ANALYSIS OF CUPREDOXIN AND CO-EXPRESSED PROTEINS OF PSEUDOMONAS AERUGINOSA LESB58 USING BIOINFORMATICS TOOLS

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Abstract

Objectives: To identify the bioinformatics tools that can be used as the easiest source to analyze the properties of proteins. This activity will also enable the researchers to easily apply and predict drug target sites and other biotechnological researches against microbial infections.

Methodolgy: Cupredoxin and the co-expressed partners' retrieved from string database are mainly responsible for electron transfer and other important functions. The current analysis was conducted to investigate the amino acids composition, structure, hydropathecity, phylogenetic analysis, aliphatic index molecular weight and some other properties of cupredoxin and its functional partners of pseudomonas aeruginosa LESB58 using bioinformatics tools.

Results: The analysis showed that some of the functional partners of cupredoxin are unstable. These protein located in periplasmic space and cytoplasm. Leucine is the most abundant amino acid followed by alanine. Majority of amino acids possess negative charge. Phylogenitically all these proteins have common ancestor.

Conclusion: Bioinformatics tools can easily be applied to study the characteristics and comparison of different proteins.

Keywords: Cupredoxin, Amino Acid, Analysis, Bacteria, Bioinformatics Tools

Introduction

Azurin which is also termed as cupredoxin is copper containing proteins involved in electron transfer reactions (Vijgenboom et al. 1997). This protein in *Pseudomonas aeruginosa* transfer electron during enzymatic

reactions. Amino acid composition, molecular weight varies in this protein obtains from various species of pseudomonas. Alanine was reported as highest in quantity (Thirunavukkarasu et al. 2011).

Denitrification phenomena are anaerobic process where the terminal electrons receptors are nitrogen oxides. The function of NOR enzymes is highly reduced in case of mutation in nirQ/nirQ and the mutant strain of *Pseudomonas aeruginosa* loss the power to grow in anaerobic environment (de Boer et al. 1996, Bartnikas et al. 1997, Jüngst and Zumft 1992). Cytochrome c551 (NirM) of P. aeruginosa is an orthologous of cyt-8. This cytochrome is involved in electron transfer from cyt-bc1 to Cyt-cd-type nitrite reductase (Zumft 1997, Averill 1996, Canovas 2003).

The gene nosZ encode N_2OR in Pseudomonas stutzeri (Zumft 1997). Five more nos genes are present in nos operon i.e. nosR, nosD, nosF nosY and nosL. NosR encodes for transcription regulator, nosD, nosF and nosY are involved in ABC transporter while nosL product's is copper chaperone (Gudat et al. 1973). The pyochelin synthase (pchG) is similar to ATP binding cassette transport proteins with export function (Punj et al. 2004). PcoA gene contain multicopper oxidase domain (SufI domain; COG2132 [http://www.ncbi.nlm.nih.gov/COG]) which may be involve in conversion of Cu to Cu^{-2} which is less toxic (Hasegawa et al. 2003).

Periplasmic proteins play a significant role signaling in microorganisms. Nitrite reductase reduces nitrite to nitric oxide in soil (Honisch & Zumft 2003). Nos operons codes nosD, nosF, nosY, and nosL. Each of these involve in assembly of enzymes. NosR and nosL encodes for transcription and copper chaperon respectively while remaining nos genes code for regulatory proteins (Reimmann et al. 2001). PchG is involved in reducing thiazoline to thiazolidine ring (Jorda & Yeates 2011). The role of some microbial enzymes to convert high toxic material to less toxic helps in survival of pathogens. PcoA has a multicopper domain that might convert Cu^{+1} to Cu^{+2} leaving it less toxic. Phylogenetic analysis reported in the current is also similar to other studies (Canovas et al. 2003).

Therefore, the current_analysis was designed to study the properties of azurin proteins and their functional partners using bioinformatics tools.

Materials and Methods

The study of microbial proteins is considered important for their practical applications as well to control their serious infections. Cupredoxin protein also known as azurin proteins produced by several genera of pseudomonas, Bordetella and some other genera. These proteins are involved in electron transfer during metabolism in living systems.

Tools used

String database was used to search the azurin of *pseudomonas aeruginosa LESB58* and its functional partners. The interactions, amino acids sequences and protein structures were also retrieved from the string database. BlastP and PROTPARM tool (http://web.expasy.org/protparam/) of ExPASy were used for amino acids composition, Formula, number of atoms, aliphatic index, hydropathecity, positive and negative residues, isoelectric point and molecular weight. Mega-6 tool and pyre-2 was used for phylogenetic analysis, amino acid composition and structure prediction of unknown proteins partners.

Results

Amino acids compositions are greatly varied from 104 of nirM to 715 of nosR. Leucin is the most predominant amino acid followed by alanine and glycine. The pH at which the net charge on the protein become zero is called isoelectric point (pI), play an important role in protein function. The pI value determined in the current analysis was maximum (7.15) for nir and lowest for aspP (4.95). Cysteine proteins play a role in the stability of protein (Zhao et al. 2011). Cysteine number in each protein of current analysis given in the figure 13. Proteins stability index in (Table 2) shows the structure stability of azurin and its functional partners of *P.aeruginosa*. The current analysis showed that some proteins are unstable. Hydrophobicity or hydrophilicity relative of a compound especially amino acids is termed as hydropathecity. Hydrophobic amino acids have large hydropathecity value while hydrophilic have low. This Property is important in predicting the regions that are exposed to surface of membrane. We have find that most of the azurin functional partners are exposed to membrane surface and have antigenic importance (Table 2). Further, most of the predicted functional partners of azurin are hydrophilic and their function is greatly affected by water.

The relative volume occupied by aliphatic side chain of Leucin, isoleucine, alanine and valine is called aliphatic index. High aliphatic index was observed for azurin and its predicted functional partners (Table 2). Amino acid composition of each of these proteins is given in the table Figure. Molecular Phylogenetic analysis by Maximum Likelihood method (Jones et al. 1992 & Tamura 2013).

S .No.	Gene	Product	No. of a.a
1.	nir	nitrite reductase precursor	568 aa
2.	nirM	Cytochrome c-551 precursor; Electron donor for cytochrome cd1 in nitrite and nitrate respiration.	104 aa
3.	hemE	Uroporphyrinogen decarboxylase; Catalyzes the decarboxylation of four acetate groups of uroporp.	355 aa
4.	nos Z	Nitrous-oxide reductase; a part of a bacterial respiratory system.	639 aa
5.	aspP	Adenosine diphosphate sugar pyrophosphatase.	205 aa
6.	nirQ	Regulatory protein NirQ; Activator of nitrite and nitric oxide reductase.	260 aa
7.	nosR	Regulatory protein NosR; Transcriptional activator of the nitrous-oxide reductase gene nosZ.	715 aa
8.	pcoA	Copper resistance protein A precursor.	614 aa
9.	rpoS	RNA polymerase sigma factor RpoS; involve in initiation factors that promote the attachment.	334 aa
10.	pchG	pyochelin biosynthetic protein PchG.	349 aa

Table 1. Symbols, amino acid number and function of azurin and its functional partners.

Protein	azu	Nir	nirQ	nirM	pchG	rpoS	pcoA	nosR	aspP	nosZ	hemE
Location	P.S*	P.S*	Cyto*	P.S*	Cyto	Cyto	P.S*	Cyto	Cyto	P.S*	Cyto
M.W (KD)	16.008	62.653	28.904	10.966	37.966	38.235	68.073	80.269	23.133	70.96	38.822
No.bp	447	1707	783	315	1050	1005	1445	2148	618	1920	1068
Formula	$C_{703}H_{1130}N_{18}{}_{8}O_{217}S_{10}$	$C_{2806}H4_{398}N_{7}{}_{68}O_{832}S14$	$C_{1278}H_{2029}N_{3}{}_{67}O_{378}S1_{0}$	$C_{491}H_{773}N_{30}$ $O_{144}S_{5}$	$C_{1675}H_{2683}N_{4}{}_{85}O_{487}S_{9}$	$C_{1680}H_{2724}N_{4}{}_{82}O_{523}S_{6}$	$C_{2985}H_{4620}N_{8}{}_{54}O_{895}S_{40}$	$C_{3648}H_{5700}N_{9}{}_{94}O_{1001}S_{29}$	$C_{1026}H1_{609}N{}_{295}O_{300}S_{8}$	$C_{3133}H_{4904}N_{8}{}_{81}O_{942}S_{30}$	$C_{1732}H_{2710}N_{4}{}_{78}O_{505}S_{16}$
No. of atoms	2248	8818	4062	1548	5339	5415	9394	11367	3238	9891	5441
Positive	14	66	28	10	33	47	62	77	22	78	34
Negative	15	65	37	10	43	60	79	80	34	89	40
High	Leu	Ala	Leu	Ala	Leu	Leu	Gly	Leu	Leu	Gly	Ala
P.I*	6.39	7.75	5.31	6.55	5.14	5.26	5.74	6.64	4.95	6.05	5.79
Hydropathecity	-0.067	-0.391	-0.166	-0.056	-0.097	-0.547	-0.445	0.071	-0.229	-0.366	-0.075
Aliphatic Index	86.96	82.06	92.12	85.58	107.74	95.75	69.63	102.21	96.05	78.09	36.56
Status	Stable	Stable	Stable	Stable	Unstable	Unstable	Stable	Unstable	Unstable	Stable	Stable
P.I*, isoelectric	point	P.S,	periplasmic	space	Cyt* ,	cytoplasm					

Table 2: Functional partners of azurin and their characteristic determined of bioinformatics tools

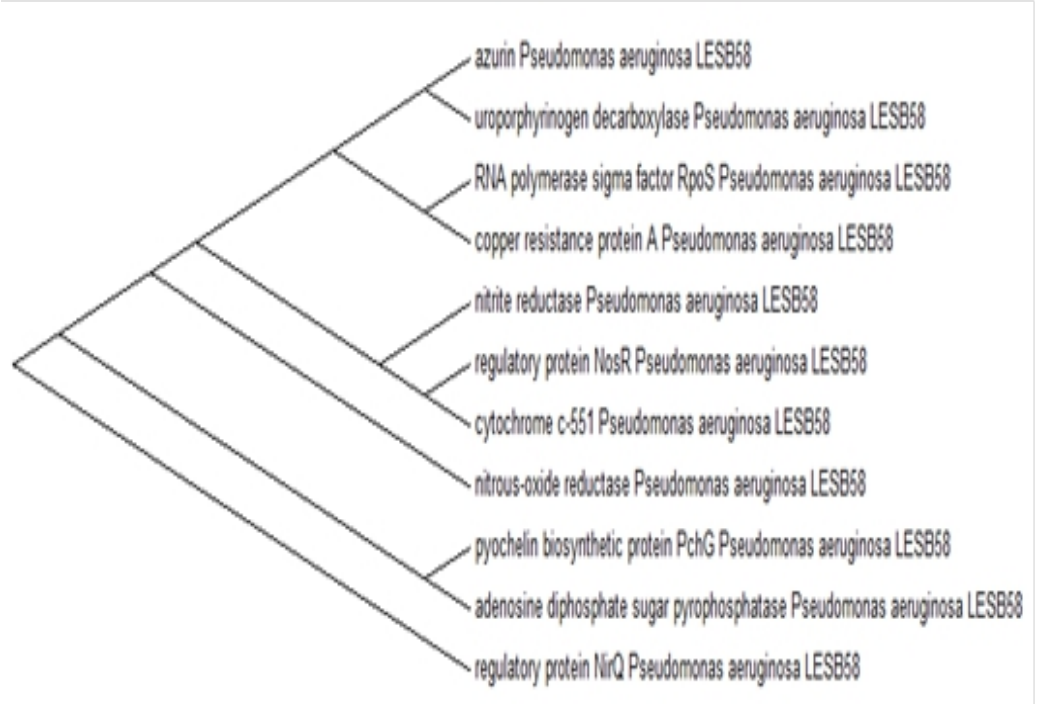

Fig: 1 phylogenetic analysis of azurin and its predicted functional partners.

Protein	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu	Met	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr	Total
adenosine diphosphate sugar pyrophosphatase	8.3	2.0	6.3	10.2	3.4	8.8	2.9	3.9	1.5	11.7	2.0	2.0	5.4	4.4	9.3	2.9	2.0	9.3	2.4	1.5	205
azurin	7.4	2.0	7.4	2.7	4.1	7.4	2.7	2.7	8.1	12.2	4.7	4.7	3.4	4.1	1.4	8.8	6.8	7.4	0.7	1.4	148
copper resistance protein A	8.1	0.2	8.1	4.7	3.1	10.5	3.7	2.9	2.9	9.0	6.4	3.1	6.0	2.1	7.2	5.7	5.7	5.2	2.0	3.3	614
cytochrome c-551	17.3	1.9	4.8	4.8	1.9	8.7	1.0	2.9	8.7	9.6	2.9	2.9	6.7	5.8	1.0	3.8	3.8	6.7	2.9	1.9	104
nitrite reductase	8.3	0.4	6.7	4.8	2.6	7.9	3.0	5.3	7.6	7.7	2.1	3.5	6.3	4.0	4.0	6.3	6.0	7.9	1.9	3.5	568
nitrous-oxide reductase	7.2	1.7	8.1	5.8	4.1	8.8	3.3	5.0	6.3	6.9	3.0	4.1	4.7	1.9	5.9	5.3	5.5	8.5	1.4	2.7	639
pyochelin biosynthetic protein PchG	14.6	1.4	5.2	7.2	2.6	8.0	1.4	2.3	0.0	16.0	1.1	1.1	6.3	4.3	9.5	6.0	2.3	7.4	2.3	0.9	349
regulatory protein NirQ	11.5	1.9	6.9	7.3	2.7	7.7	2.7	3.1	1.5	13.5	1.9	0.8	5.4	4.6	9.2	2.7	5.4	6.9	1.2	3.1	260

regulatory protein NosR	**10.9**	**2.1**	**4.5**	**6.7**	**5.0**	**6.9**	**1.5**	**4.2**	**2.7**	**13.3**	**1.3**	**1.8**	**5.2**	**4.6**	**8.3**	**4.6**	**3.2**	**8.0**	**2.1**	**3.2**	**715**
RNA polymerase sigma factor RpoS	**5.7**	**0.3**	**7.2**	**10.8**	**2.7**	**4.8**	**2.4**	**5.4**	**5.1**	**13.5**	**1.5**	**2.7**	**4.8**	**3.9**	**9.0**	**6.0**	**6.3**	**5.7**	**0.9**	**1.5**	**334**
uroporphyrinogen decarboxylase	**11.8**	**0.8**	**5.9**	**5.4**	**3.7**	**9.3**	**2.3**	**4.2**	**3.1**	**10.4**	**3.7**	**2.3**	**5.4**	**3.9**	**6.5**	**5.4**	**4.2**	**6.8**	**1.4**	**3.7**	**355**
Avg.	**9.5**	**1.2**	**6.6**	**6.3**	**3.5**	**8.2**	**2.6**	**4.1**	**4.1**	**10.8**	**2.8**	**2.7**	**5.5**	**3.6**	**6.9**	**5.3**	**4.7**	**7.3**	**1.7**	**2.7**	**390**

Table 3 Amino acid composition in azurin and its functional partners.

Discussion

Analysis of microbial proteins proved to be very economical as a therapeutic agent for some type of cancers as well for control of infection for drug designing. Azurin in combination with anticancer drugs left a very strong synergistic effect on oral cancer cells carcinoma. The present analysis revealed that most members in the present investigation are negatively charged. Leucine is the most abundant amino acids followed by Alanine. Similar results were also obtained in other studies (Canovas et al. 2003). Amino acid cysteine residues pair create a specific cross link (Disulfide Bridge) in proteins. These bridges play a central role in proteins stability (Jorda & Yeates 2011). At a certain pH the pI value influence the solubility of molecules. Molecules such as proteins are amphoteric that have both acidic and basic functional groups. The overall charge on proteins depends on the number of positive, negative, neutral, or polar amino acids in nature, Proteins have positive charge at a pH below their pI and negative at a pH above their pI value thus help in separation of proteins on a polyacrylamide gel by using a isoelectric focusing, which uses a pH gradient to separate proteins. The pI value calculated in the current analysis lying between 4.95-7.75 for Nir and AspP respectively. The 2-D polyacrylamide gel electrophoresis also work in the first step using isoelectric focusing.

Analysis of aliphatic index is significant due to hydrophobicity of aliphatic amino acids and give information about stability of proteins at high temperature, and also the denaturants i.e. urea. Gly, Ala, Glu, and Leu give information about stability [Table 2] (Zhao et al. 2011). The pI of protein give a clear link with subcellular localization, length, ecology, of proteins and taxonomy of organisms [17]. All those proteins having instability index less than 40 were placed as stable, and larger than 40 were unstable given in table 2.

Conclusion

The azurin and its predicted functional partner's involve in electron carrier during stress as well other important biological reactions. These proteins can also be used for treatment of some cancers. The current analysis showed that variations are present in amino acid sequence, molecular weight, aliphatic index isoelectric point. Bioinformatics tools provide a cheap and unexpansive time saving opportunity for analysis of biological molecules. Similar procedure can be adopted in future to study the important proteins mostly involve in virulency of microorganisms. Such analysis helps the drug designers and investigators to design effective drugs against such protein to prevent the possible infections. Further analysis is required to investigate the functional partners and their use as an alternative for cancers treatment.

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