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Study on Variability Assessment and Evolutionary Relationships of Glutamate Racemase in *Pseudomonas* Species

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Abstract: *Pseudomonas* species is known to cause multiple nosocomial infections in patients and results in high morbidity and mortality rates (10%). The greatest obstacle in treating patients infected with the *Pseudomonas* species is the widespread emergence of antibiotic resistance. Hence, there is an urgent need to develop new compounds which can be effective against *Pseudomonas* species and possibly remain tolerant to drug resistance. The enzyme glutamate racemase plays an important role in cell wall synthesis of bacteria and as a rate limiting step, thus it is an excellent target for the designing of new class of antibacterial agents. The objective of this study is to investigate the variations in sequences of glutamate racemase, a potential drug target across the all 31 species of *Pseudomonas.* Sequence variability and conservation for functional motif identification is helpful for identifying evolutionarily important residues with functional significance; subsequently these results of variable sites were supported by entropy profile obtained from protein variability server using Shannon entropy. Phylogenetic profile among the different *Pseudomonas sp.* having fully/highly conserved residues was observed, suggesting possible functional similarities between them. The variation analysis in conserved and non-conserved region of the sequence can be used to predict the binding site for target specific drug discovery.
Key words: *Pseudomonas*, glutamate racemase, nosocomial infections, variability analysis, active site, phylogenetic tree.

1 Background

Multi-drug resistant bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumonia* and *Acinetobacter baumanni* infect frequently patients with compromised immune system. These multi-drug resistant pathogens are the major threats to human health, and responsible for an overwhelming number of hospital-associated infections. *Pseudomonas* is a Gram-negative bacterium measuring 0.5-0.8 μm, rod-shaped and polar-flagella bacteria, it is isolated from many natural niches like soil, marine habitats, plants, animals, and humans (Lewenza *et al.*, 2005). In *Pseudomonas sp.*, *P. aeruginosa* is increasingly recognized as an emerging opportunistic pathogen of clinical relevance. Several different epidemiological studies indicate that antibiotic resistance is increasing in clinical isolates (Van *et al.*, 2003). It is the third most-common pathogen associated with hospital-acquired infections. These infections are due to extra cellular virulence factors (Vasil *et al.*, 1986) and cell associated factors (Woods *et al.*, 1983). Almost 80% bacterial infections are due to biofilm state. Mostly *Pseudomonas* infection comprises of three stages: (a) attachment of bacteria and colonization; (b) local tissues infection; and (c) disseminated systemic disease (Woods *et al.*, 1983).

Emergence of multi-drug-resistant bacteria with high morbidity rates of *P. aeruginosa* makes it important to identify the potential drug targets which are effective against it. The inhibition of essential cell wall targets such as glutamate racemase (cofactor independent) provides a great opportunity to design the next generation of antibiotics which is absent in humans. It catalyzes the rate limiting step of the bacterial cell wall biosynthetic pathway and inhibiting this enzyme can restrict the bacterial growth. Glutamate racemase is an enzyme which belongs to *Asp/Glu* racemase super family that converts L- or D-glutamate to D- or L-glutamate respectively. D-glutamate conversion by glutamate racemase is important for the phase-I peptidoglycan cell wall synthesis. It consists of one domain which belongs to *Asp/Glu/Hydration* family, which has been biochemically and structurally characterized in a range of Gram-positive and -negative bacteria where the variation is found in structural level like

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tail-tail dimer, mixed form and head-head dimer formation (Lundqvist *et al.*, 2007). It has been observed that structure of glutamate racemase from *Aquifex pyrophilus* clearly shows that the Asp10, Cys73, Cys184, His186, and Glu152 residues are situated in the active site (Hwang *et al.*, 1999). The evolutionarily related (homologous) sequence already known in protein and functionally/structurally important residues can be identified by considering their degree of conservation across the family of aligned sequence (Zvelebil *et al.*, 1998). Here we explored the residues that may play an important role in binding of various analogues of glutamate racemase, which may help us to find the mechanism of binding and interaction of these analogues in divergent physiologic conditions. Some of the substrate based inhibitors for glutamate racemase have already known such as L-serine O-sulfate, D-N-hydroxyglutamate and aziridino-glutamate (Fisher, 2008). In this study, glutamate racemase sequences in *Pseudomonas* species were collected from databases followed by their multiple sequence alignment, phylogenetic analysis and functional residual identification. The detection of conserved residues will be useful in identifying functionally significant residues. Over the period of time pathogens have developed multi-drug resistance against the available antibiotics, this issue has prompted the research to find novel drug compound which can inhibit the growth of these evolved pathogens. There is evidence for sequence and structural level annotation of evolutionary events in various biological entities ranging from restriction endonucleases to proteins through nucleotides of nuclear and mitochondrial genomes (Singh and Pardasani, 2009; Singh *et al.*, 2008; Singh *et al.*, 2009; Konstantin *et al.*, 2011). These functional and evolutionary annotations could help provide insights into the existence, association and interpretation of various biological and biochemical phenomenon latent in biological data.

The aim of this study is to identify the variation/conservativity across the all 31 sequences of diversified range of *Pseudomonas* species for glutamate racemase target. The variability assessment and phylogenetic relationship will be helpful with identifying the evolutionary conserved and functionally important residues even in the absence of structural information. However, functional domain variation studies are also significant in microbes (Gupta *et al.*, 2011). Current study will shed light on target-ligands interactions and enhance our understanding of underlying mechanism and its basis for designing novel compounds.

2 Methodology

2.1 Dataset

The sequences of glutamate racemase protein of 31 *Pseudomonas* species were downloaded from NCBI database and *Pseudomonas* database (http://www.pseudomonas.com/) (Supplementary Table).

2.2 Multiple sequence alignment and motif identification

These glutamate racemase sequences (31 in total) were first checked manually for their completeness and ambiguity. Thereafter sequences were aligned using PRALINE tool (Simossis *et al.*, 2003) for optimizing progressive multiple sequence alignment (MSA) as a first step to assess sequence conservation of individual residue/motifs as well as applying hydrophobic scale of each residue. PROSITE web server was used to identify the unique conserved motifs as a signature in glutamate racemase sequences (http://www.expasy.ch/PROSITE/). To support and validate the results, protein variability server was used to identify entropy profile (variation) in particular site using Shannon entropy (default value is 1) (Shannon, 1948), and Simpson and Wu-Kabat entropy methods (Wu and Kabat, 1970).

2.3 Phylogenetic studies

Phylogenetic studies were carried out using MEGA v5.0 tool (Kumar *et al.*, 2008), where 1000 replicates were given to the evolutionary relationship using Neighbor-Joining method. The evolutionary distances were computed using the Juke-Cantor method by eliminating all gaps and missing data. The phylogenetic tree was constructed on the basis of the aligned complete sequences of various species of *Pseudomonas*.

2.4 Likelihood analysis

Branch lengths estimates were obtained by using maximum likelihood (ML) method. The best probabilistic model of sequence evolution was determined with the program MODELTEST 3.07 (Posada and Crandall 1998) using the Akaike information criterion (AIC) (Akaike, 1974). ML searches for the best trees were performed with the program Treefinder (Jobb *et al.*, 2004). The parameters of the model and the ML tree were then determined in an iterative way. Initial parameter values were those estimated by MODELTEST 3.07; those values were used for a first round of heuristic search starting with a neighbor-joining (NJ) tree and using tree bisection-reconnection (TBR) branch-swapping. Parameters were then re-estimated on the resulting tree and used for another round of heuristic search. The process was repeated until the convergence of all parameters. Bootstrap support (BPs) were estimated after 250 replicates by using the best estimated parameters, a NJ starting tree, and TBR branch-swapping.

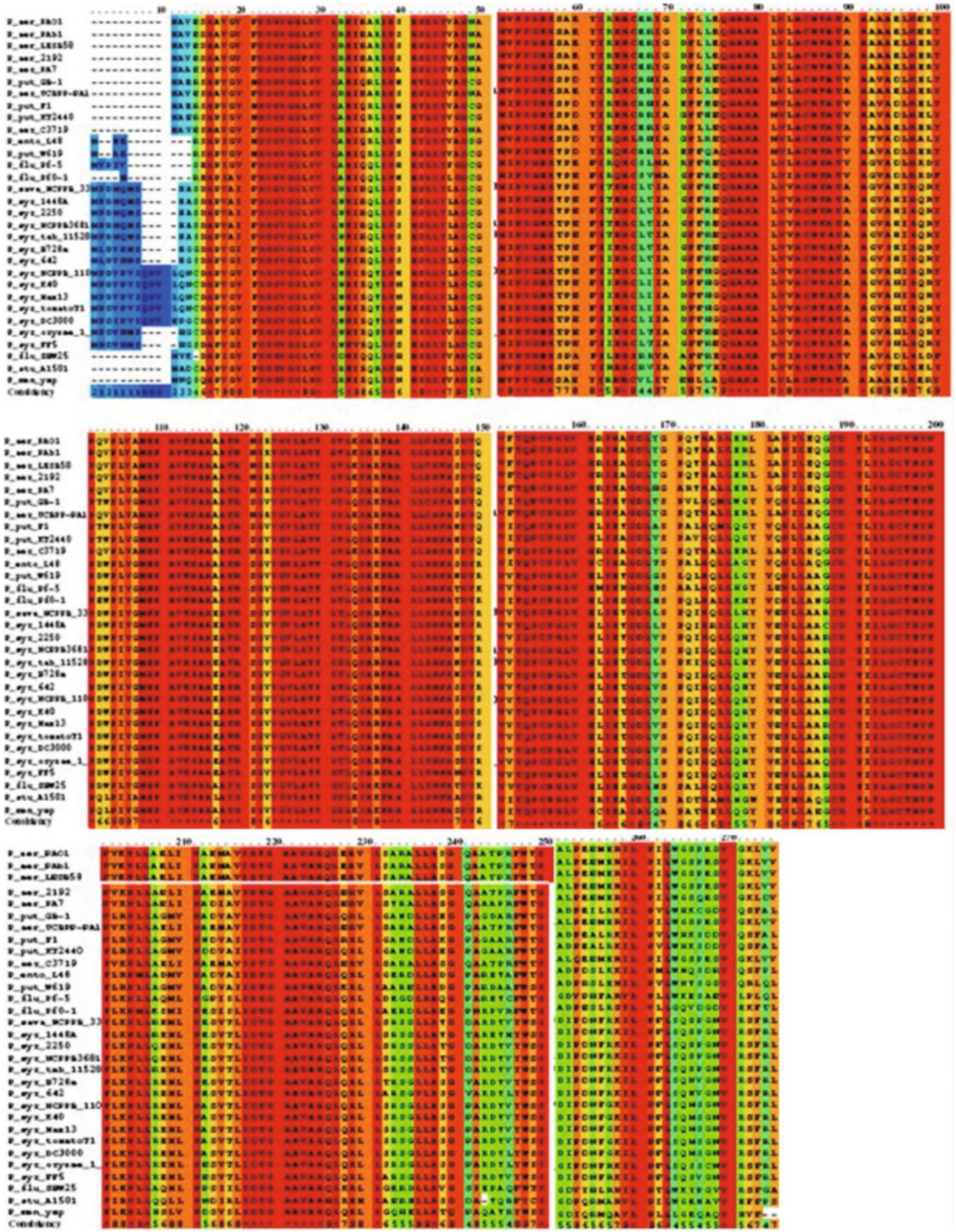

Fig. 1 MSA of the glutamate racemase protein in *Pseudomonas sp.* The dark to light gradient represents highly conserved to variable region.

3 Discussion

MSA results (Fig. 1) obtained from PRALINE showed that major variation occurred in *P. aeruginosa PA7* and *P. Fluorescence pf01* where 14 and 8 amino acid variations were recorded respectively. Identified changes that have occurred (Table 1) might be affecting the binding efficacy for the concerned species. Important residues found involved in these changes are mainly comprising of Ala, Val, and Pro. All these studies indicate that *P. aeruginosa* strains are highly conserved except some changes and specifically *P. syringae* and *P. putida* species are highly conserved. In *P. aeruginosa* Cys75 and Cys186 are identified to be critical for catalytic activity based on the literature (Fisher, 2008; Gupta *et al.*, 2011; Hwang *et al.*, 1999; Zvelebil *et al.*, 1998), and Asp12, Ser13, Val42, Pro43, Tyr44, Gly45, Cys75, Asn76, Thr77, Thr119, Thr122, Val150, Cys186, Thr187, His188 are predicted to belong to the active site. However, out of these, Val42, Pro43, Thr119 and Val150 have not been previously identified as a component in active sites of glutamate racemase of the other species. This novel information would help to design new compounds or their derivatives by actively changing their positions while performing molecular modeling and simulation studies.

These results are supported by the analysis demonstrated by protein variability server which interprets the entropy profile (variability) in 31 sequence of glutamate racemase from *Pseudomonas* species. This analysis was based on variability by all three methods including Shannon diversity index (H), Simpson diversity index (D) and Wu-Kabat variability coefficient (W) (Fig. 2). These three variation results demonstrate that the major variation sites are positions 4-18, 21-32, 35-51, 53-66, 75-78, 85-99, 105-157, 159-186, 202-204, 213-225, and 228-265. The most conserved residues found are at positions Met1, Leu33, Leu34, Ile52, Gly67, Ala70, Ala74, Thr79, Ala80, Ala84, Pro100, Val102, Pro104, Leu158, Pro173, Thr187, Ala212, and Leu226-227. According to literature, *Aquifex pyrophilus* clearly showed that the Asp10, Cys73, Cys184, His186, and Glu152

Table 1 Different strain of *Pseudomonas* with their respective variability in amino acids

No.	*Pseudomonas species*	Variation in amino acids
1	*P. aeruginosa* PA7	Val3Ala, Ala7Pro, Ser30Ala, Glu66Arg, Tyr159His, Pro161Ala, Glu62arg, Ala172Gly, Glu203Asp, Met204Ile, Ala223Thr, Thr234Ala, Pro257Gln, Val264Asp
2	*P. aeruginosa* PAb1	Thr187Asp
3	*P. aeruginosa* 2192	Leu18Phe
4	*P. aeruginosa* UCBPP-PA1	Met11Phe
5	*P. aeruginosa* C3719	Pro243Gln
6	*P. fluorescence* Pjo1	Glu12Gly, Ser13Val, Val4Tyr, Pro43Gly, Tyr44Glu, Cys75Thr, Val150Leu, Cys185thr
7	*P. putida* F1	Glu12Ala, Val148Arg, Glu151Leu
8	*P. putida* KT2440	Val142Leu, Asn76Met
9	*P. syringe*	Glu152Cys

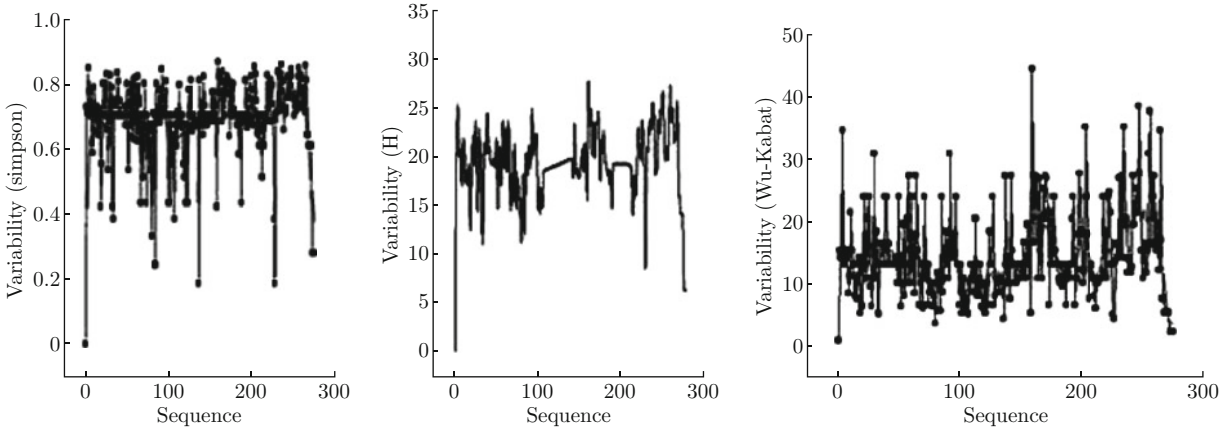

Fig. 2 Variability graph (in order of Simpson diversity index D, Shannon entropy H and Wu-kabat variability coefficient W) displays the entropy profile with peaks indicating those sites which exhibit the largest variation among the member of dataset.

residues were situated in the active site (Hwang *et al.*, 1999) having important role in catalysis mechanism. All these relative positions of the active site residues were confirmed by the mechanism given by Glavas and Tanner (2003), and suggested that both cysteins were neutral thiols (Tanner *et al.*, 1993). Structure positions will be conserved and functional positions will vary if we are able to interpret the protein having similar structure and different functions and variations between the ligand and substrate will be directly proportional to degree of variation (Magliery *et al.*, 2005). Consequently the results explore the sequence variability of target gene which will be helpful to understand the functional variability in Asp/Glu racemase families of protein with similar structures.

These motifs in proteins are helpful to understand and predict the functional classes. Significantly the PROSITE database identified two motifs, which are unique to glutamate racemase from all 31 species of *Pseudomonas* (Table 2). The motifs and patterns were VLACNTATA, [IVA]-[LIVM]-x-C-x(0,1)-N-[ST]-[MSA]-[STH]-[LIVFYSTANK] (positions 72-80) and LILGCTHYPFV, [LIVM](2)-x-[AG]-C-T-[DEH]-[LIVMFY]-[PNGRS]-x-[LIVM] (positions 182-192) (where x is any amino acid, C is an active site residue), which are present in *Pseudomonas sp.* The two motifs, *i.e.* Aspartate-Glutamate racemase 1 (PS00923) and Aspartate-Glutamate racemase 2 (PS00924) encoded aspartate glutamate racemase super family, involved in racemization are highly conserved across the all 31 species of *Pseudomonas*, which are responsible for molecular function (glutamate racemase activity, and isomerase activity) and biological processes (cellular cell wall organization, regulation of cell shape and peptidoglycan biosynthesis process). MSA and hydrophobicity measured by PRALINE tool is represented in Fig. 1 and Fig. 3, showing the conservation pattern of amino acid sequences from all *Pseudomonas* species.

Table 2 PROSITE results with the 9-mer pattern and the variable positions in *Pseudomonas sp.*

Pseudomonas species	Group name	Length and position	Consensus pattern
P. aeruginosa PAO1, *P. aeruginosa* C3719, *P. aeruginosa* PA7, *P. aeruginosa* PACS2, *P. aeruginosa* UCBPP-PA14, *P. aeruginosa* LESB58, *P. entomophila* L48, *P. mendocina* ymp, *P. putida* GB-1, *P. putida* F1, *P. putida* KT2440, *P. putida* W619, *P. stutzeri* A1501	1) Aspartate-Glutamate racemase 1 (PS00923) 2) Aspartate-Glutamate racemase 2 (PS00924)	1) 9 & 72-80 2) 11 & 182-192	[IVA]-[LIVM]-x-C-x(0,1)-N-[ST]-[MSA]-[STH]-[LIVFYSTANK] [LIVM](2)-x-[AG]-C-T-[DEH]-[LIVMFY]-[PNGRS]-x-[LIVM]
P. fluorescens Pf0-1	1) Aspartate-Glutamate racemase 1 (PS00923) 2) Aspartate-Glutamate racemase 2 (PS00924)	1) 9 & 70-78 2) 11 & 180-190	[IVA]-[LIVM]-x-C-x(0,1)-N-[ST]-[MSA]-[STH]-[LIVFYSTANK] [LIVM](2)-x-[AG]-C-T-[DEH]-[LIVMFY]-[PNGRS]-x-[LIVM]
P. fluorescens Pf-5	1) Aspartate-Glutamate racemase 1 (PS00923) 2) Aspartate-Glutamate racemase 2 (PS00924)	1) 9 & 74-82 2) 11 & 184-194	[IVA]-[LIVM]-x-C-x(0,1)-N-[ST]-[MSA]-[STH]-[LIVFYSTANK] [LIVM](2)-x-[AG]-C-T-[DEH]-[LIVMFY]-[PNGRS]-x-[LIVM]
P. fluorescens SBW25	1) Aspartate-Glutamate racemase 1 (PS00923) 2) Aspartate-Glutamate racemase 2 (PS00924)	1) 9 & 71-79 2) 11 & 181-191	[IVA]-[LIVM]-x-C-x(0,1)-N-[ST]-[MSA]-[STH]-[LIVFYSTANK] [LIVM](2)-x-[AG]-C-T-[DEH]-[LIVMFY]-[PNGRS]-x-[LIVM]
P. savastanoi pv. savastanoi NCPPB 3335, *P. syringae pv. phaseolicola* 1448, *P. syringae pv. syringae* B728a, *P. syringae pv. syringae* 642, *P. syringae pv. syringae* FF5, *P. syringae pv. aesculi str.* 2250, *P. syringae pv. aesculi str.* NCPPB3681, *P. syringae pv. tabaci* ATCC11528, *P. syringae pv. oryzae str.* 1_6	1) Aspartate-Glutamate racemase 1 (PS00923) 2) Aspartate-Glutamate racemase 2 (PS00924)	1) 9 & 78-86 2) 11 & 188-198	[IVA]-[LIVM]-x-C-x(0,1)-N-[ST]-[MSA]-[STH]-[LIVFYSTANK] [LIVM](2)-x-[AG]-C-T-[DEH]-[LIVMFY]-[PNGRS]-x-[LIVM]

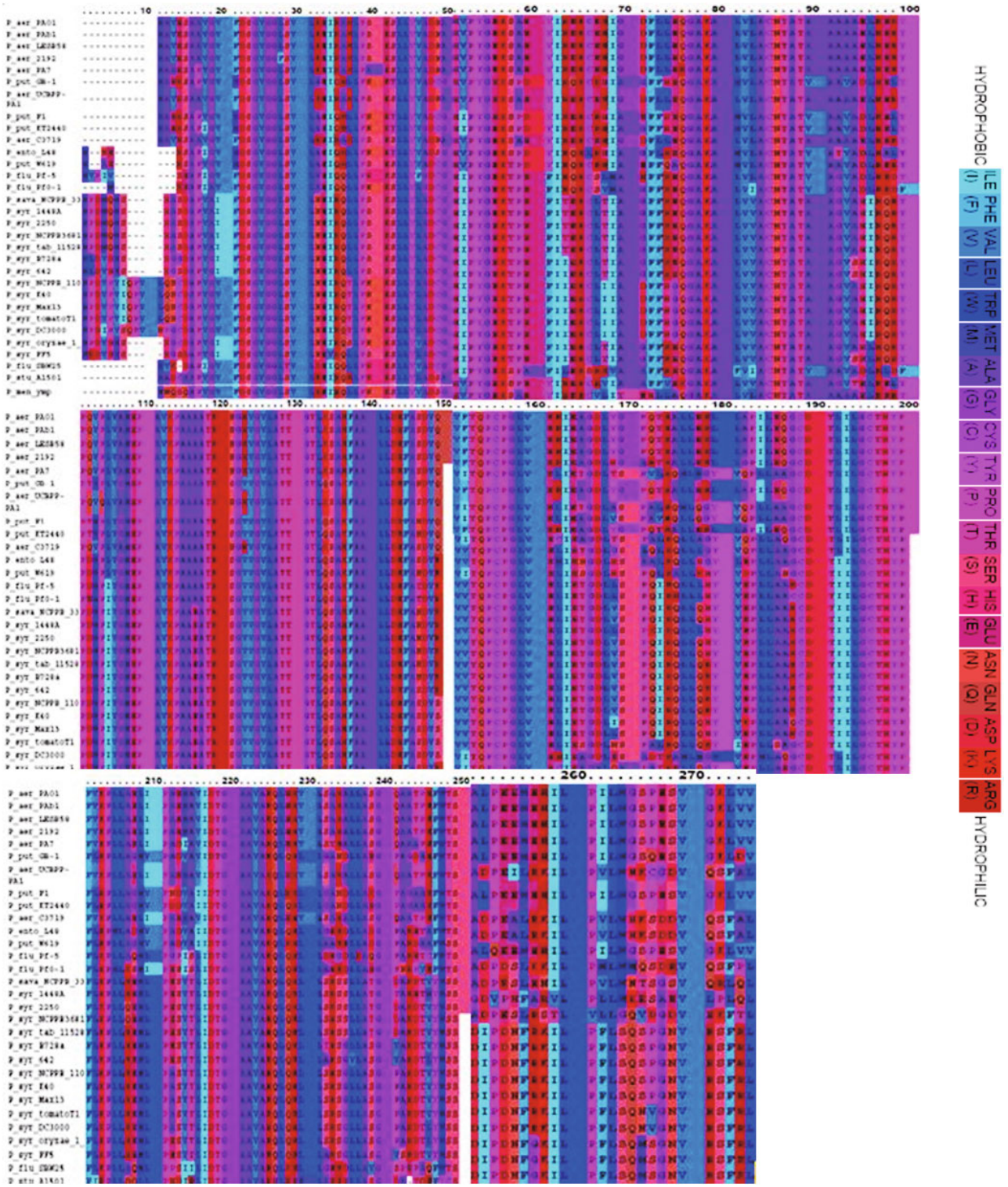

Fig. 3 The different colors indicated the variability range of each position of glutamate racemase in 31 *Pseudomonas sp.* The light to dark gradient represents hydrophobic to hydrophilic amino acid residues.

Phylogenetic tree of glutamate racemase for 40 species (31 *Pseudomonas sp.* and 9 reference species) was also constructed (Figs. 4(a) and 4(b)) using MEGA v5.0 with 100 bootstrap replicates which helped in elucidating the evolutionary relationship of the various functional sites present in *Pseudomonas* species. The phylogenetic tree showed that *P. aeruginosa, P. fluorescence, P. putida* were grouped together and these

were distantly related with the other known species, implying that these are resistant to known antibacterial drugs and more effective drugs against these species need developing.

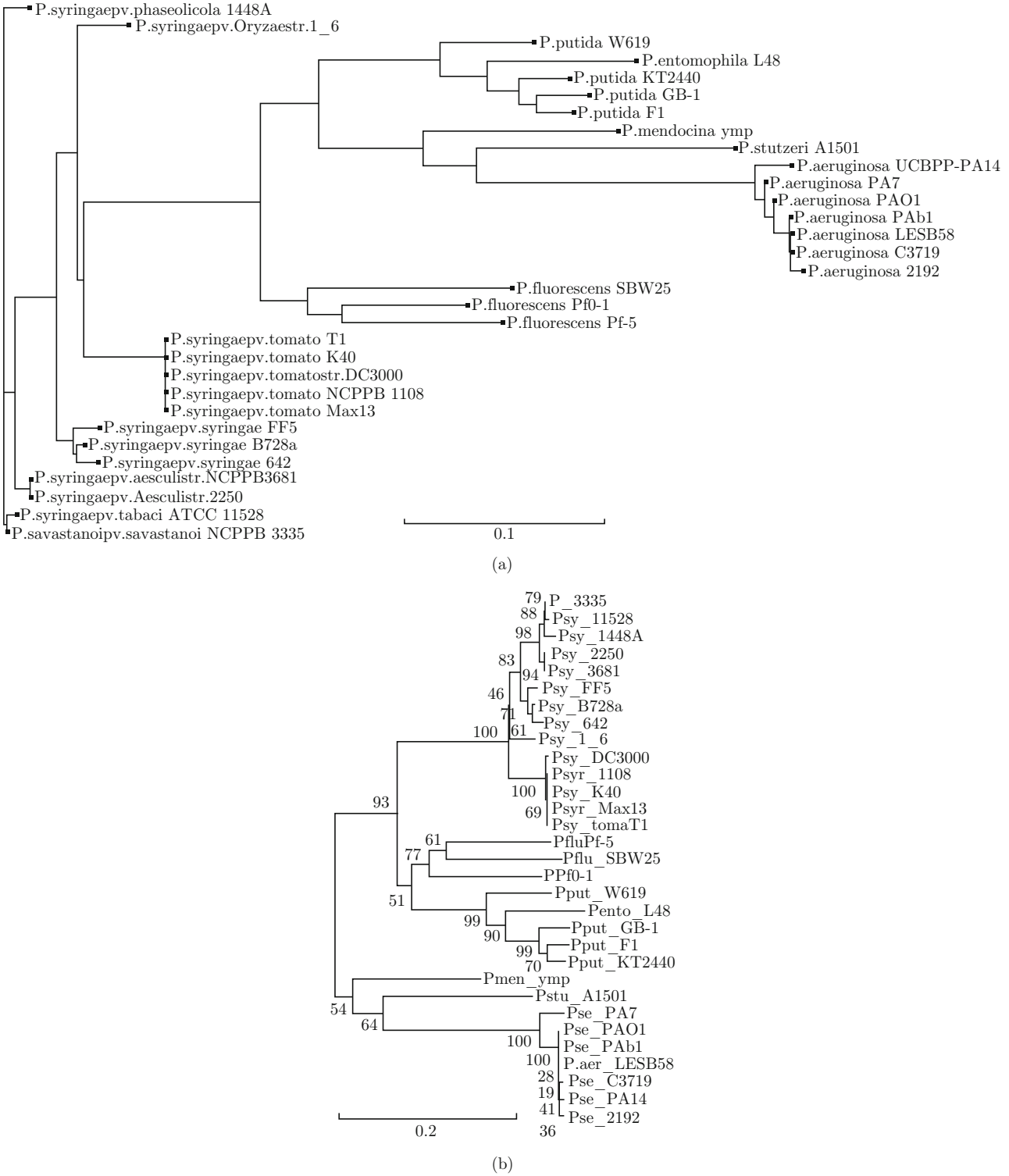

Fig. 4 (a) The cladogram of glutamate racemase for different *Pseudomonas* species. (b) Neighbour joining phylogenetic tree of glutamate racemase from 31 *Pseudomonas* species and reference species, and robustness of individual nodes of the tree was determined by using MEGA v5.0 with 100 bootstrap value.

Different parts of genome with different functional constraints are expected to evolve at different rates. Thus, comparative genomics promises to offer a comprehensive study of distinct patterns and processes of molecular evolution (Singh, 2008). Phylogenetic tree generated through heuristic search parameters estimated by MODELTEST and ML methods gave similar trends except positions of some species are either shuffled or interchanged from previous NJ tree (Fig. 5). Positions in the later are statistically more robust as the modeling parameters are being estimated through refined process of model selection using AIC and ML method. Few positions in the likelihood analysis tree were found in more suitable place than the previous one such as the position of P.aer_2192 which looks more accurate in Fig. 5. Similarly for some other species such as Psy 642, P.Syr_Max13, and P.aer_LESB58 positions and BPs are better in evolutionary reference (Fig. 5). The identification of specificity and functionality determining positions (Tables 3 and 4) will be useful for the design of inhibitors with altered binding specificity.

Docking and simulation studies could provide novel insights for species specific molecules (Chauhan *et al.*, 2012). Comparative information regarding the variability and conservation of amino acids among multiple species could be an excellent source of information and it is believed that generated information is biologically meaningful. Amino acid conservation and variability information for molecular function and biological processes for the specific locations amongst various *Pseudomanas* species could provide clues for the design of unique or common compounds for single or a group of species. Consequently, variable analysis of functional regions of target implicates the importance of target specific drug discovery for the treatment of invasive infections.

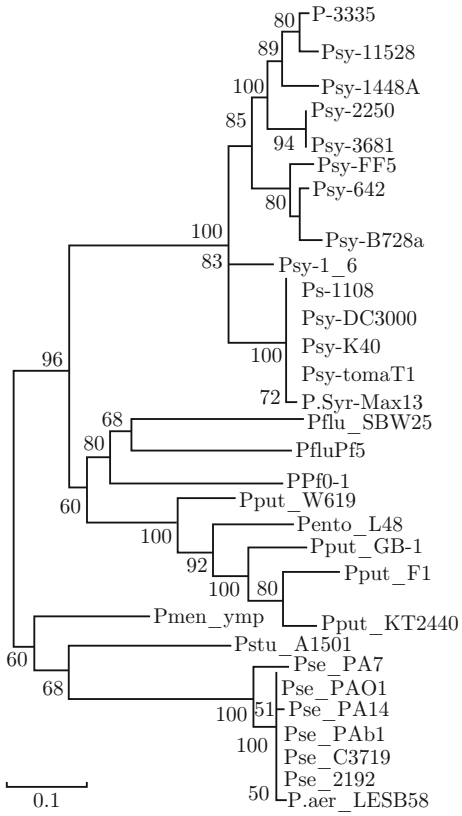

Fig. 5 Phylogenetic tree reconstructed through likelihood analysis. Branch lengths estimates were obtained by using ML method. The best probabilistic model of sequence evolution was determined with the program MODELTEST using the AIC. The process of parameter estimation was repeated until the convergence of all parameters. Bootstrap support was estimated after 250 replicates by using the best estimated parameters, a NJ starting tree and TBR branch-swapping.

Table 3 Variability analysis by using Shannon entropy

Position	Consensus	Variability	Position	Consensus	Variability	Position	Consensus	Variability	Position	Consensus	Variability
1	M	0	71	E	2.365	141	V	1.595	209	T	1.94
2	A	2.081	72	V	1.875	142	A	2.525	210	G	1.875
3	D	2.063	73	L	1.94	143	T	2.162	211	A	1.618
4	V	2.733	74	A	1.224	144	Q	1.94	212	A	1.246
5	S	2.243	75	C	1.94	145	P	1.769	213	V	1.875
6	A	1.94	76	N	1.633	146	C	1.94	214	A	1.769
7	S	2.1	77	T	1.769	147	P	1.769	215	T	1.515
8	V	2.043	78	A	1.279	148	G	1.789	216	Q	1.814
9	G	1.658	79	T	1.618	149	L	1.814	217	L	1.875
10	V	1.717	80	A	0.835	150	V	1.94	218	A	2.498
11	D	2.33	81	A	1.769	151	E	1.875	219	R	1.954
12	D	2.056	82	A	1.848	152	C	2.194	220	A	2.24
13	S	1.992	83	V	1.735	153	I	1.875	221	L	1.789
14	G	1.618	84	A	0.813	154	E	1.789	222	Q	2.392

Continued

Position	Consensus	Variability	Position	Consensus	Variability	Position	Consensus	Variability	Position	Consensus	Variability
15	V	1.985	85	T	2.365	155	A	2.278	223	A	2.372
16	G	1.595	86	L	1.724	156	G	1.94	224	R	2.37
17	G	1.94	87	R	1.875	157	D	2.108	225	R	2.125
18	L	1.982	88	E	1.982	158	L	1.098	226	L	1.03
19	S	1.098	89	R	2.213	159	I	3.095	227	L	0.612
20	V	1.366	90	Y	1.854	160	E	2.525	228	A	2.015
21	L	1.94	91	P	1.875	161	P	2.214	229	R	2.161
22	R	2.365	92	Q	2.743	162	G	2.577	230	G	1.956
23	E	1.94	93	V	2.143	163	T	2.417	231	Q	2.539
24	I	1.875	94	P	1.956	164	R	1.789	232	A	2.086
25	S	2.239	95	L	2.207	165	A	2.539	233	L	2.548
26	V	2.522	96	V	1.982	166	L	2.118	234	A	2.124
27	L	1.741	97	P	2.385	167	L	1.875	235	T	2.731
28	L	1.279	98	M	1.94	168	Q	2.459	236	R	2.303
29	P	1.789	99	E	1.94	169	I	2.266	237	F	2.563
30	S	2.531	100	P	1.224	170	R	2.525	238	A	1.722
31	E	1.814	101	A	1.94	171	Q	2.412	239	T	1.886
32	S	2.265	102	V	1.159	172	Q	2.246	240	S	1.848
33	L	1.098	103	K	1.875	173	P	1.473	241	A	1.93
34	L	1.03	104	P	1.366	174	L	2.374	242	L	2.525
35	Y	1.94	105	A	1.595	175	L	1.94	243	P	2.216
36	V	2.189	106	K	1.8606351	176	Y	2.125	244	E	2.264
37	A	2.108	107	A	1.8644353	177	V	2.355	245	S	2.589
38	D	1.94	108	E	1.8682354	178	G	2.086	246	S	2.35
39	L	2.55	109	V	1.8720356	179	C	1.94	247	E	2.773
40	L	2.385	110	L	1.8758357	180	D	1.94	248	I	2.616
41	H	1.94	111	A	1.8796358	181	T	1.875	249	I	2.143
42	V	2.189	112	K	1.883436	182	L	1.769	250	L	1.875
43	P	1.94	113	A	1.8872361	183	I	1.94	251	P	2.108
44	Y	1.94	114	E	1.8910363	184	L	1.652	252	I	2.13
45	G	1.94	115	V	1.8948364	185	G	1.789	253	L	1.917
46	E	1.94	116	L	1.8986366	186	C	1.94	254	W	2.056
47	K	1.94	117	A	1.9024367	187	T	1.224	255	I	2.606
48	S	2.043	118	C	1.9062368	188	H	1.94	256	L	2.872
49	P	1.769	119	N	1.910037	189	Y	1.94	257	P	2.241
50	Y	2.365	120	T	1.9138371	190	P	1.789	258	F	2.514
51	Y	1.94	121	A	1.9176373	191	F	1.94	259	S	2.521
52	I	1.279	122	T	1.9214374	192	C	2.335	260	V	2.108
53	R	1.769	123	A	1.9252376	193	T	2.385	261	Q	2.162
54	E	2.278	124	A	1.9290377	194	P	1.54	262	S	2.33
55	R	1.704	125	A	1.9328378	195	L	1.875	263	L	2.036
56	E	1.683	126	V	1.936638	196	L	1.789	264	G	2.792
57	F	2.251	127	A	1.9404381	197	A	2.207	265	N	2.267
58	I	2.521	128	T	1.9442383	198	L	2.616	266	–	1.595
59	I	1.784	129	L	1.9480384	199	L	2.213	267	–	1.595
60	E	1.967	130	R	1.9518385	200	P	1.93	268	–	1.418
61	R	2.459	131	E	1.9556387	201	P	1.769	269	–	1.418
62	F	1.94	132	R	1.9594388	202	L	1.863	270	–	1.418
63	L	1.719	133	Y	1.963239	203	D	2.683	271	–	1.418
64	T	2.541	134	P	1.9670391	204	E	2.505	272	–	0.637
65	E	2.207	135	Q	1.9708393	205	A	2.146	273	–	0.637
66	Q	2.056	136	K	1.9746394	206	L	1.615	274	–	0.637
67	G	1.224	137	A	1.9784395	207	I	1.94	275	–	0.637
68	A	1.769	138	E	1.9822397	208	E	1.515			
69	K	1.94	139	V	1.9860398						
70	A	1.246	140	R	1.956						

Table 4 Conserved amino acid residues with their positions in glutamate racemase sequences from 31 *Pseudomonas sp.*

Alignment position	Residues (conserved)	Alignment positions	Residues (conserved)
6	A	132	L
12	D	133	D
13	S	134	R
14	G	135	F
15	V	136	A
16	G	138	D
17	G	139	V
20	V	141	V
21	L	143	T
23	E	144	Q
28	L	145	P
29	P	146	C
31	E	147	P
33	L	148	G
54	L	149	L
55	Y	150	V
67	D	151	E
68	H	153	I
69	P	154	E
70	Y	156	G
72	G	158	L
74	E	164	R
79	K	167	L
81	I	173	P
87	R	175	L
91	P	179	C
98	M	180	D
99	E	181	T
100	P	183	I
101	A	184	L
102	V	185	G
103	K	186	C
104	R	188	H
105	A	189	Y
106	A	190	P
108	A	191	F
109	T	194	P
110	R	196	L
112	G	201	P
114	V	207	I
115	G	208	D
116	V	209	T
117	L	210	G
118	A	211	A
119	T	212	A
120	T	213	V
121	G	214	A
122	T	215	R
123	L	217	L
125	S	221	L
126	A	228	A
128	F	240	S
129	A	250	L
130	A	253	L
131	L	260	V

4 Conclusion

The study investigated the conservation and hyper variation of MSA of glutamate racemase in all 31 *Pseudomonas* species to identify sequence homology and evolutionary conserved residues which have functional importance. The motifs analyzed from the MSA were compared with the PROSITE database and we found 2 motifs which are unique to binding. *In-silico* study investigated each amino acid residue present in motifs that are in relation with the ligand, which will further helpful in drug binding and designing the inhibitors against *Pseudomonas* infections. Conserved amino acids of glutamate racemase in 31 *Pseudomonas* species can prove to be critical in development of effective antibacterial compounds against the pathogens resistant to currently existing drugs. Consequently, these variant analyses can help in the further study to know the potency between receptor and ligand binding in drug designing against these species, which could be of therapeutic relevance.

Electronic Supplementary Material

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s12539-013-0181-x and is accessible for authorized users.

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