue the project based biology courses successfully. The significance of this study is the capability of students in completion of the biology project with the usage of only computer and internet and not to depend upon expensive wet lab study.

Keywords: bioinformatics, in silico, project, computer, COVID

1. Introduction

Modern biological experiments are done using techniques such as microbiology, polymerase chain reaction [1, 2], SDS-PAGE and zymogram analysis [3]. Unfortunately, after COVID lockdown many of the universities and colleges teaching biology based courses faced the difficulty in conducting project based learning courses. In our university, we also faced the similar problem. 5 students from the Department of Biotechnology, Kalasalingam Academy of Research and Education used modern bioinformatics tools, databases and software such as NCBI, PROTPARAM, SOPMA, BLAST, CLUSTAL OMEGA, SWISS-MODEL server, PATCHDOCK, PYMOL and CARD to carry out the research work. NCBI genome database was used by the students for the preliminary searching of the protein sequences.

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The major research objectives of this study is to retrieve the antibiotic resistance determinant protein sequences and to predict primary, secondary, tertiary structures of proteins. Other research objectives included in this study are local and multiple alignment of protein sequences and finding the conserved domains in the protein sequences along with the docking of protein structures with ligands. NCBI (National Centre for Biotechnology Information) was used for retrieving the protein sequences. PROTPARAM tool was used by the students to study the primary structure of protein sequences. SOPMA (Self Optimized Prediction Method with Alignment tool) was used by the students for predicting the secondary structure of protein sequences. BLAST (Basic Local Alignment Search Tool) was used by the students to examine the sequence similarity and identity of protein sequences against the available databases. CLUSTAL OMEGA tool was employed by the students to do multiple sequence alignment and analysis of protein sequences. SWISS- MODEL server was used for automated homology modeling of protein 3D-structures. PATCHDOCK server was used for the docking studies of protein with antibiotic. PYMOL tool was used to see the tertiary structure of proteins. CARD (The Comprehensive Antibiotic Resistance Database) was used for finding out the closest of relative of study proteins. Thus, by using such modern bioinformatics tools, software and databases, the students were successfully able to complete the phase I of the mandatory project work course despite without access to wet lab experiments. We have used the modern bioinformatics tools, databases and software to solve the problem of dependence upon expensive wet lab biology experiments which could not be done during lockdown period as the educational institution is out of bounds for the students due to COVID outbreak.

2. Literature Review

The importance of continuing education in particular biology based course during the COVID-19 pandemic has been a real challenge [4]. Many universities and colleges have reimaged the new pedagogical possibilities post-Covid-19 outbreak [5].The effects of blended and project-based learning are felt post-Covid-19 outbreak [6]. COVID-19 has opened the flood gates for e- Learning based projects [7]. In country such as China where COVID-19 outbreak started, online teaching has been recommended for project based courses [8]. Many strategies have been created by the teachers to engage remote learners in biomedical education after the outbreak [9]. Many universities and colleges have closed due to corona virus disease. This had impact upon education and mental health of students and academic staffs who were unable to continue traditional lab based courses [10]. COVID-19 has come as blessing in disguise and became a catalyst for educational change [11]. Many biology based educational institutions in the world have switched over to e-based projects [12]. In India, COVID-19 had great impact on higher education [13]. Our study is significant because there are no significant earlier published reports where bioinformatics was used for remote learning for project based courses. We have used the modern bioinformatics tools, databases and software which are alternatives to expensive wet lab biology experiments. The existing wet lab facilities are expensive and are out of bounds for students Post-COVID outbreak.

3. Methodology

The NCBI genome database was used by the students for the initial search and retrieval of protein sequences [14]. The primary structural features of protein sequences were analyzed using PROTPARAM tool [15]. The similarity and identity of the protein sequences was determined by the students using BLAST tool [16]. Multiple sequence alignment and motif analysis of protein sequences were done by the students using CLUSTAL OMEGA tool [17]. Conserved Domain Database was employed by the students for searching the conserved domains in the protein sequences [18]. The students predicted three dimensional structures of proteins using Swiss-Model server [19]. PATCHDOCK server was used by the students for the docking studies of protein with antibiotic. Later, the students used PYMOL tool to display the tertiary structure of proteins [20]. Finally, the students used CARD (The Comprehensive Antibiotic Resistance Database) for finding out the closest of relative of study proteins.

4. Result and Discussion

A. Protparam Analysis

The students used the Protparam homepage and FASTA sequence was entered and the parameters were computed. The results were obtained as displayed in the figure [Fig. 1].

Properties	A	B	C	D	E	F	G	H	I
Number of amino acids	712	214	381	284	291	241	347	338	162
Theoretical pI	5.37	8.79	8.74	9.69	8.91	7.74	5.17	5.99	10.47
Stability index (II) value	39.99 (Stable)	45.96 (Unstable)	33.62 (Stable)	27.75 (Stable)	23.94 (Stable)	19.84 (Stable)	38.65 (Stable)	42.96 (Unstable)	33.76 (Stable)
Aliphatic index value	84.33	81.17	87.64	121.87	88.97	95.98	98.33	103.79	144.94
GRAVY value	-0.268	-0.233	-0.045	0.512	-0.199	-0.061	0.057	-0.054	1.013

A-WP_022649503.1 MULTISPECIES: penicillin-binding protein activator

B -WP_085929697.1 MULTISPECIES: penicillin-binding protein activator LpoB

C -WP_022646807.1 MULTISPECIES: cephalosporin-hydrolyzing class C beta-lactamase ACT- 24

D -WP_022647060.1 MULTISPECIES: beta-lactamase regulator AmpE

E -WP_025368620.1 MULTISPECIES: class A extended-spectrum beta-lactamase CTX-M-2

F -WP_032492430.1 MULTISPECIES: subclass B1 metallo-beta-lactamase KHM-1

G -WP_000913396.1 MULTISPECIES: rod shape-determining protein MreB

H -WP_003860400.1 MULTISPECIES: rod shape-determining protein MreC

I - WP_003860402.1 MULTISPECIES: protein rod shape-determining MreD

GRAVY -Grand average of hydropathicity

Fig. 1 : Predicted physicochemical and biological properties of β-lactam resistance determinants and related proteins

B. SOPMA Analysis

The students used the SOPMA homepage and FASTA sequence was entered and the parameters were computed. The following results such as the number of β strands, number of α helices, other percentage and total residues was obtained [Fig. 2].

Protein sequence ID	Number of β-strands (%)	Number of α-helices (%)	Others (%)	Total residues
WP_022649503.1	53(7.44%)	291(40.87%)	368(51.69%)	712
WP_085929697.1	13(6.07%)	80(37.38%)	121(56.55%)	214
WP_022646807.1	21(5.51%)	145(38.06%)	215(56.43%)	381
WP_022647060.1	3(1.06%)	214(75.35%)	67(23.59%)	284
WP_025368620.1	19(6.53%)	141(48.45%)	131(45.02%)	291
WP_032492430.1	18(7.47%)	86(35.68%)	137(56.85%)	241
WP_000913396.1	29(8.36%)	149(42.94%)	169(48.7%)	347
WP_003860400.1	21(6.21%)	123(36.39%)	194(57.4%)	338
WP_003860402.1	5(3.09%)	109(67.28%)	48(29.63%)	162

WP_022649503.1 MULTISPECIES: penicillin-binding protein activator

WP_085929697.1 MULTISPECIES: penicillin-binding protein activator LpoB

WP_022646807.1 MULTISPECIES: cephalosporin-hydrolyzing class C beta-lactamase ACT- 24

WP_022647060.1 MULTISPECIES: beta-lactamase regulator AmpE

WP_025368620.1 MULTISPECIES: class A extended-spectrum beta-lactamase CTX-M-2

WP_032492430.1 MULTISPECIES: subclass B1 metallo-beta-lactamase KHM-1

WP_000913396.1 MULTISPECIES: rod shape-determining protein MreB

WP_003860400.1 MULTISPECIES: rod shape-determining protein MreC

WP 003860402.1 MULTISPECIES: protein rod shape-determining MreD

Fig. 2: Secondary structure summary of the β-lactam resistance determinants and related proteins

C. BLASTp Analysis

BLASTp stands for Basic Local Alignment. The BLAST output can be delivered in a variety of formats.

Protein sequence ID	Sequences producing significant alignments	E-value
WP_022649503.1	Penicillin-binding protein activator(Enterobacter hormaechei)WP_063861684.1	0
WP_085929697.1	MULTISPECIES: penicillin-binding protein activator LpoB [*Enterobacter*] WP_085929697.1	$1e^{-153}$
WP_022646807.1	ACT family cephalosporin-hydrolyzing class C beta-lactamase [*Enterobacter hormaechei*] wp_126490135.1	0
WP_022647060.1	MULTISPECIES: beta-lactamase regulator AmpE [*Enterobacteriaceae*] WP_023299333.1	0
WP_025368620.1	Extended spectrum beta-lactamase CTX-M [*Escherichia coli*] AYD75455.1	0
WP_032492430.1	Subclass B1 metallo-beta-lactamase [*Cellvibrio sp.*] HCS63136.1	$3e^{-146}$
WP_000913396.1	Rod shape-determining protein mreB [*Escherichia coli* CFT073] AAN82446.1	0
WP_003860400.1	Rod shape-determining protein MreC [*Enterobacter hormaechei*] WP_148386311.1	0
WP_003860402.1	MULTISPECIES: rod shape-determining protein MreD [*Enterobacteriaceae*] WP_010436160.1	$1e^{-106}$

WP_022649503.1 MULTISPECIES: penicillin-binding protein activator

WP_085929697.1 MULTISPECIES: penicillin-binding protein activator LpoB

WP_022646807.1 MULTISPECIES: cephalosporin-hydrolyzing class C beta-lactamase ACT- 24

WP_022647060.1 MULTISPECIES: beta-lactamase regulator AmpE

WP_025368620.1 MULTISPECIES: class A extended-spectrum beta-lactamase CTX-M-2

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WP_000913396.1 MULTISPECIES: rod shape-determining protein MreB

WP_003860400.1 MULTISPECIES: rod shape-determining protein MreC

WP_003860402.1 MULTISPECIES: protein rod shape-determining MreD

Fig. 3: BlastP analysis of β-lactam resistance determinants and related proteins

The students used BLAST for identifying species, and sequence comparison. From BLASTp, the students concluded that the list of hits starts with the best match. E-value represents the expected number of change alignments, the smaller the E- value the better the match [Fig. 3].

D. Multiple Sequence Alignment and Motif Analysis

The students found that H-X-H-X-D motif, a characteristic zinc-binding motif, is present in Multispecies: beta-lactamase regulatory AmpE and Multispecies: subclass B1 metallo beta lactamase KHM-1(wp_022647060.1 and wp_032492430.1). S-X-N motif ,the penicillin binding protein specific motif is present in Multispecies: penicillin binding protein activator and Multispecies: class A extended spectrum beta lactamase CTX-M-2 (wp_022649503.1 and wp_025386201).S-V-V, a motif specific to Class-D β- lactamase, is present in Multispecies: Rod shape determining protein mreD (wp_003860402.1).Y-X-N is the characteristic motif is present in Multispecies: penicillin binding protein activator LpoB and Multispecies: cephalosporin-hydrolyzing class C beta- lactamase

ACT-24 (wp_085929697.1 and wp_022646807.1).K-X-G motif involved in active site function and regulation is present in Multispecies: penicillin binding protein activator and Multispecies: cephalosporin hydrolyzing class C beta lactamase ACT-24 and Multispecies: penicillin-binding protein activator (wp_022649503.1 and wp_022646807.1). S- X-X-K with an active serine known for destroying the beta lactam ring of the antibiotic is present in Multispecies: penicillin binding protein activator (wp_022649503.1) [Fig. 4].

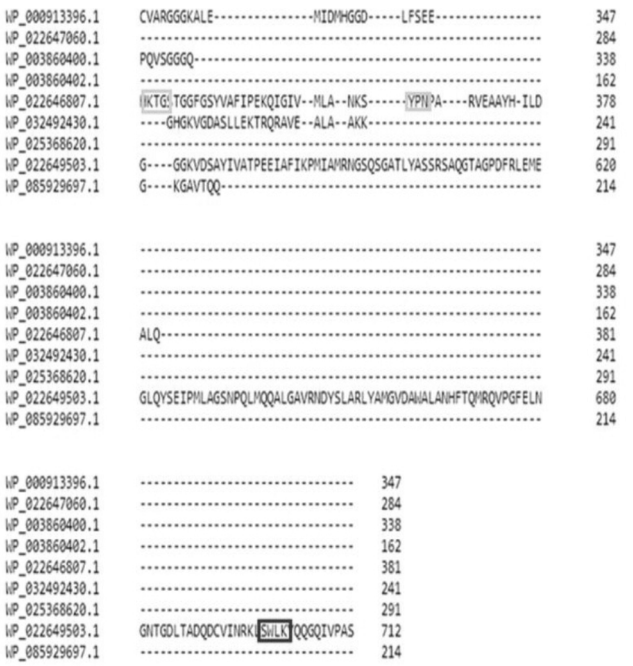

Fig.4:Motif analysis of the beta lactam resistant protein

E. Homology Modelling Analysis and docking studies

The homology model predicted by the students gave the essential structural features namely the α-helix, β structure and an active serine known for destroying the beta lactam ring of the antibiotic. The beta lactamase domain containing proteins had shown greater level of interaction with the penicillin substrate specificity. The interacting side chains are represented as balls and sticks. The sequence alignment and template structure are then used by the students to produce structural model of the target. The accuracy of the structures generated by homology modelling is highly dependent on the sequence identity between target and template [Fig. 5].

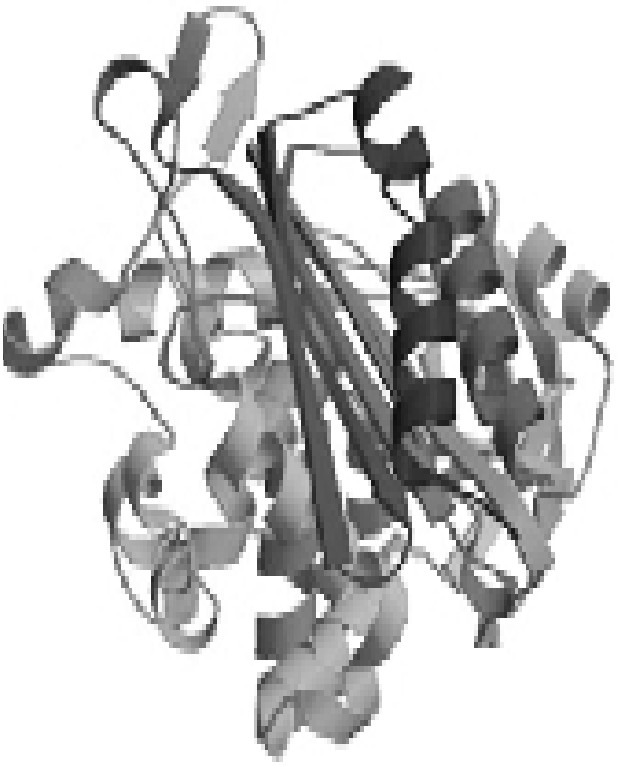

Fig.5 : Three dimensional protein structure of beta-lactam resistant determining protein.

F. PatchDock Analysis

PatchDock uses a geometry based biomolecular docking software. The students gave input as two molecules of protein and antibiotic. The output obtained by the students showed a list of potential protein-antibiotic complexes. The input protein (Receptor) and penicillin (Ligand) in PDB format was uploaded by the students. The protein PDB format was retrieved from the Swiss-model server and the penicillin PDB format was retrieved using Pubchem by the students. The PDB format molecules in the receptor molecule and ligand molecule were readied by the students. The default RMSD value for this parameter is 4.0Å.

A web page that represents the top 20 solutions of docked protein-antibiotic complex was automatically generated. The result was represented in a table. The geometric score, the interface area size and the rigid transformation of the solution were obtained. The PDB file of the protein-antibiotic complex was downloaded as a compressed file contains the top scoring solutions[Fig.6].

Molecular Docking Algorithm Based on Shape Complementarity Principles

[About PatchDock] [Web Server] [Download] [Help] [FAQ] [References]

Receptor	Ligand	Complex Type	Clustering RMSD	User e-mail	Receptor Site	Ligand Site	Distance Constraints
model_011.pdb	tclcactvs0009s8vDy.pdb	Default	4.0	imjashin@gmail.com	-	-	-

Solution No	Score	Area	ACE	Transformation	PDB file of the complex
1	4974	595.60	-170.05	0.44 -1.00 -1.24 8.19 18.87 12.26	result.1.pdb
2	4966	584.40	-116.90	2.88 1.09 1.76 8.20 18.53 13.52	result.2.pdb
3	4700	558.30	-116.56	3.09 -1.00 -1.01 8.02 19.13 15.24	result.3.pdb
4	4524	510.20	-128.34	-1.39 1.17 2.91 7.62 21.39 18.67	result.4.pdb
5	4496	567.50	-235.14	1.61 1.39 -0.20 8.15 16.35 4.49	result.5.pdb
6	4368	497.30	-137.31	-2.70 -1.23 -1.10 7.25 21.63 19.30	result.6.pdb
7	4358	593.30	-228.27	2.06 -1.02 2.90 7.55 15.99 5.60	result.7.pdb
8	4118	577.40	-188.68	-0.04 1.14 1.94 9.19 17.99 10.37	result.8.pdb
9	4058	618.10	-192.28	-2.75 1.14 1.12 8.03 16.43 8.70	result.9.pdb
10	3988	438.50	-144.94	-1.82 0.84 1.17 7.66 24.18 23.54	result.10.pdb
11	3984	433.80	-148.33	2.21 1.13 2.27 5.36 22.58 22.88	result.11.pdb
12	3970	473.90	-216.48	2.82 1.11 2.80 21.31 -3.45 19.23	result.12.pdb
13	3958	589.70	-228.09	1.37 -1.19 -1.81 8.37 17.70 8.76	result.13.pdb
14	3946	488.60	-181.27	0.85 -0.50 -0.02 3.19 22.82 24.01	result.14.pdb
15	3902	467.70	-216.96	0.28 -1.19 -0.56 21.46 -2.91 18.87	result.15.pdb
16	3894	449.90	-156.59	0.10 -1.50 2.78 20.32 -3.63 26.05	result.16.pdb
17	3892	420.70	-114.29	1.83 -0.53 1.00 10.11 24.00 13.27	result.17.pdb
18	3836	433.60	-97.21	0.43 -0.09 -2.44 6.60 16.80 33.62	result.18.pdb
19	3834	465.10	-120.73	1.43 -0.15 -2.10 10.34 22.51 14.22	result.19.pdb
20	3826	409.00	-130.82	-3.10 -0.26 -2.87 -3.10 10.89 31.40	result.20.pdb

Fig.6. Beta-lactamase (WP_022646807.1 MULTISPECIES: cephalosporin-hydrolyzing class C beta-lactamase ACT- 24) docked with penicillin

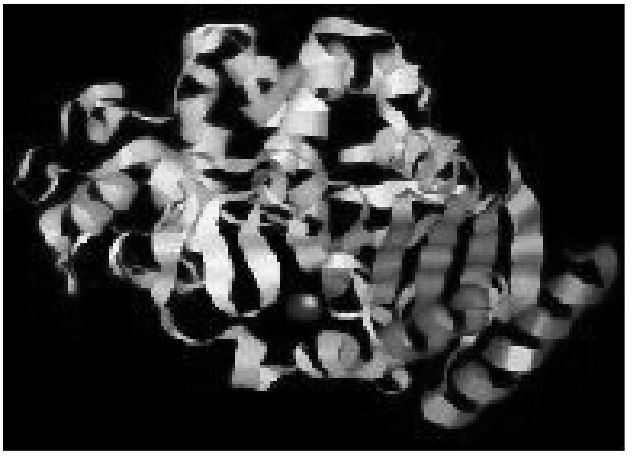

Fig.7: Docking of tertiary structure of beta-lactam resistant determining protein to penicillin.

A. Docking of Beta-lactam resistant protein to penicillin

The students finally concluded the project by docking the beta-lactam resistant protein to penicillin [Fig. 7].

Thus, by using such modern bioinformatics tools, software and databases, the students were successfully able to complete the phase I of the mandatory project work course despite without access to wet lab experiments.

A. Qualitative comparative analysis of in silico bioinformatics and wet lab experiments

The following advantages of bioinformatics were observed by our students over wet lab experiments:

1. Less consumption of time to do the experiment
2. No usage of expensive wet lab consumables
3. Less dependence upon lab resources during the lockdown period
4. Highly statistically significant when compared to wet lab experiments

B. Student feedback of in silico bioinformatics and wet lab experiments

The students were asked to give feedback. All the students were found to be more comfortable with in

Fig. 8: Student performing the experiment in his home with the help of his laptop

silico bioinformatics mode of project as they were able to do the experiment in comfort in their home [Fig. 8]. All the students were able to offer practical solution to the research objectives as evident from the results. All the students were able to manage the time properly.

4. Conclusion

After COVID-19 lockdown, many universities, colleges and schools have adapted technology for teaching, learning and students' engagement [21]. Similarly, our students also have adapted bioinformatics technology for completing the project. There are wide branches of biology oriented courses such as physiotherapy has adapted technology based student research projects during the COVID-19 lockdown [22]. Our students have also adapted the modern bioinformatics tools, databases and software such as NCBI, PROTPARAM, SOPMA, BLAST, CLUSTAL OMEGA, CDD, SWISS-MODEL server, PATCHDOCK, PYMOL and CARD to carry out the research work. NCBI was used by the students as it provides access to biomedical and genomic information. PROTPARAM tool used by the students helped them to compute physical and chemical parameters of the protein. SOPMA tool used by the students helped them to compute secondary structural features of protein. BLAST tool used by the students helped them to identify the similar protein sequences in NCBI database. CLUSTAL OMEGA used by the students helped them to do alignment of multiple protein sequences. CDD was used by the students to identify conserved domains in the protein sequences. SWISS-MODEL server used by the students helped them to automated modeling of protein structure. The students used shape complementarity principles in PATCHDOCK to perform the docking experiments. PYMOL used by the students helped them to visualize the structures of proteins. Like our case study, online teaching-learning in higher institutes of education such as universities and colleges during the lockdown period of COVID-19 pandemic has become common [23]. Similar to our study, information technology has been employed by an institute for IT bachelor capstone project during lockdown [24]. Since, the role of online teaching tools has been important during the lockdown of COVID-19, we also used modern in silico tools for this capstone project [25]. Many secondary school students have also used tool such as citizen science to facilitate practical and online science learning as done by us with help of bioinformatics tools [26]. This method of doing the project has resulted in the phase I completion of the project course successfully. This methodology of usage of freely available tools, databases and software will help students worldwide to pursue the project based biology courses successfully. Moreover, a complex study problem such as antibiotic resistance was studied without spending any chemical reagents and other wet lab requirements. Hence, we suggest the usage of in silico tools for complex research projects such as capstone projects offered by the institutions all over the world. But, we believe that classical wet lab experiments will further enhance our knowledge about the study such as antibiotic resistance in bacteria.

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