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Halophilic bacterial inoculants for improving plant growth and biocontrol.

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ABSTRACT

Bacteria are able to synthesis a wide range of metabolites with fungicidal capabilities. The antagonistic potential of soil bacterial strains (S1 Bacillus farraginis, S2 Paenibacillus cookii) was assessed by the dual culture technique against some plant pathogenic fungi, i.e. Alternaria alternata, Alternaria brassicae and Fusarium oxysporum. The result indicated that bacterial species exhibited varying degree of biocontrol potential against all tested fungi. Out of two bacterial species, S1 Bacillus farraginis showed effective biocontrol potential against all tested fungi with reduction of 82-96% in fungal colony diameter. S1 Bacillus farraginis showed almost complete inhibition against pathogenic fungi such as Alternaria alternata, Alternaria brassicae whereas S2 Paenibacillus cookii was least effective. The experimental results exhibit the antifungal activity of bacterial species and indicate the possibility of using these bacterial species as antifungal agents against these fungal species. Subsequently, in the present study, an antagonistic strain was isolated from the soil of the agricultural field, Mathura refinery and college campus and identified as S1 Bacillus farraginis and S2 Paenibacillus cookii based on the biochemical test. The strain showed the higher antifungal activity against seven tested fungal pathogen belonging to various taxonomic groups on dual culture plates. Furthermore, the strain produced volatile antimicrobial compounds which have strong fungal growth inhibitory effect. The strain also showed high chitinase, cellulose, glucanase and protease activities. The hyphal morphologies of Alternaria alternate, Alternaria brassicae, and Fusarium oxysporum were significanty destroyed by the crude enzymes and butanol extract from the culture supernatant and the affected hyphae showed abnormal bending, tip curling and irregular branching. Hence S2 Paenibacillus cookii is considered as a potential biocontrol agent of the soil borne fungi causing plant diseases which is an important prospective of the present study.

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INTRODUCTION

Agriculture research is more concerned now days about the concept of bacterial inoculants than chemicals products. There is a genuine interest in developing the bacterial products that are reliable and eco-friendly [1,2]. Worldwide consciousness on the harmful effects associated with the continuous use of chemicals, for example, overall damage to the ecology and health hazards, has smooth way for development and use of this microbe based technology. Two major components in developing this technology are the understanding of the plant growth promoting microorganisms and bio-control against phyto-pathogens. The bacterial traits involved in these activities, include nitrogen fixation, phosphate solubilization, iron sequestration, synthesis of phytohormones, modulation of plant ethylene levels, and control of phytopathogenic microorganisms [3, 4].

Biocontrol seems to be a reliable alternative to synthetic chemicals, which have raised serious concerns of plant diseases. Filamentous Fungi cause serious problems in plant by producing mycotoxins and potentially allergenic spores, causing spoilage of plant and plant products that is costly as well as sometimes dangerous. *Alternaria alternata*, *Alternaria brassicae* and *Fusarium oxysporum* species are commonly found as contaminants in foods throughout drying and subsequent storage [3, 4]. Filamentous fungi,mainly *Alternaria alternata, Aternaria brassicae and Fusarium spp.* produced mycotoxins, secondary metabolites, under the appropriate environmental conditions. Antifungal agents produced by microorganisms may be used as biocontrol agent. Antifungal metabolites produce by bacteria like *S1 Bacillus farraginis and S2 Paenibacillus cookii* have been investigated for their antifungal properties.

Bacillus species, gram-positive bacteria, are good biological control agents (BCA) for, their ability to produce different types of antimicrobial compounds, such as antibiotics (e.g. bacilysin, iturin, mycosubtilin), siderophores and to induce growth and defense responses in the host plant [1-4] . However, gram-negative bacteria have gained significant attention for antagonistic activity. Fungal pathogens such as *Alternaria alternata, Alternaria brassicae ansd Fusarium oxysporum* cause severe plant

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disease,limiting plant yields as well as quality of the product. Moreover, they have wide host spectra causes diseases in economically important agricultural crops worldwide. These fungal phytopathogens are difficult to control not only because of their wide host spectra but also because of their soilborne nature. To cope with this, chemical fungicides are generally used in higher doses. The synthetic fungicides applied to the soil or to the aerial portions of plants not only destroy the environment, but also contaminate the ground water bodies and may also enter the human food chains. Therefore, the alternate measures are required for the durable and environment friendly control of the soilborne fungal pathogens. Different computational methods have been used to find interaction with microbial proteins and properties of compounds [5-14]. The biological control offers a way to repress the fungal phytopathogens . The use of antagonistic microorganisms may not only control the plant pathogens more efficiently, but are also safer for the environment. The aim of this work was to evaluate biocontrol potential of different soil bacterial species *S1 Bacillus farraginis and S2 Paenibacillus cookii* against plant pathogenic fungi.

METHOD

Three nutrient agar Petri plates were prepared at concentrations of 0.15%, 0.20 and 2.5% of NaCl. The bacterial populations were isolated from halophilic soil of different areas collected from Mathura Refinery(R), College Campus (H.C.S.T.)(C), Agricultural land(A).The bacterial counts were calculated on the basis of serial tenfold dilution by spread plate using one gram of soil and appropriate dilution The 15 test tubes were taken and marked as follows: $(R,10^{-1})$, $(R\ 10^{-2})$, $(R,10^{-3})$, $(R,10^{-4})$, $(R,10^{-5})$, $(C,10^{-1})$, $(C,10^{-2})$, $(C,10^{-3})$, $(C,10^{-4})$, $(C,10^{-5})$, $(A,10^{-1})$, $(A,10^{-2})$, $(A,10^{-3})$, $(A,10^{-4})$, $(A,10^{-5})$. Serial dilution was performed by taking 10 ml of distilled water in each tube R, C and A marked 10^{-1} tube. Also 9 ml of distilled water was added in rest (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}) tubes. One gram of soil samples from each site (R, C, A) were weighed and mixed with distilled water in the respective tubes $(R,10^{-1})$, $(C,10^{-1})$ and $(A,10^{-1})$. After mixing, 1ml of mixture was transferred from each 10^{-1} tube into 10^{-2} respective tube and mixed properly. This

serial dilution step was repeated for all tubes up to 10^{-5}. Furthermore, the Colony Forming Units (CFU) was enumerated on the nutrient agar medium (NAM) whereas, the relative abundance of dominant bacteria from soil sample was determined. Furthermore, the petri plates with Nutrient Agar (NA) solutions were prepared and autoclaved for 15 minutes at 121˚C and at pressure of 15 psi. After autoclaving, the NA solution was cooled and poured in six petri plates named as (A, 10^{-4}), (A, 10^{-5}), (C, 10^{-4}), (C, 10^{-5}), (R, 10^{-4}) and (R, 10^{-5}) in the laminar flow in order to solidify. All petri plates were incubated at 30˚C for 24 to 48 hrs. Shape and size of the colonies were observed and CFU of each plate & Occurrence percentage of each colony were calculated.

Formula used:

a) Dilution factor, dF = 1/no. of dilution
b) Colony forming unit, CFU =(Average no. of colony/1gm)*dF
c) Occurrence percentage ,OC = (same type of colony in 1 plate /total no. of colony)*100

Phenotypic characterization of the isolate was performed by subjecting the bacterial isolate to cultural (oxygen requirement), morphological (colony morphology and pigmentation), microscopic (Gram staining, cell shape, size and arrangement of cells), biochemical (utilization of different carbon sources and enzyme activity) and physiological characterization (temperature, pH, salt tolerance and antibiotic sensitivity) by standard procedures. Effects of different temperatures were analyzed by incubating the plates at different temperatures of 37°C, 28°C and 4°C. Furthermore, the effects of pH were measured by preparing nutrient agar for 12 plates and adjust pH using pH meter; 3 plates for each pH 3, 4, 5 & 8. Plates were incubated at 37°C for 24 hours. Biochemical analysis of the bacteria was performed by amylase production test (or starch hydrolysis), sugar mannitol fermentation, hydrolysis of gelatine, a protein (production of gelatinase), urease test, catalase activity, methyl red test, H_2S test and citrate test. Three different funguses e.g. *Alternaria alternate Alternaria brassicae* and *Fusarium oxysporum* were selected for analyzing inhibition by halophilic bacteria. ***Alternaria alternata*** has been recorded causing leaf spot and other diseases on over 380 host species. It is opportunistic pathogen on numerous hosts causing leaf spots, rots and

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blights on many plant parts. It can also cause upper respiratory tract infections and asthma in people with sensitivity. ***Alternaria brassicae*** is a plant pathogen able to infect most *Brassica* species including important crops such as brocoli, cabbage and oil seed rape. It causes damping off if infection occurs in younger plants and less severe leaf spot symptoms on infections of older plants. ***F. oxysporum*** strains are ubiquitous soil inhabitants that have the ability to exist as saprophytes, and degrade lignin and complex carbohydrates associated with soil debris. They are also pervasive plant entophytes that can colonize plant roots and may even protect plants or be the basis of disease suppression.

Table 1: Total number of bacterial colonies formed for soil sample from Agricultural land (A).

Strain	Number of colony	OC	Shape & Size
A1	2	8.69	Medium
A2	3	13.043	large size
A3	12	52.17	hollow pin point
A4	3	0.57	Small
A5	3	0.57	feather
			like
Total	23		

RESULTS

The soil samples from Agricultural land (A) formed total 23 bacterial colonies with maximum OC of 52.17 for A3 with colony size of 12 (Table 1). The sample from Campus (H.C.S.T.) (C) formed total 147 bacterial colonies with maximum OC of 74.83 for C3 with colony size of 110 (Table 2). The maximum number of total colonies was observed for soil sample from Mathura Refinery (R) with total bacterial colonies of 398 with maximum OC of 48.99 with number of colonies of 195 for R2 (Table 3).

Table 2: Total number of bacterial colonies formed for soil sample from Campus (H.C.S.T.) (C).

Strain	Number of colony	OC	Shape & Size
C1	1	0.68	Circular
C2	30	20.41	very fine
C3	110	74.83	very small pin

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			point
C4	1	0.68	plane
C5	5	3.40	Star like
Total	147		

Table 3: Total number of bacterial colonies formed for soil sample from Mathura Refinery(R).

Strain	Number of colony	OC	Shape & Size
R1	2	0.50	large star
R2	195	48.99	Medium
R3	50	12.56	Pinpoint
R4	1	0.25	star like
R5	105	26.38	very small pin point
Total	353		

Table 4 represents the gram staining results for the different soil samples and depicted that R1, R3, R4, C1, C3 and C5 produced negative results with gram stain. On the other hand, R2, R5, C2, C4 and A1 produced positive results with gram stain.

Table 4: Gram staining results for three bacterial samples.

Strain	Shape	Gram Stain
R1	Round	negative
R2	Round	positive
R3	chain like	negative
R4	chain like	negative
R5	Round	positive
C1	Round	negative
C2	round	positive
C3	rod shaped	negative
C4	-	positive
C5	rod shaped	negative
A1	branched	positive

Study of Physiological Effects on Bacteria showed that best growth was observed at 37^{o}C for the bacterial colonies and no growth was observed at 4^{o}C. Optimizing condition for pH revealed that the best growth was maintained at pH 6 and pH 8.On the other hand, no growth was appeared at pH 4 and pH 5.

Further the biochemical tests for two bacterial species soil bacterial strains *S1, Bacillus farraginis* and *S2, Paenibacillus cookii* were performed. A typical positive

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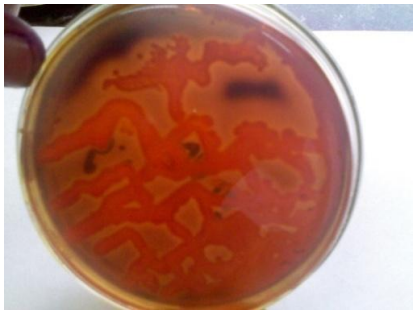
starch hydrolysis reaction (i.e. clear zone surrounding microbial colonies) is shown by both strains.



S1

S1

Fig. 1 Amylase production test

The hydrolysis Of Gelatin (Production of gelatinase) shown that all the three test tubes were solidified after placing in refrigerator. No gelatine hydrolysis occurs. Hence both strains were Negative in result.

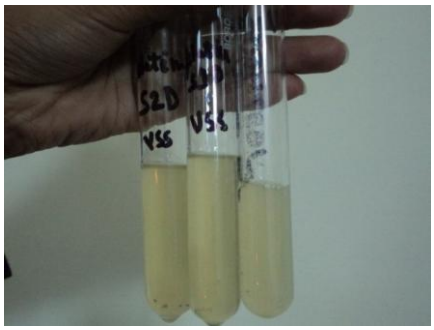


Fig. 2 Production of gelitinase

Furthermore, the mannitol fermentation by both Bacterial Strains S1 and S2 did not show orange coloration of the medium, hence result was negative.

Fig. 3 Mannitol fermentation by bacteria.

In addition, in urease test both strains S1 and S2 did not show a deep pink coloration of medium hence negative result.

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Fig. 4 Urease test

On the other hand, in catalase activity test both the bacterial strains S1 and S2 were positive for the catalase test.

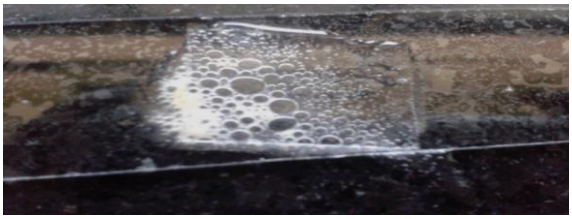

Fig. 5 Catalase activity test.

Furthermore, in the H_2S Test strains S1 and S2 didn't produced any black precipitate (which depicts H_2S production) hence they showed negative result.

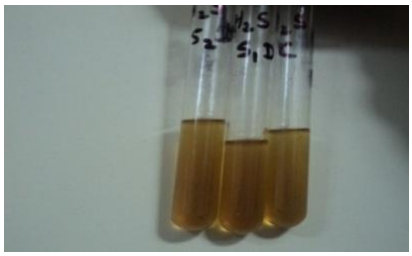

Fig. 6 H_2 S test

In citrate test, strains S1 and S2 shows +ve and –ve result respectively.

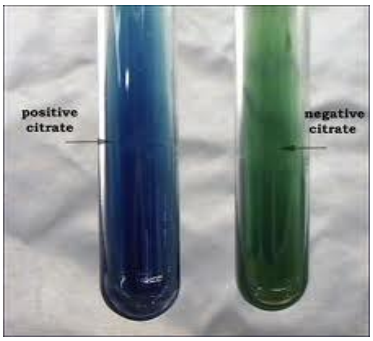

Fig. 7 Citrate test

Table 5: Biochemical Test

Biochemical Test	**Sample 1**	**Sample 2**
Amylase Production Test	+ve	+ve
Hydrolysis of Geletin Test	-ve	-ve
Catalase Test	+ve	+ve
Urease Test	-ve	-ve
Mannitol Test	-ve	-ve
H_2S Test	-ve	-ve
Citrate Test	+ve	-ve

Two bacterial species were screened for their antifungal activity against different fungi viz. *Alternaria alternata, Aternaria brassicae, and Fusarium oxysporum*. Experimental results showed that all tested bacterial species show varying degree of

biocontrol potential against fungal strains. *S1 Bacillus farrraginis* showed effective biocontrol potential against all tested fungi with reduction of 82-96% in fungal colony diameter. While in case of *S2 Paenibacillus cookii*, colony diameter of *Alternaria alternate and Alternaria brassicae* was effectively reduced up to 93% and 76% respectively.

DISCUSSION

The antagonism between different microbial strains can be expressed by the production of metabolites, competition and direct parasitism, pathogen enzyme activity is also associated with reduction of induced resistance . Several microbial agents have been used for biological control of plant pathogen. In the present study *S1 Bacillus farraginis* showed highest antifungal activity against *Alternaria alternata* (94%). While in case of *S2 Paenibacillus cookii* diameter of *Fusarium oxysporum,* was reduced up to 88-98% while S1 *Bacillus farraginis* and S2 *Paenibacillus cookii* moderately inhibit *Alternaria brassicae* growth 61% and 64% respectively. Although different microorganisms have been utilized as control agents, antibiotics produced by bacteria isolated from the soil contribute to the suppression or inhibition of the growth of phytopathogens. The presented report exhibit the antifungal activity of bacterial species and indicate the possibiliity of using these bacterial species as a biological agent to control these pathogenic fungi. However, biological agents tested in this study should be investigated extensively for plant safety before commercialization.

CONCLUSION

The bacterial strains that are described in this work have potential as biological control agents. The strains of ***S1 Bacillus farraginis*** and ***S2 Paenibacillus cookii*** are the most promising due to their ability to produce endospores that can remain in the soil during long periods and can be produced easily at an industrial level with a low cost. This last is important for product preparations in which it is possible to incorporate antifungal filtrates stable to heat and pH changes to control the disease in its early state and for the production of spores to be inoculated into the soil to prevent future infections in the cultivars. The results obtained will be used

for the design of new environmentally friendly methodologies for the control of ***Alternaria alternata*** and ***Alternaria brassicae*** without using chemical compounds that contaminate agricultural soils.

SIGNIFICANCE

Biopesticides are typically microbial biological pest control agents that are applied in a manner similar to chemical pesticides. In order to implement these environmentally friendly pest control agents effectively, it can be important to pay attention to the way they are formulated and applied. They can also be used to control internal seed–borne fungal pathogens as well as fungal pathogens that are on the surface of the seed. Many biofungicidal products also show capacities to stimulate plant host defenses and other physiological processes that can make treated crops more resistant to a variety of biotic and abiotic stresses.

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Molecular Docking of Phytochemicals of Adhatoda vasica with the potential drug targets of Asthma – An Ayurinformatics Approach

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ABSTRACT

The traditional Ayurveda medicines and herbal drugs are derived from medicinal plants and are consumed by the human civilization from over 5000 years ago, as these are time tested therapies over a long period of time they will have no serious side effects. In India, we have an ocean of medicinal plants and treasures of knowledge (in the form of Ayurveda) for the perfect use of those medicinal plants. Charak Samhita and Shusrut Samhita of Ayurveda states Vasak (Adhatoda vasica) as the remedy for Asthma. So a molecular (docking) level study to identify the potential drug candidate for Asthma was conducted and we have found that all most all phytochemicals of Adhatoda vasica interacts with the identified drug targets of Asthma. Further, in silico, in vitro and in vivo study of phytochemicals of Adhatoda vasica and target proteins of Asthma may pave the way for a potential drug candidate for Asthma with fewer side effects. Our study shows that Adhatoda vasica phytochemicals are having good drug like nature, and passes almost all drug filters. When the phytochemicals of Adhatoda vasica were docked with the target proteins of Asthma (IL-3, IL-5, IL-4, IL-13, TNF- α (Tumor necrosis factor-alpha) Vasicolinone shows the best binding affinity of -6.9, -6.6, -8.7, -7.6, -5.9, -6.4 Kcal/Mol respectively.

INTRODUCTION

The burden of asthma is immense, with more than 300 million individuals currently suffering from asthma worldwide [1]. Global Burden of Disease Study shows 489,000 deaths attributable to asthma annually [2]. with a global economic burden of asthma costs (direct and indirect) exceed US$6 billion in USA itself [3]. By 2050 approximately 20 million people in India and one billion people globally is expected to get affected by Asthma [4]. A review of current medicines reveals another horrific story of adverse side effects with increase severity and

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life-threatening asthma exacerbations, premature death, as well as asthma-related deaths. Because of many such side effects, FDA Panel banned two Popular Asthma Drugs viz. formoterol and salmeterol [5]. Modern pharmaceutical companies are shifting towards plant derived compounds, which has less side effects compared to synthetic compounds. The global market for plant-derived drugs was worth $22.1 billion in 2012 and expected to be $26.6 billion in 2017 [6]. So there is an urgent need to go for plant derived drugs for Asthma.

MATERIALS AND METHOD

Target proteins of Bronchial Asthma were identified using the KEGG Pathway database [7] and various references were collected on the identified drug targets for Asthma. The PDB files of the target proteins for asthma were retrieved using the RCSB-PDB server [8] and energy minimized using the gromos96 force field of the SwissPDB viewer [9]. Compounds *of Adhatoda vasica* were retrieved from the Knapsack Family Plant Database [10] and the lower energy conformer was calculated using the MMFF94 force field of the Marvin Sketch software [11 &12]. Toxicity assessment, ADME/Tox and Drug likeliness of the compounds were calculated using the preADMET tool [13] and OSIRIS Property Explorer [14]. The Biological activity of the compounds was accessed using the Schrodinger Quick Prop utility. Finally Molecular docking studies were carried out between the identified Target protein of Asthma and the screened ligands *of Adhatoda vasica* using the AutoDock Vina, already used for docking the compounds against proteins [15-22].

RESULTS & DISCUSSION

Active site prediction of the target proteins of asthma

Table 1. Results of predicted active sites of the target proteins (The predicted best two active sites based on the simple score from the DogSiteScorer)

SL.NO	PDB ID	Pocket Description		
		Pocket No	Volume [Å^3]	Simple Score
1.	1A8M	P0	606.04	0.28
		P1	434.93	0.21
2.	1BBN	P0	711.30	0.46
		P1	544.06	0.32
3.	3VA2	P0	1361.06	0.63
		P1	809.59	0.49
4.	1EOT	P0	529.09	0.39
		P1	223.94	0.09
5.	1JLI	P0	599.36	0.42
		P1	198.59	0.12
6.	3BPO	P0	1465.58	0.59
		P1	1108.96	0.60

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Virtual Screening of *Adhatoda* phytochemicals

Table 2. Results for Toxicity Assessment of Phytochemicals of Adhatoda Vasica

Compounds	hERG inhibition risk	Carcino_Mouse	Carcino_Rat	Osiris Result			
				Mutagenicity	Tumeroenicity	Irritant	Reproductive effect
Anisotine	Medium	-ve	-ve	N	Y	N	N
Deoxyvasicinone	Medium	-ve	-ve	N	N	N	N
Peganine	Low	-ve	-ve	N	N	N	N
Vasicinol	Low	-ve	-ve	N	N	N	N
Vasicinone	Low	-ve	-ve	N	N	N	N
4,2'-Dihydroxychalcone 4-glucoside	High	+ve	-ve	N	N	N	N
Adhatodine	Medium	-ve	-ve	N	Y	N	N
Peganidine	Low	-ve	-ve	N	N	Y	N
Vasicinolone	Low	-ve	-ve	N	N	N	N
Vasicoline	Medium	-ve	-ve	N	N	N	N
Vasicol	Low	-ve	-ve	N	N	N	N
Vasicolinone	Medium	-ve	-ve	N	N	N	N

Table 2 shows that all compounds except **4,2'- Dihydroxychalcone 4-glucoside** shows high risk for hERG inhibition, which will hamper the normal electrical activity in the heart, causing **cardiac arrhythmia** also it shows carcinogenic effect on rat models, so **4,2'- Dihydroxychalcone 4-glucoside** has been screened out in Toxicity Assessment of Phytochemicals of *Adhatoda Vasica*

Table 3. Results of Druglikeliness of phytochemicals derived from Adhatoda vasica.

Compounds	CMC-Rule	MDDR-Rule	Lipinski's Rule
Anisotine	Qualified	Mid-structure	Suitable
Deoxyvasicinone	Qualified	Mid-structure	Suitable
Peganine	Qualified	Mid-structure	Suitable
Vasicinol	Qualified	Mid-structure	Suitable
Vasicinone	Qualified	Mid-structure	Suitable
4,2'-Dihydroxychalcone 4-glucoside	Qualified	Drug-like	Suitable
Adhatodine	Qualified	Mid-structure	Suitable
Peganidine	Qualified	Mid-structure	Suitable
Vasicinolone	Qualified	Mid-structure	Suitable
Vasicoline	Qualified	Mid-structure	Suitable
Vasicol	Not qualified	Nondrug-like	Suitable
Vasicolinone	Qualified	Mid-structure	Suitable

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Table 3 shows that Vasicol does not fulfill the CMC Rule and Lead like rule for drug likeliness, hence it has been filtered out based on the drug likeliness filters.

Table 4. Results of ADME properties phytochemicals of Adhatoda vasica.

Compounds	BBB	Caco2	HIA	MDCK	Pgp Inhibition	PPB
Anisotine	0.55	21.33	96.34	15.54	Y	75.21
Deoxyvasicinone	1.64	26.68	99.97	55.36	N	49.23
Peganine	0.54	29.32	95.47	278.65	N	80.33
Vasicinol	0.49	21.24	90.43	26.11	N	68.72
Vasicinone	0.52	20.70	94.86	3.50	N	70.03
4,2'-Dihydroxychalcone 4-glucoside	0.06	12.80	73.66	4.36	N	84.02
Adhatodine	0.25	25.86	95.94	2.64	N	77.95
Peganidine	0.58	22.95	95.35	80.89	N	40.15
Vasicinolone	0.36	21.11	91.63	1.71	N	58.16
Vasicoline	0.45	57.10	100.00	14.47	N	90.19
Vasicol	2.17	0.38	96.91	41.70	Y	90.40
Vasicolinone	0.39	47.07	98.57	26.46	N	85.94

Table 4 states that ADME properties of vasicol shows Pgp inhibition and strong plasma protein binding capacity (>90), hence it has been filtered out based on ADME properties. HIA (Human Intestinal Absorption) analysis shows all most all compounds are well absorbed compounds (HIA 70 ~ 100 %), however 4, 2'-Dihydroxychalcone 4-glucoside lowest HIA among all compounds of Adhatoda vasica. Caco2 cell permeability analysis shows all compounds has moderate permeability (4 ~ 70) except Vasicol which shows very low permeability 0.38 (> 4). The BBB shows all compounds have moderate BBB and vasicol has high BBB which is undesired property of non CNS active drug.

Table 5. Other OSIRIS Properties of phytochemicals of Adhatoda vasica

Compounds	ClogP	SOLUBILITY	mW	TPSA	DRUG LIKENESS	DRUG SCORE
Anisotine	2.55	-3.49	349.0	71.0	0.6	0.53
Deoxyvasicinone	1.25	-2.06	186.0	32.67	3.85	0.94
Peganine	0.22	-1.53	188.0	35.83	3.95	0.96
Vasicinol	-0.12	-1.24	204.0	56.06	3.41	0.96
Vasicinone	0.39	-1.66	202.0	52.9	4.61	0.96
4,2'-Dihydroxychalcone 4-glucoside	0.62	-3.13	402.0	136.6	-4.95	0.41
Adhatodine	2.38	-3.36	335.0	53.93	-0.02	0.5
Peganidine	0.91	-1.79	244.0	52.9	2.51	0.55
Vasicinolone	0.05	-1.36	218.00	73.13	4.39	0.96
Vasicoline	-0.02	-1.48	206.0	66.56	2.57	0.93
Vasicol	2.72	-3.2	291.0	18.84	4.4	0.85
Vasicolinone	2.89	-3.33	305.0	35.91	4.92	0.83

Table 5 shows all of the phytochemicals of *Adhatoda vasica* except 4, 2'-Dihydroxychalcone 4-Glucoside,Adhatodine and Peganidine has high drug score more than 0.7

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Docking Results

Table 6. Binding Affinity (Kcal/Mol) in the Docked Complex of Target Protein and Ligands

Ligands	TARGET PROTEINS					
	IL-3	IL-4	IL-5	IL-13	EOTAXIN	TNF-α
Deoxyvasicinone	-4.5	-5.2	-7.1	-5.8	-4.7	-4.6
Peganine	-4.5	-5.0	-6.2	-5.8	-4.7	-4.4
Vasicinol	-5.2	-5.6	-6.0	-6.2	-4.9	-5.0
Vasicinone	-4.5	-5.8	-6.9	-6.1	-4.9	-7.4
Vasicinolone	-4.9	-5.6	-6.4	-6.4	-5.3	-4.8
Vasicoline	-6.3	-5.2	-7.2	-6.7	-5.7	-6.0
Vasicolinone	-6.9	-6.6	-8.7	-7.6	-5.9	-6.4

Table 7. Binding Affinity (Kcal/Mol) in the Docked Complex of Target Protein active sites and Ligands

Ligands	TARGET PROTEINS											
	IL-3		IL-4		IL-5		IL-13		EOTAXIN		TNF-α	
	P0	P1	P0	P1	P0	P1	P0	P1	P0	P1	P0	P1
Deoxyvasicinone	-3.4	-4.6	-4.2	-3.4	-5.3	-3.4	-3.7	-3.4	-3.6	-2.8	-3.3	-2.9
Peganine	-3.2	-4.4	-2.9	-3.4	-5.4	-4.2	-3.4	-3.3	-3.3	-3.0	-3.0	-3.3
Vasicinol	-3.3	-4.6	-3.4	-3.3	-5.1	-4.9	-3.7	-3.2	-3.4	-2.8	-3.1	-3.1
Vasicinone	-3.2	-4.3	-3.2	-3.5	-5.2	-3.5	-3.7	-3.5	-3.6	-3.0	-3.2	-3.2
Vasicinolone	-3.3	-4.6	-3.5	-3.4	-4.7	-4.2	-3.8	-3.4	-3.6	-2.8	-3.2	-3.0
Vasicoline	-4.7	-5.1	-4.2	-4.1	-6.3	-4.5	-4.6	-4.0	-4.2	-3.7	-4.7	-4.0
Vasicolinone	-4.9	-5.1	-4.2	-4.2	-6.5	-4.7	-4.9	-3.9	-4.5	-3.9	-4.8	-4.1

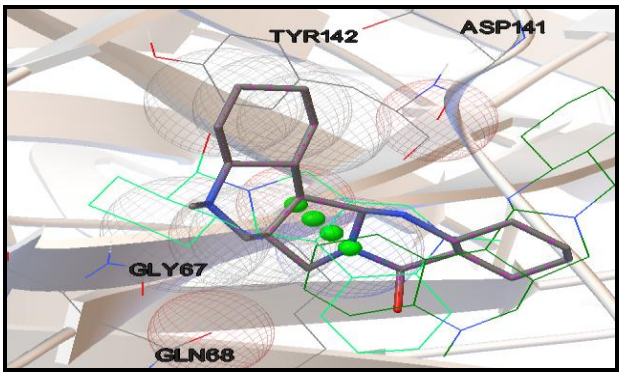

Fig. 1 Visualization of target protein 1A8M with Vasicolinone ligand possessing a minimal binding affinity and the known residue in the docked complex are shown

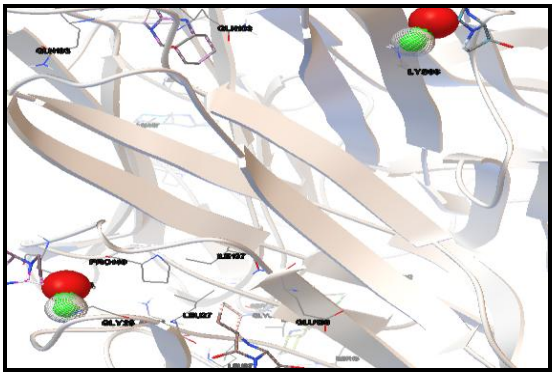

Fig. 2 Visualization of target protein 1A8M with Vasicolinone ligand possessing a minimal binding affinity and the known residue in the docked complex are shown

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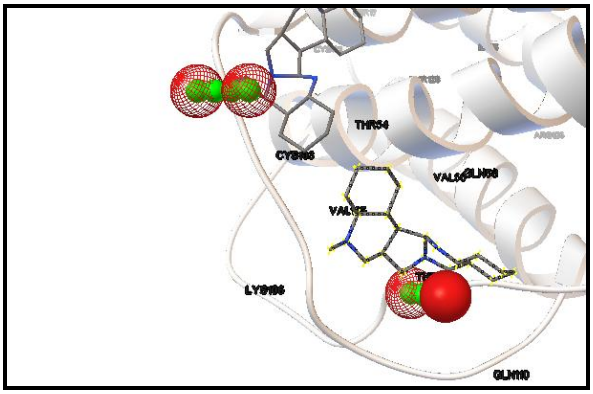

Fig. 3 Visualization of target protein 1BBN with Vasicolinone ligand possessing a minimal binding affinity and the known residue in the docked complex are shown

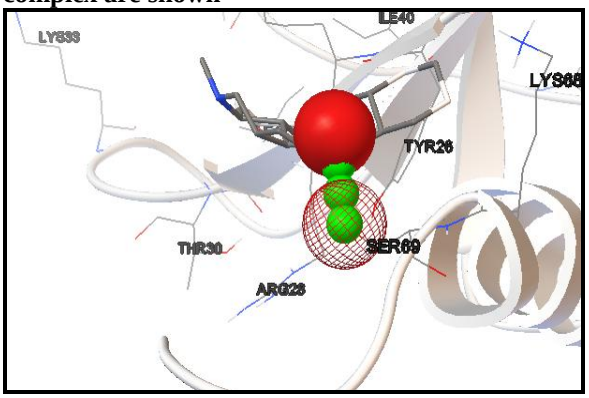

Fig. 4 Visualization of target protein 1EOT with Vascicolinone ligand possessing a minimal binding affinity and the known residue in the docked complex are shown

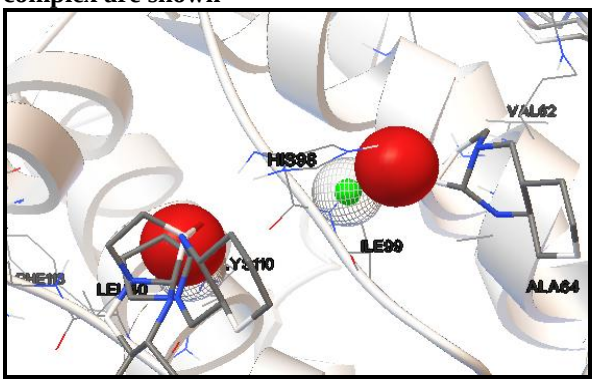

Fig. 5 Visualization of target protein 1JLI with Peganine ligand possessing a minimal binding affinity and the known residue in the docked complex are shown

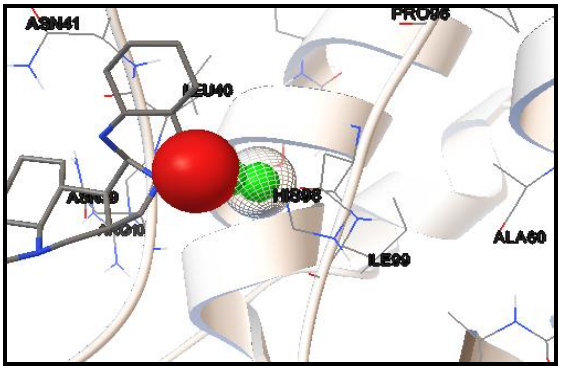

Fig. 6 Visualization of target protein 1JLI with Vasicolinone ligand possessing a minimal binding affinity and the known residue in the docked complex are shown

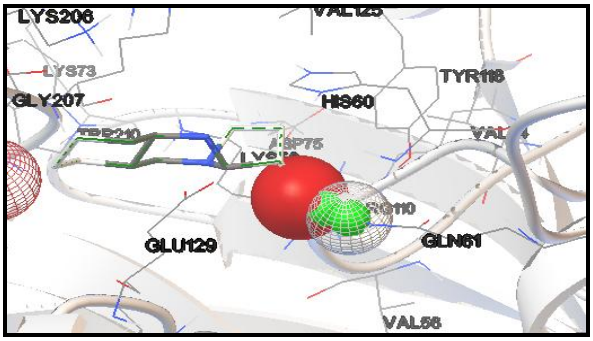

Fig. 7 Visualization of target protein 3VA2 with Peganine ligand possessing a minimal binding affinity and the known residue in the docked complex are shown

CONCLUSION

In India we have an ocean of medicinal plants and treasures of knowledge (in the form of Ayurveda) for the perfect use of those medicinal plants. Charak Samhita and Shusrut Samhita of Ayurveda states *Vasak (Adhatoda vasica)* as the remedy for Asthma. So a molecular (docking) level study to identify the potential drug candidate

for Asthma was conducted and we have found that all most all phytochemicals of *Adhatoda vasica* interacts with the identified drug targets of Asthma. Further study *in silico*, *in vitro* and *in vivo* on phytochemical of *Adhatoda vasica* and target proteins of Asthma may pave the way for a potential drug candidate for Asthma with fewer side effects.

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Interaction Study of Different Coloring Agents present in Commercial Hair Dye Products with Coil 1A of Keratin Protein Type-I Cuticular Ha4 (KRT34)

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ABSTRACT

Different coloring agents are present in the commercial hair dye products which have been used to color hair. Apart from chemical coloring dyes, the natural coloring dye henna is also available to provide color to hair. In this paper we are reporting the interaction study of different coloring agents with the coil 1A of the hair keratin protein type I cuticular Ha4 (gene KRT34). We used molecular docking methods to provide information about binding affinity of different coloring agents with the hair keratin protein. We found that the commercial chemical coloring agents sodium metabisulfite and ammonium hydroxide, and the natural coloring agent catechin have higher binding affinities for the keratin protein.

INTRODUCTION

Currently, hair dyes are most commonly used to provide black, red, brown, golden and blue hair colors. These dyes interact with hair proteins and react to produce effect on hair texture [1]. Several commercial hair dyes are available in the market which are quite commonly used to color hair professionally and many people choose to do it at home. Many companies, such as L'Oréal, have been producing several at-home dye kits. These products are generally classified by their level of permanence. Their strength ranges from a rinse, which easily washes out after one shampoo, to permanent, which cannot be washed out with shampoo. Main colorant's ingredients present in these commercial hair dye products are: isoascorbic acid, polyglyceryl-4 oleyl ether, oleic acid, oleyl alcohol, phenyl methyl pyrazolone, sodium metabisulfite, fragrance/perfume, monoethanolamine (MEA), polyglyceryl-2 oleyl ether, resorcinol, water, trideceth-2 carboxamide MEA, pentasodium pentetate, 2-

Amino-3-hydroxypyridine, ethanol/SD Alcohol 40, ammonium hydroxide, hexylene glycol, PEG-2 oleamine, ammonium acetate, sodium diethylaminopropyl cocoaspartamide, paminophenol and 2-methylresorcinol [2]. However, there are some potential problems associated with these chemical hair dye agents such as, using hair dye can harm a baby if you are pregnant, a link for hair dye and cancer, hair loss, burning, redness, itchy, raw skin and face swelling and possible trouble breathing [2-7]. Therefore, it is necessary to understand the effect of hair dye agents interaction with hair and its component i.e. keratin hair protein. Keratin is one family of fibrous structural protein and key structural component of human hair. There are two types of hair/microfibrillar keratin, I (acidic) and II (neutral to basic) [8]. Among them, keratin, type I cuticular Ha4 is a protein that in humans is encoded by the *KRT34* gene. It is an acidic protein and belongs to the intermediate filament family. Experimental techniques have been implemented to detect interaction of dye compounds on hair proteins [1, 9]. In addition, many computational methods, e.g. docking, have been also used to predict potential affinity of compounds with proteins [10-15]. Therefore, in this work we are reporting the use of molecular docking methods for finding interaction of commercial hair dye agents and natural hair dye agents with the coil 1A region of the keratin protein KRT34.

MATERIALS AND METHOD

Protein input file and model building

The amino acid sequence of the coil 1A region (35 residues ranging from K99 to R133) of the keratin protein type I cuticular Ha4 (gene KRT34) was downloaded from UniProt protein database (http://www.uniprot.org/) with UniProt ID O76011 (KRT34_HUMAN) and saved in fasta format. Then, the sequence of the coil 1A of KRT34 was submitted to SWISS-MODEL server (http://swissmodel.expasy.org/) for model building by homology modeling method. After the homology modeling, the predicted model of the coil 1A of KRT34 was submitted to ProSA-web (prosa.services.came.sbg.ac.at/prosa.php), a protein structure analysis server [16]. ProSA-web determines the quality of the protein model and matches the predicted 3D model with the experimentally (X-ray and NMR) determined 3D structures of the proteins.

Coloring agents in commercial hair dye

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Information for different coloring agents and their ingredients present in the commercial products of cosmetic companies were obtained from internet [2]. Their 3D structures were obtained from PubChem Compound Database (https://pubchem.ncbi.nlm.nih.gov/). In addition, the structure of the natural plant coloring agent available in henna (*Lawsonia inermis*) i.e. catechin (PubChem CID: 9064) was also obtained from PubChem for comparison.

Docking of coloring agents with KRT34

The molecular docking of the coloring agents was performed with the modeled structure of the keratin protein (KRT34) using iGEMDOCKv2.1 software [17]. We selected the *Drug Screening* platform of iGEMDOCK for docking of compounds. The following parameters were selected for docking, such as Population size: 200, Number of generations: 70 and Number of solutions: 3. The coloring compounds were sorted at the end of docking process based on their interaction energies and fitness values produced by the docking via iGEMDOCKv2.1 software.

RESULTS AND DISCUSSION

SWISS-MODEL predicted putative homology 3D model of the coil 1A region of the KRT34 keratin protein (35 residues ranging from K99 to R133, Fig 1 by Discovery Studio http://accelrys.com/products/collaborative-science/biovia-discovery-studio/). This model has been developed using 1gk7 (chain A) as template pdb with sequence identity of 42.86 % .

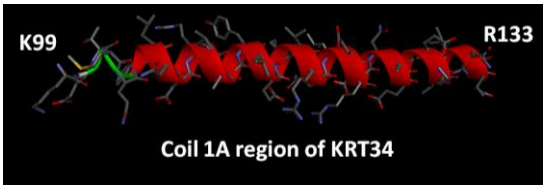

Fig. 1 The 3D model coil 1A region of the KRT34 keratin protein.

This 3D model was further submitted to ProSA server for protein structure analysis. ProSA server predicted that our 3D model matches with the protein structures determined by NMR method and produced low Z-score of -1.18 (Fig. 2).

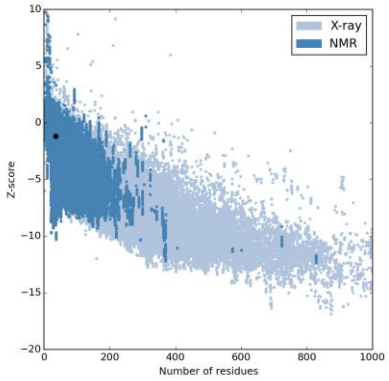

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Fig. 2 ProSA-web result of KRT34 coil 1A region homology 3D model; the black dot represents the model Z-score.

We used this predicted 3D model for our further docking interaction study. Subsequently, the 3D model was submitted to molecular docking study by iGEMDOCKv2.1 software. The docking software iGEMDOCK found lowest fitness value for the coloring agent sodium metabisulfite (-36.51 kcal/mol) followed by ammonium hydroxide (-16.37 kcal/mol), present in the commercial hair dye products (Table 1). The natural compound catechin was in 3rd rank among the coloring agents with fitness value of -4.46 kcal/mol, moreover compare to the commercial hair dye agents it may produce less side effects on hair (Fig. 3, Table 1).

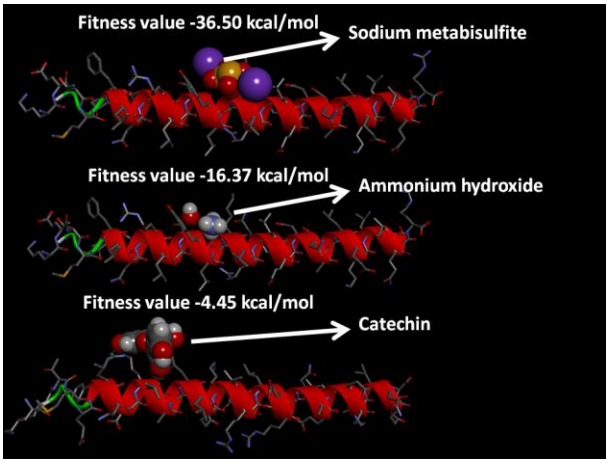

Fig. 3 Docking of coloring agents sodium metabisulfite, ammonium hydroxide and catechin with KRT34 coil 1A region.

Therefore, docking analysis predicts that commercial dye agent sodium metabisulfite has higher affinity for the hair keratin protein than the natural hair dye henna and chemically most efficient ingredients in the hair dye. On the other hand, the natural hair dye henna may produce less side effect on hair.

Table 1: iGEMDOCK fitness value (in kcal/mol) of coloring agents with keratin protein KRT34 coil 1A region.

RANK	FitnessValue	LigandName
1	-36.51	Sodium metabisulfite
2	-16.37	Ammonium hydroxide
3	-4.46	Catechin
4	44.02	Oleamine
5	99.11	Monoethanolamine
6	140.70	Oleyl alcohol
7	145.71	Pentasodium pentetat
8	155.36	Hexylene glycol
9	161.69	Ammonium acetate
10	230.22	Isoascorbicacid
11	236.26	OleicAcid
12	338.35	Polyglyceryl-2 oleyl

Interaction analysis shows that the sodium metabisulfite binds with the keratin protein by making hydrogen bond with the K115 amino acid residue, ammonium hydroxide with the S111 amino acid residue and natural dye

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catechin interacts with the keratin protein by making hydrogen bonds with the R108 and Y112 amino acid residues (Fig. 4).

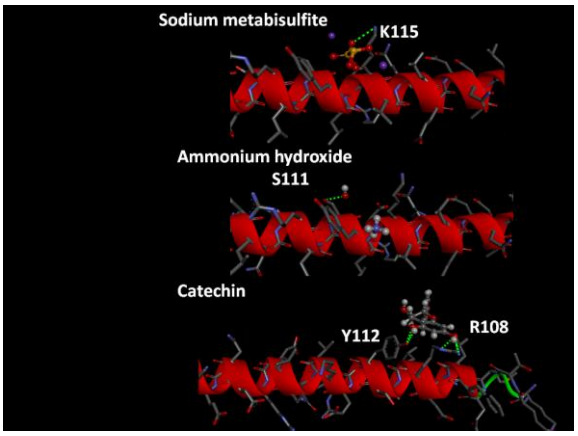

Fig. 4 Hydrogen bonding of coloring agents with keratin protein KRT34 coil 1A region.

Sodium metabisulfite (Fig. 5) is seen as an ingredient primarily in hair care products, such as formulas for permanent waves, hair bleaches, hair dyes, colors and tints, and also in some bath and skin care products (CosmeticsInfo.org).

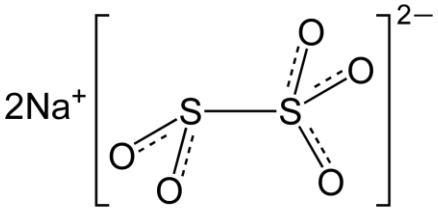

Fig. 5 Sodium metabisulfite

Catechin is a flavan-3-ol, a type of natural phenol and antioxidant. It is a plant secondary metabolite. It belongs to the group of flavan-3-ols (or simply flavanols), part of the chemical family of flavonoids (Fig. 6).

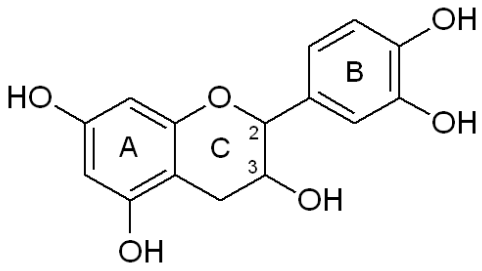

Fig. 6 Catechin

CONCLUSION

We obtained the homology 3D model of coil 1A region of the keratin protein KRT34 which provides useful information about the structure of the coil region of the keratin protein. Furthermore, molecular docking study provides detailed insight about binding affinity of different coloring agents with the hair keratin protein. The commercial chemical coloring agents sodium metabisulfite, ammonium hydroxide and natural coloring agent catechin display good binding affinity for the coil 1A region of the keratin protein. This study reveals the application of molecular docking methods to select best coloring agents among many based on their binding affinity toward hair keratin and will provide a clue for interaction of best dye ingredients with hair.

This study may help the cosmetologist to design the most efficient contents of the hair dye.

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A Comprehensive Study on Grid Performance Enhancement and Server Fault Penalty Minimization on JPPF Grid for *E.coli* Genome Sequence Alignment Problem

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ABSTRACT

Recent advancement in computational analysis in the arena of genomics and proteomics has necessitated the use of high performance computing architectures such as grid, cluster and parallel computing. In this paper an attempt has been made to study the performance enhancement by optimization of RL/NT load balancing algorithm followed by node provisioning and minimization of performance penalty caused by hardware failure of the grid server. An E.coli genome sequence alignment (using PAM 120 substitution matrix) was performed on the grid We have achieved a performance gain of 13.79 times with just 8 processing nodes in comparison to serial execution time. The grid has been made fault tolerant by introducing peer server and performance penalty has been reduced 3.72 times by introducing additional peer grid server.

INTRODUCTION

The rapid growth in genomics and proteomics arena has led to the development of incipient generation of distributed systems such as Grid Computing. Grid is extensively used in computational biology and bioinformatics like comparative genome analysis [1]. Performance of grid depends on the appropriate selection of load balancing algorithm at grid server [2]. JPPF [3] Grid middleware[4] has been used in our experimental grid with 8 nodes/ 3 servers and the sequence alignment of E.coli genome was executed. Two load balancing algorithm i.e Reinforcement Learning (RL) and Node Threads (NT) Algorithm has been studied. Job execution time of sequence alignment has been studied for all possible parameters of

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both load balancing algorithm and seedup factor has been calculated [5]. We have studied performance enhancement by parameter adjustment of load balancing, node provisioning. Finally the grid has been made fault tolerant[5] by adding two peer grid servers. The performance penalty caused by the server failure has been studied and minimized by addition of more peer servers.

MATERIALS AND METHOD

The Server[CPU (4 Cores): Intel (R) Core ™ i7-337 3.4 GHz, 8MB RAM] , Nodes [CPU (2 Cores): Intel (R) Core 2 Duo 3.4GHz, 2GB RAM], Interconnection Network [Gigabit Ethernet 1000Mbps, D-Link Switch 24-Port 10/100/1000],OS [Windows 7], Grid Middleware[JPPF 5.0.1], JDK 1.7, Apache Ant, version 1.9.4 and Sequence data [1.7 MB E.coli genome and 2 KB DNA polymerayse III,alpha subunit sequence], Grid Enabled Sequence Alignment Program supplied in JPPF Sample pack 5.0.1.

We have submitted a sequence alignment (E.coli genome with DNA polymerayse III,alpha subunit using PAM120 matrix) job from a client(g_c) to our grid server(g_s) and upon completion of the job we have noted the total job execution time (Θ_{Tik}) through the JPPF Admin UI.We have analysed two load balancing algorithms i.e. Reinforcement learning and Nodethreads algorithm. For Nodethreads algorithm we have used 2,4,6,8 and 10 threads per grid node and recorded the total execution time for each thread values. The network communication overheads L_{gsgk} (the task delivery time over head from the grid server gs to a grid node gk), L_{gkgs} (the task result delivery time over head from a grid node gk to the server gs), L_{gcgs} (job submission time over head from the client to the grid server gs), L_{gsgc} (the result delivery from the server gs to the client gc), has been recorded for each run of sequence alignment. The ΔL has been computed as max ($L_{gsgk}+L_{gkgs}+\Theta_{Tik}$) and Θ_{ES} (time it takes to run a sequence alignment on grid) has been computed as $L_{gcgs} + L_{gsgc} + \Delta L$. The Θ_{E1} is the time it takes to run an entire a sequence alignment on a single processing unit has also be noted down. The speedup factor[5] has been calculated as SpeedUp Factor (S) = Θ_{E1}/ Θ_{ES} which is derived from Amdal's law. The same has been repeated for RL algorithm. In case of RL algorithm two parameters i.e. performance cache size and performance variation

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threshold has been studied. The cache size studied were 1000,2000,3000,4000 and 5000.For each cache size we have studied performance variation threshold of 0.000,0.001 (default),0.010,0.10,1 and 10.For each combination of parameters in both the algorithms we conducted three runs of sequence alignment and taken the mean job execution time and mean of network communication overhead. In each algorithm the best performance configuration has been used for comparing the algorithms. The best Load balancing algorithm has been further optimized by node provisioning. In node provisioning we have studied creation of slave nodes (1 to 10) for each grid node. The grid has been made fault tolerant by adding peer servers and performance penalty has been studied and performance penalty has been minimized by additional peer servers.

RESULTS AND DISCUSSION

The sequence alignment job submitted from client. The Θ_{ES} has been measured for RL load balancing and Node thread load algorithm. The result is shown in Table 1 & 2 and Figure 1 & 2 , where as Θ_{E1} also measured and found to be 79483.6667 *ms*.

Table 1. Performance Analysis of NT Algorithm

Thread #	Θ_{Tik}	$L_{gsgk}+L_{gkgs}$	$L_{gsgs}+L_{gsgc}$	ΔL	Θ_{ES}	$(S) = \Theta_{E1}/\Theta_{ES}$
2	17004.00	7.17	3.00	17011.17	17015.50	4.67
4	10805.66	2.17	1.33	10807.83	10809.17	7.35
6	9126.00	2.25	43.38	9128.25	9171.63	8.66
8	8076.00	1.29	6.31	8077.29	8083.60	9.80
10	8086.33	46.61	13.50	8132.94	8146.44	9.75

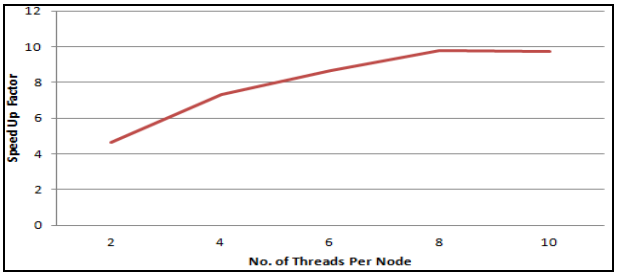

Fig. 1 Graphical representation of Speedup Factor with increase in number of threads in Grid Nodes using Node Thread Algorithm at Grid Server.

Table 2. Performance Analysis of RL Algorithm

Performance Cache Size	Performance variation Threshold	Θ_{Tik}	$L_{gsgk}+L_{gkgs}$	$L_{gsgs}+L_{gsgc}$	ΔL	Θ_{ES}	$(S) = \Theta_{E1}/\Theta_{ES}$
1000	0.000	7004.00	0.67	66.08	7004.67	7070.75	11.24
	0.010	7303.00	0.14	0.03	7303.14	7303.17	10.88
	0.1	32105.00	17.00	11.00	32122	32133	2.47
	1	32635.00	54.00	19.27	32689	32708.27	2.43
	10	32168.00	20.00	0	32188	32188	2.46
	0.001(default)	7352.00	0.66	52.03	7352.66	7404.69	10.73
2000	0.000	6887	6.085	34.31	6893.09	6927.39	11.49
	0.010	6917.5	0.175	0.145	6917.68	6917.82	11.48
	0.1	26286.17	9.515	5.69	26295.69	26301.38	3.022
	1	32416.5	37.75	9.76	32454.25	32464.01	2.44
	10	32159.75	19	3.5	32178.75	32182.25	2.469
	0.001(default)	7435.5	0.405	26.025	7435.905	7461.93	10.65
3000	0.000	6770.00	11.5	2.54	6781.5	6784.04	11.72
	0.010	7532.00	0.21	0.26	7532.21	7532.47	10.55
	0.1	20467.3333	2.03	0.38	20469.36	20469.74	3.88
	1	32198.00	21.5	0.25	32219.5	32219.75	2.46
	10	32151.50	18.00	7	32169.5	32176.5	2.47
	0.001(default)	7519.00	0.15	0.02	7519.15	7519.17	10.57

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4000	0.000	6804.167	6.09	1.475	6810.26	6811.73	11.67
	0.010	8084.5	0.215	0.13	8084.72	8084.85	9.83
	0.1	26321	11.815	1.14	26332.82	26333.96	3.01
	1	32205.33	20.05	1.99	32225.38	32227.37	2.46
	10	32420.58	22.15	5.18	32442.73	32447.91	2.45
	0.001(default)	7225.67	0.27	0.025	7225.937	7225.96	10.99
5000	0.000	6838.33	0.68	0.41	6839.013	6839.42	11.62
	0.010	8637.00	0.22	0	8637.22	8637.22	9.20
	0.1	32174.67	21.6	1.9	32196.27	32198.17	2.46
	1	32212.67	18.6	3.73	32231.27	32235	2.47
	10	32689.67	26.3	3.36	32715.97	32719.33	2.43
	0.001(default)	6932.33	0.39	0.03	6932.72	6932.75	11.46

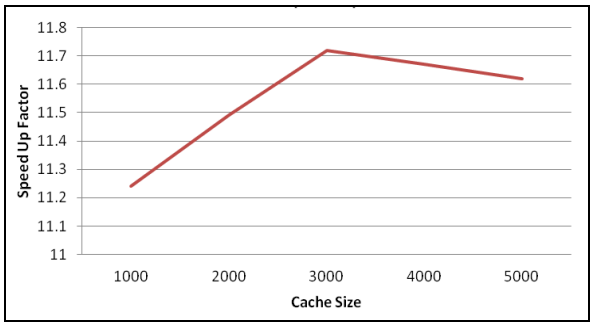


Fig. 2 Graphical representation of Speedup Factor with increase in cache size in Reinforcement Learning Algorithm

Table 3. Performance Analysis of Node Provisioning in RL Algorithm

No. of slave Nodes	Θ_{Tik}	$L_{gsgk}+L_{gkgs}$	$L_{gcgs}+L_{gsgc}$	ΔL	Θ_{ES}	(S) = Θ_{El}/Θ_{ES}
1	5720.00	13.68	29.20	5733.68	5762.89	13.79
2	5798.00	14.75	20.87	5812.75	5833.62	13.62
3	5917.67	3.98	189.28	5921.64	6110.93	13.00
4	6110.00	28.63	354.98	6138.63	6493.62	12.24
5	6687.33	21.61	229.33	6708.95	6938.28	11.45
6	6635.00	173.57	389.80	6808.57	7198.37	11.04
7	7888.33	10.08	1.4433	7898.41	7899.86	10.06
8	17565.33	46.85	69.29	17612.18	17681.47	4.49
9	31101.33	256.89	120.68	31358.23	31478.90	2.52
10	55224.33	109.85	79.34	55334.18	55224.33	1.43

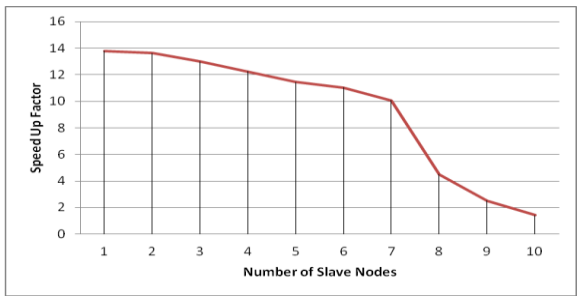
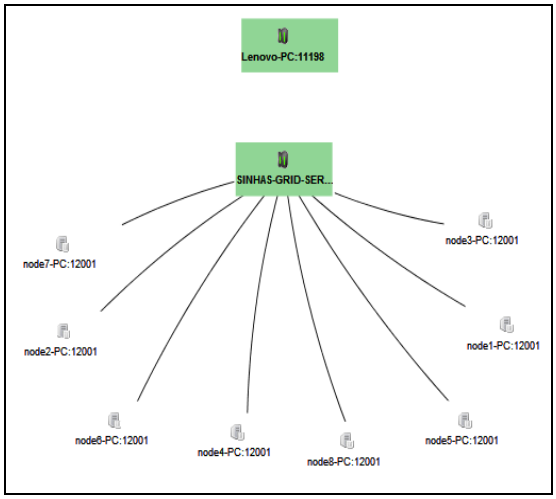
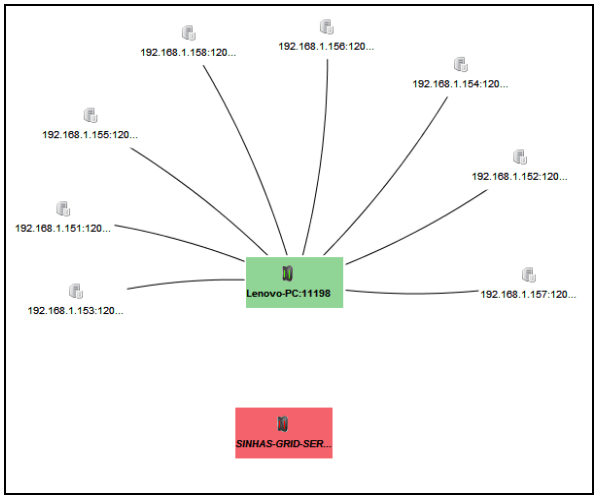


Fig. 3 Graphical representation of Node Provisioning on RL Algorithm optimized configuration. Speedup Factor is shown with increase in number of slave nodes in each node



4(a)



4(b)

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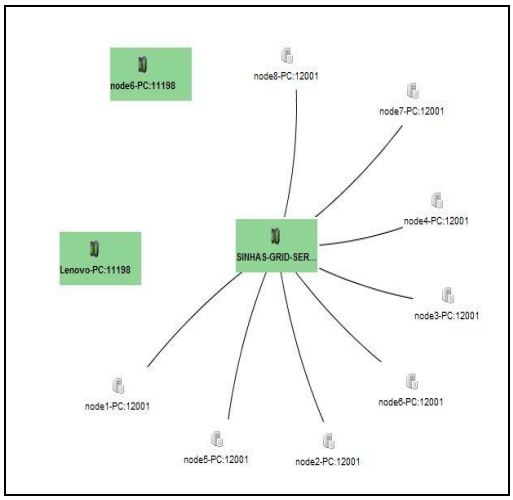
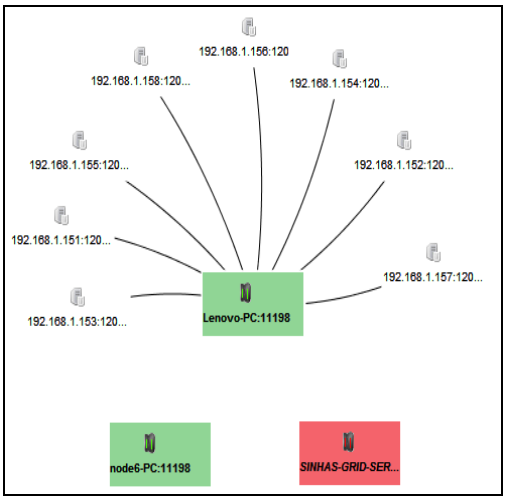


4(c)



4(d)

Fig. 4 Failure in grid server has increased Θ_{Tik} from 6770 *ms* to 27939.50 *ms* but with a additional peer server Θ_{Tik} has reduced from 27939.50 ms to 13244 *ms* which means 3.72 times penalty minimization

CONCLUSION

Performance of grid depends on the configuration of grid by the appropriate selection of load balancing strategy. Performance of load balancing algorithm depends on appropriate adjustment of parameters of load balancing. In the result section it can be found that we have achieved a Speed Up of 11.72 times with just 8 processing nodes as compared to serial execution time in Reinforcement Learning load balancing algorithm by adjustment of load balancing parameters and further enhancement of speedup of 13.79 with node provisioning with RL algorithm. The grid has been made fault tolerant less performance penalty, penalty has been reduced 3.72 times by introducing additional peer grid server.

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Short notes on comparison of Molecular docking, Molecular Dynamics and QM/MM methods

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ABSTRACT

Molecular Modeling methods such as Molecular Docking, Molecular Dynamics, Computational Chemistry tools and QM/MM are frequently used in solving Biological, Biochemistry, Molecular Chemistry problems and in the field of Drug Discovery. In combined application, these methods are very powerful to predict and to determine the interaction between the compound and DNA or Protein. In this paper, we are giving shorts notes about advantages and limitation of these methods which may help to select the kind of method to study the interaction between the compound and DNA or protein.

INTRODUCTION

Molecular Modeling methods such as Molecular docking, Molecular Dynamics, Gaussian and QM/MM etc. have been extensively used to determine the interaction between small compounds and proteins or DNA and also characterization of the chemical compounds. Several reports are available in literature to describe their applications in solving biological problems and chemical compounds characterization [1-8]. Here, in this short communication, we are describing the advantage and limitation of these methods.

Molecular Docking

Molecular docking determines the affinity of the ligand molecule towards a target whose 3D structure is known. During the docking process, the ligand generates multiple binding geometries (binding modes) in relation to the receptor. Only the stable conformation of the ligand binds with the receptor [9]. The most important goals of molecular docking are: 1. Characterization of the binding sites **2.** Positioning of the ligand into the binding site

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(Fig. 1) and **3.** Evaluating the strength of interaction for a specific ligand receptor complex [10].

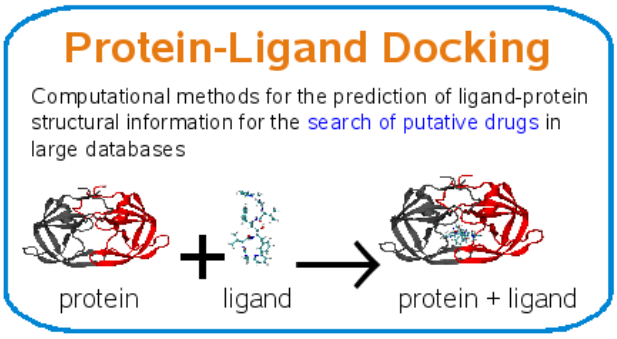

Fig. 1 Binding of Ligand with Protein. (Source: http://docking.cis.udel.edu/images/science/protein_ligand_docking.png)

To achieve these precise objectives, various docking programs that implement new algorithms have been developed [10]. Therefore, based on the change in conformations of the ligand and protein, two categories of docking process are in use: (1) Rigid docking and (2) Flexible docking.

Rigid docking

In rigid docking, the protein molecule is considered to be rigid and while the ligand is allowed to change its conformation and orientation to evaluate the binding pose and affinity. The ligand produces all possible translations and rotations to bring about all feasible alterations and establish minimal energy stable bound conformation at binding site pocket of protein [11].

Flexible docking

The flexible docking is also known as soft docking where both the protein and ligand molecules are allowed to undergo conformational flexibility to produce a better protein-ligand interaction. The mechanism of action of soft docking employs the principle of lock-and-key binding theory proposed by Emil Fischer in 1890. As per this hypothesis, ligand molecule fits into a specific binding site of the receptor protein. In addition, an explicit coherent induced-fit model of protein - ligand binding was elaborated by Koshland in 1958. In this model, both protein and ligand are flexible and undergo conformation changes to form perfect fit minimal energy complex.

Several softwares are available to perform docking. These include DOCK [12], AUTODOCK4.0 [13], GOLD [14], FlexX [15] etc. Improvements in docking algorithms have increased the speed and accuracy of these programs. All the docking programs are

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associated with scoring function for finding interaction during the course of protein and ligand interactions.

Energy function for molecular docking

Molecular docking simulations entail different energy evaluation steps. The energy function determines the non-covalent interactions within proteins, with solvent & ligands and predicted stability of a protein. The forcefield terms have the van der Waals force, 1-4 interaction, and coulombic electrostatics energies.

Force field Energy

Forcefield energies are described by the total potential energy of the system of particles and calculated using the AMBERand CHARMM.

$\Delta G = E_{forcefield} + E_{solvation} - E_{reference} - T\Delta S$ (*Entropy*)

The force field in docking is defined by both bonded and non-bonded terms. The bonding terms include covalent bonds, hydrogen bonds while the non-bonded terms include the long-range electrostatic and van der Waals forces. The total energy can be written as follows:

$E_{total} = E_{covalent} + E_{noncovalent}$

where the components of the covalent and noncolvalent contributions are given by the following summations:-

$E_{covalent} = E_{bond} + E_{angle} + E_{dihedral,}$

$E_{noncovalent} = E_{electrostatic} + E_{vanderwaals,}$

The total force field parameters are

$$\Delta G = \Delta G_{\mathrm{vdW}} \sum_{ij} \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} \right) + \Delta G_{\mathrm{hbond}} \sum_{ij} \left(\frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}} + E_{\mathrm{hbond}} \right)$$

$$+\Delta G_{\mathrm{elec}} \sum_{ij} \frac{q_i - q_j}{s(r_{ij}) r_{ij}} + \Delta G_{\mathrm{tor}} N_{\mathrm{tor}} + \Delta G_{\mathrm{sol}} \sum_{icj} S_i V_j \mathrm{e}^{\left(\frac{-r_{ij}^2}{2\sigma^2}\right)}$$

Along with Flex scoring function: f is a penalty function for deviations from ideal geometry for each kind of interaction, and f* is a function penalizing for lipophilic interactions deviating from an ideal separation distance.

$$\Delta G = \Delta G_0 + \Delta G_{rot} \cdot N_{rot} + \Delta G_{H-bond} \sum_{H-bonds} f(\Delta R, \Delta\alpha) + \Delta G_{ionicinteractions} \sum_{ionicinteractions} f(\Delta R, \Delta\alpha) + \Delta G_{aromatic} \sum_{aromaticinteractions} f(\Delta R, \Delta\alpha) + \Delta G_{lipophilic} \sum_{lipophilicinteractions} f^*(\Delta R)$$

(Source: http://cnx.org/content/m11456/latest/flexxscore.jpg)

However, these scoring functions still have some limitation to provide accurate docking results.

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Problems in molecular docking and virtual screening

Molecular docking programs are widely used for performing docking simulations between the lead compounds and the receptor molecules. The ligands can be obtained from databases and screened against the structure of the receptor. However, the main drawback in the docking process is to provide flexibility to the receptor molecule. Solvation parameters are also incorporated to improve the docking efficiency. However further refinement is needed to improve the accuracy of docking results. Selection of the force field poses another challenge in molecular docking. The force field parameters such as energy calculations for bonding and non-bonding atoms need to be improved. Although, the molecular docking is able to provide clues about effective molecules from diverse set of compounds, there is need to further verify the results via analyzing docking mode, r.m.s.d. from original coordinates and position for binding of the lead compounds. In addition, the results may conflict with more number of compounds having same affinity for desired receptor.

Advantage and Disadvantage

Drug–DNA interactions have been extensively studied in the recent past. Number of techniques have been employed to decipher these interactions. DNA is a major target for a wide range of drugs that may specifically or non-specifically interact with DNA and affect its functions. Interaction between small molecules and DNA are of two types, covalent interactions and non-covalent interactions. Three major modes of non-covalent interactions are electrostatic interactions, groove binding and intercalative binding. Here, i am summarizing the techniques used for finding the interactions between drugs and DNA and possible outcomes which are reported in the literatures. Interaction of different probes have been studied using different computational techniques such as Molecular docking, Quantum Mechanics/Molecular Mechanics (QM/MM), Classical and Monte Carlo molecular dynamics methods.

Molecular Docking based DNA-Probes interaction

Molecular Docking

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Special notes:

Advantage of Molecular docking:

1: Quickly predicts the binding mode and binding site on DNA for our probes.

2: Different conformations of the probes are easily ranked based on binding energies and make the analysis very easy.

Disadvantage of Docking:

1: Docking gives rough estimation of binding energy (not accurate) between DNA-Probes interaction as DNA flexibility is not taken into account.

2. Effect of explicitly solvent is not take into account during the docking process.

3: There is no salt effect during the DNA-Probes docking simulation.

Molecular docking plays an important role in establishing the interaction between different molecules and helps in structure based drug discovery. Docking is usually performed between small molecules (Ligand) and a target macromolecule like protein and DNA. The use of automated molecular docking software helps in predicting the binding mode of the ligand, formation of energetically favorable complex and predicting binding affinity. Molecular docking provides information about the mechanism of binding of various drugs. Molecular docking also corroborates experimental results and helps in understanding the mechanisms of interaction.

Molecular docking programs are commonly employed in determining the binding mode of various drugs where intercalators and groove binders are easily distinguished. Figure 1 shows the docking of the probe with the DNA. Here, probe interacts with the DNA at different positions and generates corresponding free energy of binding. The best binding mode of the probe is selected based on the lowest binding energy on the specific position of the DNA (Fig. 2).

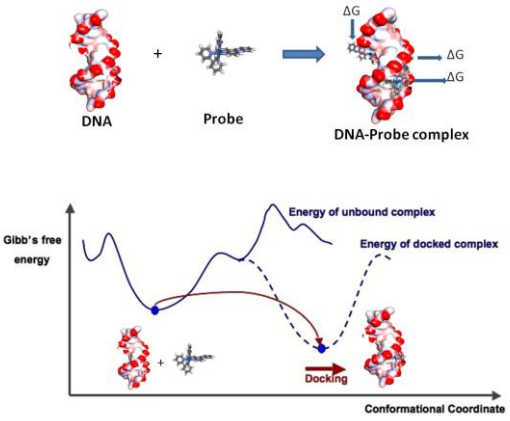

Fig. 2 Example of Docking process where probe binds at the DNA and results in generating lower binding energy complex.

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Conversion of Binding energy into calculated binding constant:

Δ G (binding) = -R*T*ln Kb

Δ G (binding) = -2.303 R*T*$\log_{10}$ Kb

Kb = $10^{-\Delta G/(2.303RT)}$

Substituting $R = 1.987 \times 10^{-3}$ kcal mol^{-1} deg^{-1} and T = 298.15 K (corresponding to 25°C)

$\mathbf{K_b=10^{-\Delta G/1.36}}$

Molecular Dynamics (MD) and Quantum Mechanics /Molecular Mechanics (QM/MM) approaches for DNA-Probe interaction

The structural and dynamic effects of DNA-Probe interaction can be addressed by simulations of DNA-Probe complex in explicit solvent which is the advantage on docking. Here, we are discussing the state-of-art of DNA-Probe complex molecular dynamics simulations. The reported work explained why a critical analysis of the MD trajectories is required to assess their reliability, and estimate the value and limitations of MD which are as follows:

Molecular dynamics is a computer simulation method where the DNA-probe flexibility can be maintained. Also, the effect of solvent can also produced on the system. MD provides facility to choose the environmental conditions for DNA-Probe simulations such as variation of salt concentration and variation of Temperature which may be correlated with the experimental conditions. The MD method used to generate variation in the DNA-Probe interactions along with the MD simulation time which can be analyzed at different time interval (Fig. 3). There are some pros and cons associated with MD method:

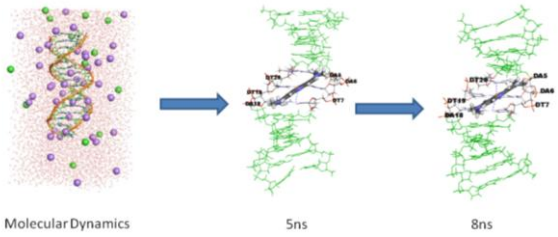

Fig. 3 Interactions during the MD simulation time

Energy equation of Molecular Dynamics simulation:

The generalized potential energy (V(F)) terms comprise in the Molecular dynamics simulation are :

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$$V(\mathbf{r}) = \sum_{\text{bonds}} \overset{(1)}{K_b}(b - b_0)^2 + \sum_{\text{angles}} \overset{(2)}{K_\theta}(\theta - \theta_o)^2 + \sum_{\text{dihedrals}} \overset{(3)}{K_\chi}(1 + \cos(n\chi - \delta)) + \sum_{\text{nonbonded-pairs},i,j} \left[\overset{(4)}{\frac{q_i q_j}{4\pi e_o r_{ij}}} - \varepsilon_{ij} \left\{ \left(\frac{R_{\min ij}}{r_{ij}} \right)^{\overset{(5)}{12}} - 2 \left(\frac{R_{\min ij}}{r_{ij}} \right)^{6} \right\} \right]$$

Energy dependencies on:

1. Bond length
2. Bond valence angle
3. Bond dihedral angle
4. Non-bonded electrostatic interactions
5. Non-bonded van-der Waals interactions

Advantage of Molecular Dynamics simulation:

1. More accurate than docking simulation for describing DNA-probe interaction.
2. Explicitly solvent effect can be produced during MD simulation.
3. Salt effect can be described during the simulation.
4. DNA flexibility can be easily maintained in MD simulation which produces improvement in the DNA-probe interactions such as hydrogen bonding.
5. Time evolution of different classical properties can be described such as Temperature, potential energy, Hydrogen bonding, RMSD etc. between DNA and Probe which is not possible in docking process.

Disadvantage of Molecular Dynamics simulation:

1. Information about accurate force field file is needed for running the MD simulation such as we have limitation of Osmium probe force fields parameters.
2. No straightforward information about binding energy and binding constant of DNA-probe interaction. Such limitations of MD can be solved by using combined Quantum Mechanics /Molecular Mechanics (QM/MM) approach.
3. Simulation time is very long and very expensive.

Combined quantum-mechanics/molecular-mechanics (QM/MM) approaches have become the method of choice for finding the interaction between the metal complex and DNA. QM/MM method is very accurately able to predict the binding mode and interaction between the probe and DNA.

QM/MM

Introduction

In principle, there are several techniques for monitoring in vitro binding of drugs to the double-helical DNA such as spectroscopy,

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voltammetry and molecular mechanic calculations (Molecular Modeling). In fact, molecular mechanic force fields, based on quantum mechanics, have been used to determine structural and energetic properties of isolated molecules and can be used to examine molecule–molecule and molecule–biomolecule interactions. The critical point of DFT, HF or ab initio calculations for large biomolecules is accessed to obtain accurate and reliable results.

Thehybrid **QM/MM** (quantummechanics/molecular mechanics) approach is a molecular simulation method that combines the strengths of the QM (accuracy) and MM (speed) approaches, thus allowing for the study of chemical processes in solution and in proteins. In this way, the computational chemistry techniques, especially the hybrid methods (ONIOM, Own N-layer Integrated molecular Orbital molecular Mechanics) based on combination of several theoretical approaches, have been developed by Morokuma and co-workers [16] for large biomolecule systems. ONIOM is a powerful and systematic method that divides the system into onion-like layers. The chemically important region is treated with accurate high level Quantum Mechanics (QM) method and the rest of the layers are treated with computationally less intensive lower level QM (for example, semi-empirical) or Molecular Mechanics (MM) methods. This balance between efficiency and accuracy makes the ONIOM method a more practical tool in the study of large systems than a single ab initio or MM method.

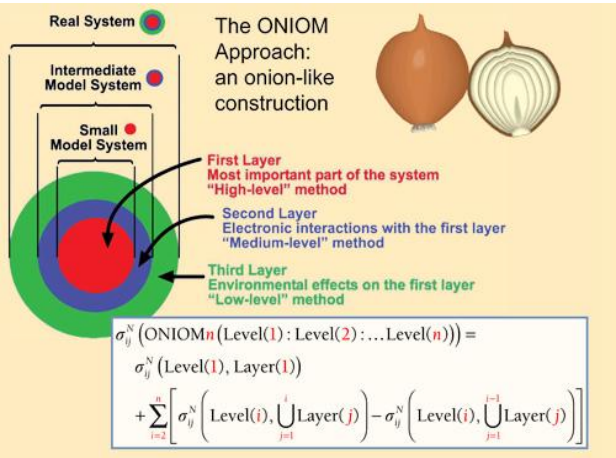

Fig. 4: ONIOM method [16]

The interaction energy produced by QM/MM calculation is described as:

$$\Delta E_{(\text{Interaction energy})} = E_{(\text{DNA-Probe complex})} - E_{(\text{DNA})} - E_{(\text{Probe})}$$

Major Advantage of QM/MM

1. The hybrid **QM/MM** (quantum mechanics/molecular mechanics)

approach is a molecular simulation method that combines the strengths of the QM (accuracy) and MM (speed) approaches.

2. It is accurately able to predicts π-π stacking and hydrogen bonds interactions between DNA and probe.
3. Solvent effect can be produced by the Polarizable Continuum Model (PCM) implemented in Gaussian software (solvent effect=dielectric constant). However, single drawback is associated with QM/MM is that there is no salt effect during the calculation.

Major Disadvantage of QM/MM

1. No salt effect.
2. Simulation time is very long.

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