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ORIGINAL PAPER

Polar Characterization of Antifungal Peptides from APD2 Database

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Abstract The increase in the number of pathogens due to fungi that are tolerant to therapies does not grow at the same speed than the advance on new antifungal drugs. In this sense, it is imperative to find anti-fungi peptides that are not detrimental to mammalian cells and have an effective toxicity to fungi. In this work, we use a method called polarity index, to identify anti-fungi peptides with an efficiency of 70 %. This method already published, initially identified selective antibacterial peptides from APD2 Database, and was characterized by developing a comprehensive analysis of the polar dynamics of a peptide from its linear sequence. Discriminating tests showed that in addition to being efficient in this identification, it was also good at rejecting other classifications of peptides found in that same database.

Keywords Polarity index method · Selective antibacterial peptides · Anti-fungi peptides

Abbreviations

SCAAP	Selective cationic amphipathic antibacterial peptides
APD2	Antimicrobial peptide database
QSAR	Quantitative structure activity relationships

Introduction

The anti-fungi peptides are produced by a large number of organisms, they likely arise for own protection from these pathogens as their immune response to fungal infections. The anti-fungi peptide group is the second largest in antimicrobial databases, just after antibacterial peptides. They have been experimentally isolated in mammals, bacteria, insects, and plants among others. Plants in particular are a very good source of this type of peptides [1], here a pattern has been observed in the linear sequence of the amino acid cysteine [2, 3]. However, within the anti-fungi peptides there are some that have extremely damaging action toward human beings [4–6], if we additionally consider these pathogens show a striking resistance to current antibiotics, we then have a lethal combination that will push the global health system to its limits in this decade. Today, it is not alarmist to say that these pathogens will win the battle unless we develop new drugs to tackle them, particularly anti-fungi drugs.

Undoubtedly, this scenario requires the use of tools that were unavailable 50 years ago to win the battle; we are talking about the computational-mathematical algorithms.

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Table 1 **Q**[*i,j*] Polarity matrix

	P+	P−	N	NP
P+	0.0434541926	0.0218654852	0.0822031572	0.0456684195
P−	0.0260171611	0.0146692498	0.0487129800	0.0215887073
N	0.0766675919	0.0500968732	0.1873789132	0.1120952144
NP	0.0415167436	0.0224190429	0.1162468866	0.0650428981

Incidences of 88 anti-fungal peptides with unique pathogenic action extracted from the APD2 [12]

Table 2 Polarity index method test

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
P[*i,j*] + Q[*i,j*] vector of study.	(1,1)	(1,2)	(1,3)	(1,4)	(2,1)	(2,2)	(2,3)	(2,4)	(3,1)	(3,2)	(3,3)	(3,4)	(4,1)	(4,2)	(4,3)	(4,4)
Rule # 1	✔	✔	✔	✔	✔	✔	✔	✔	✔							
Polar interaction 11 is present in any of the first 9 positions																
Rule # 2	x															
Polar interaction 16 is not present in the first position																
Rule # 3	x	x	x	x	x											
Polar iteration 14 is not present in any of the first five positions																
Rule # 4	x			x												
Polar iteration 12 is not present in the 1st or 4th positions																
Rule # 5	x	x			x										x	x
Polar interaction 13 is not present in the 1st, 2nd, 5th, 15th or 16th positions																
Rule # 5													x			
Polar interaction 6 is not present in the 13th position																
Rule # 6												x	x	x	x	x
Polar interaction 3 is not present from 12th to 16th positions																

Polarity index method rules. (✔): The polar interaction is present in the position. (x): The polar interaction is not present in the position

Based on actual experience, it seems unthinkable to calculate all possible combinatorial that can be build with small peptides, even with the current mathematical-computational algorithms there are combinatorial that is not possible to assess. Calculate yourself the peptides that can occur if you test experimentally 5 amino acids in length peptides, even for ordinary computing teams it is very complex to evaluate 13 amino acids in length peptides, the alphabet consists of 20 possible amino acids.

In such a situation, 8 years ago this research team decided to dedicate efforts to this task and build a robust algorithm that from a single physicochemical property, in this case polarity could generate a map of the initial-intermediate-final stages of a peptide, and that could display from a single reading of its amino acid linear sequence, as much information as possible about the toxic action of a peptide. The algorithm named Polarity index method [7] was previously published to effectively identify

Table 3 Polarity index matches by pathogenic action

Number of hits	Gram+ ONLY	Gram− ONLY	Gram+/ Gram−	Virus	HIV	Fungi	%	Protists	%	Parasites	Insects	Sperms	Cancer cells	Mammalian cells	Chemotaxis	SCAAP
Unique action	81	29	119	1	0	**62**	**70**	**1**	**100**	3	1	0	8	4	0	6
	213	111	518	1	0	**88**		**1**		9	2	0	20	11	0	51
Multiple action	112	36	434	19	22	**251**		**2**		12	6	1	27	43	10	0
	315	149	1711	125	88	**744**		**3**		47	21	9	141	244	39	0

Polarity index matches in both groups of peptides from APD2 [12], with unique and multiple pathogenic action on: Gram+ ONLY, Gram− ONLY, Gram+/Gram−, viruses, HIV, fungi, protists, parasites, insects, carcinogenic cells, mammalian cells, sperms, and SCAAP [7–10]. Unique action: Peptides with pathogenic action against only one group. Multiple action: Peptides with pathogenic action against two or more groups. (**%**): Percentage of hits/total peptides

SCAAP and other groups of peptides [7–11] in the APD2 Database (http://aps.unmc.edu/AP/ accessed December 19, 2012) [12]. In this paper, we present the results of the same method identifying anti-fungi peptides.

The fundamental thesis of this method states that the linear sequence of the peptide informs everything that is required to know in terms of its interaction with the pathogenic cell membrane, as the method requires "peptide training" with the desired profile. The method test was focused on its discriminating ability to evaluate the 14 subclassifications of the APD2 [12] database, showing 70 % efficiency.

Materials and Methods

Since the present work shows the identification of anti-fungi peptides from an already-published method [7], we indicate only the changes that this method requires for such identification (see Supplementary Material).

Polarity Index Method. Updates

Modifications

(i). Replacing the $\mathbf{Q}[i,j]$ matrix in the source program [7] by the Table 1, which represent the incidences of antifungal sequences with a unique pathogenic action. Table 1 considers 88 antifungal peptides extracted from the APD2 database [12].

(ii). Replacing the rule in the source program [7] by $\mathbf{P}[i,j] + \mathbf{Q}[i,j]$ vector complying with the next rule: the polar interaction 11 is present in any of the first nine positions, polar interaction 16 is not present in the first position, polar interaction 14 is not present in any of the first five positions, polar interaction 12 is not present in the 1st or 4th positions, polar interaction 13 is not present in the 1st, 2nd, 5th, 15th, or 16th positions, polar interaction 6 is not present in the 13th position, and polar interaction 3 is not present from 12th to 16th positions (Table 2).

APD2 Database Trial Data Preparation

APD2 Database [12] was classified 3636 by their *multiple* action against: 149 Gram – ONLY, 1711 Gram +/Gram− ONLY, 315 Gram + ONLY, 141 cancer cells, 9 sperms, 88 HIV, 744 fungi, 21 insects, 244 mammalian cells, 47 parasites, 3 protists, 39 chemotaxis, 0 SCAAP, 125 virus; and also 1059 by their *unique* action against: 111 Gram – ONLY, 213 Gram + ONLY, 518 Gram +/Gram− ONLY,

Table 4 Polarity index matches by linear sequence in fungi

No.	PUBMED ID	Sequence	Cytotoxicity	#1	Reference
1	16126239	AGECVQGRCPSGMCCSQFGYCGRGPKYCGR	11–904		[13]
2	12856109	AKYTGKCTKSKNECKYKNDAGKDTFIKCPKF DNKKCTKDNNKCTVDTYNNAVDCD	1–50		[14]
3	17883246	APPGARPPPGPPPPGPPPPGP	7–111	**N**	[15]
4	12644678	ATCKAECPTWDSVCINKKPCVACCKKAKFS DGHCSKILRRCLCTKEC	10		[16]
5	7893713	ATYNGKCYKKDNICKYKAQSGKTAICKCY VKKCPRDGAKCEFDSY KGKCYC	NF		[17]
6	17994764	AVRIGPCDQVCPRIVPERHECCRAHGRSG YAYCSGGGMYCN	NF		[18]
7	14661954	CIANRNGCQPDGSQGNCCSGYCHKEPGWVAGYCR	64		[19]
8	14661954	CIKNGNGCQPDGSQGNCCSRYCHKEPGWVAGYCR	64		[19]
9	14661954	CIKNGNGCQPNGSQNGCCSGYCHKQPGWVAGYCRRK	8–16		[19]
10	18212107	CLAGRLDKQCTCRRSQPSRRSGHEVGRP SPHCGPSRQCGCHMD	29–237		[20]
11	17272268	DEKGPKWKR	35	**N**	[21]
12	7628617	DGVKLCDVPSGTWSGHCGSSSKCSQQCKDREHFA YGGACHYQFPSVKCFCKRQC	1–30		[22]
13	11580275	DKLIGSCVWGAVNYTSDCNGECKRRGY KGGHCGSFANVNCWCET	0.4–4		[23]
14	14978308	DKLIGSCVWGAVNYTSNCNAECKRRG YKGGHCGSFANVNCW	6–12		[24]
15	10571855	DPQTECQQCQRRCRQQESGPRQQQYC QRRCKEICEEEEEYN	25		[25]
16	2303595	DSHAKRHHGYKRKFHEKHHSHRGYR	NF	**N**	[26]
17	20423320	DSHEERRQGRHGHHEYGRKFHEKHHSHRGY	NF		[27]
18	20423320	DSHEKRHHEHRRKFHEKHHSHRGY	NF	**N**	[27]
19	14728668	DTLIGSCVWGATNYTSDCNAECKR RGYKGGHCGSFLNVNCWCE	7–236		[28]
20	22497806	EGPVGLADPDGPASAPLGAP	3–22	**N**	[29]
21	17375322	ELCEKASQTWSGTCGKTKHCDDQCK SWEGAAHGACHVRDGKHMCFCYFNC	50–1000		[30]
22	12859949	ELPKLPDDKVLIRSRSNCPKGKV WNGFDCKSPFAFS	65–522	**N**	[31]
23	7947977	EQCGRQAGGKLCPNNLCCSQYGWCG SSDDYCSPSKNCQSNCKGGG	NF		[32]
24	12067732	ETCASRCPRPCNAGLCCSIYGYCGSG AAYCGAGNCRCQCRG	18–109		[33]
25	12067732	ETCASRCPRPCNAGLCCSIYGYC GSGNAYCGAGNCRCQCRG	35–155		[33]
26	11087945	FFPNVASVPGQVLLKKIFCAISKKC	NF	**N**	[34]
27	17272268	GFSPNLPGKGLRIS	50		[21]
28	18797515	GHHPHGHHPHGHHPHGHHHPH	0.07–14		[35]
29	16330099	GIMDSVKGVAKNLAAKLLEKLKCKITGC	73	**N**	[36]
30	16330099	GLLSSFKGVAKGVAKDLAGKLLEKLKCKITGC	10–164	**N**	[36]
31	19857508	GRILSFIKGLAEHL	0.5–50	**N**	[37]
32	12644678	GTCKAECPTWEGICINKAPCVKCC KAQPEKFTDGHCSKILRRCLCTKPC	NF		[16]
33	12811766	GVTITVKPPFPGCVFYECIANC RSRGYKNGGYCTINGCQCLR	275		[38]

Table 4 continued

No.	PUBMED ID	Sequence	Cytotoxicity	#1	Reference
34	2104802	GWLRKLGKKIERIGQHTRDASI QVLGIAQQAANVAATAR	10		[39]
35	18618271	HTPTPTPICKSRSHEYKGRCIQDMDCNAACV KESESYTGGFCNGRPPFKQCF CTKPCKRERAAATLRWPGL	80–159		[40]
36	12138110	ICIFCCGCCHRSKCGMCCKT	NF		[41]
37	19857508	ILGIITSLLKSLGKK	0.5–50		[37]
38	21167915	ITCQQVTSELGPCVPYLTGQGIP	100–400		[42]
39	9435139	KDCKTESNTFPGICITKPPCRKACI KEKFTDGHCSKILRRCLCTKPC	NF		[43]
40	19857508	KDLHTVVSAILQAL	0.5–50	**N**	[37]
41	21056078	KICERASGTWKGICIHSNDCNNQCV KWENAGSGSCHYQFPNYMCFCYFDC	2–6		[44]
42	21056078	KICERASGTWKGICIHSNDCNNQCV KWENAGSGSCHYQFPNYMCFCYFNC	2–6		[44]
43	8422949	KLCERPSGTWSGVCGNNNACKNQCI NLEKARHGSCNYVFPAHKCICYFPC	25–100		[45]
44	8422949	KLCERPSGTWSGVCGNSNACKNQCIN LEKARHGSCNYVFPAHKCICYFPC	35–100		[45]
45	8422949	KLCERSSGTWSGVCGNNNACKNQCIN LEGARHGSCNYVFPYHRCICYFPC	19–100		[45]
46	8422949	KLCQRPSGTWSGVCGNNNACKNQCIN LEKARHGSCNYVFPAHKCICYFPC	4–100		[45]
47	18241956	KQQLATEAESAGPIL	15	**N**	[46]
48	10571855	KRDPQQREYEDCRRRCEQQEPRQQ HQCQLRCREQQRQHGRG GDMMNPQRGGSGRYEEGEEEQS	25		[47]
49	9435139	KSTCKAESNTFPGLCITKPPCRKACL SEKFTDGKCSKILRRCICYKPC	NF	**N**	[43]
50	10860545	KTCEHLADTYRGVCFTNASCDDHC KNKAHLISGTCHNWKCFCTQNC	0.04–100	**N**	[48]
51	18786582	KTCENLADTYKGPCFTTGSCD	25–100		[49]
52	10860545	KTCENLSGTFKGPCIPDGNCNKHCRN NEHLLSGRCRDDFRCWCTNRC	0.34–100	**N**	[48]
53	2103443	KTCENLVDTYRGPCFTTGSCDDHCKN KEHLLSGRCRDDVRCWCTRNC	NF	**N**	[50]
54	10591099	LCNERPSQTWSGNCGNTAHCDKQCQ DWEKASHGACHKRENHWKCFCYFNC	NF		[51]
55	21736910	LMCTHPLDCSN	4–32		[52]
56	19914321	LSKFGGECSLKHNTCTYLKGGKNHVVNCGSAA NKKCKSDRHHCEYDEHHKRVDCQTPV	NF		[53]
57	10601197	MINRTDCNENSYLEIHNNEGRDTLCFANA GTMPVAIYGVNWVESGNNVVTLQFQRN LSDPRLETITLQKWGSWNPGHIHEILSIRIY	20–150	**N**	[54]
58	NO	NEMGGPLVVEARTCESQSHKFKGTCL SDTNCANVCHSERFSGGKCRGFRRRCFCTTHC	NF	**N**	[55]
59	1799696	NTCENLAGSYKGVCFGGCDRHCR TQEGAISGRCRDDFRCWCTKNC	NF	**N**	[56]
60	7647301	QICKAPSQTFPGLCFMDSSCRKY CIKEKFTGGHCSKLQRKCLCTKPC	NF		[57]
61	22032337	QKLCERPSGTWSGVCGNNGACRNQ CIRLERARHGSCNYVFPAHKCICYFPC	5–20		[58]
62	8422949	QKLCERPSGTWSGVCGNNNACKN QCINLEKARHGSCNYVFPAHKCICYFPC	70–100		[59]

Table 4 continued

No.	PUBMED ID	Sequence	Cytotoxicity	#1	Reference
63	22032337	QKLCERPSGTWSGVCGNNNACRNQ CINLEKARHGSCNYVFPAHKCICYFPC	10–15		[58]
64	7780308	QKLCERSSGTWSGVCGNNNACKNQ CINLEGARHGSCNYIFPYHRCICYFPC	5–11		[60]
65	8422949	QKLCQRPSGTWSGVCGNNNACKNQCIR LEKARHGSCNYVFPAHKCICYFPC	5–100		[59]
66	10447467	QNNICKTTSKHFKGLCFADSKCRKVCIQEDK FEDGHCSKLQRKCLCTKNC	NF		[61]
67	9507071	QQCGRQASGRLCGNRLCCSQWGYCGSTA SYCGAGCQSQCR	0.6–7		[62]
68	20626828	RECKAQGRHGTCFRDANCVQVCEKQA GWSHGDCRAQFKCKCIFEC	5–20		[63]
69	1495489	RECKTESNTFPGICITKPPCRKACISEKF TDGHCSKLLRRCLCTKPC	NF		[64]
70	18618271	RHRHCFSQSHKFVGACLRESNCENV CKTEGFPSGECKWHGIVSKCHCKRIC	6–117		[65]
71	11451958	RILSILRHQNLLKELQDLAL	12–119	**N**	[66]
72	1528633	RKFHEKHHSHREFPFYGDYGSNYLYDN	NF		[67]
73	1528633	RKFHEKHHSHRGYRSNYLYDN	NF	**N**	[67]
74	2303595	RKFHEKHHSHRGYR	NF	**N**	[26]
75	2303595	RKFHEKHHSHRGY	NF		[26]
76	10571855	RQRDPQQQYEQCQKHCQRRETEPRHMQ TCQQRCERRYEKEKRKQQKRY EEQQREDEEKYEERMKEEDN	2–50		[68]
77	11101813	RTCENLADKYRGPCFSGCDTHCT TKENAVSGRCRDDFRCWCTKRC	NF		[69]
78	18611251	RTCESQSHRFKGTCVRQSNCAAV CQTEGFHGGNCRGFRRRCFCTKHC	6–20		[70]
79	1995329	RVCMKGSAGFKGLCMRDQNCAQV CLQEGWGGGNCDGVMRQCKCIRQC	NF	**N**	[71]
80	9715910	RVCMKGSQHHSFPCISDRLCSNECVKEE GGWTAGYCHLRYCRCQKAC	NF		[72]
81	22032337	RYCERSSGTWSGVCGNSGKCSNQCQRLE GAAHGSCNYVFPAHKCICYYPC	25	**N**	[58]
82	22032337	RYCERSSGTWSGVCGNTDKCSSQCQRL EGAAHGSCNYVFPAHKCICYYPC	20–25	**N**	[58]
83	18618271	RYCLSQSHRFKGLCMSSSNCANVCQT ENFPGGECKADGATRKCFCKKIC	5–11		[65]
84	10512732	SKYGGECSVEHNTCTYLKGGKDHIV SCPSAANLRCKTERHHCEYDEHHKTVDCQTPV	26–96		[73]
85	18943919	SYFSAWAGPGCNNHNARYSKCGCSNIG HNVHGGYEFVYQGQTAAAYNTDNCK GVAQTRFSSSVNQACSNFGWKSVFIQC	NF		[74]
86	23193597	TFPKCAPTRPPGPKPCDINNFKSKFWHIWRA	4	**N**	[75]
87	20721297	VTCDVLSFEAKGIAVNHSACALHCIAL RKKGGSCQNGVCVCRN	4–8		[76]
88	8535393	YGPGDGHGGGHGGGHGGGHGNGQGGGH GHGPGGGFGGGHGGGHGGGGRGGGGS GGGGSPGHGAGGGYPGGHGGGHHGGYQTHGY	NF	**N**	[77]

Subject sequences identified by polarity index method in APD2 database http://aps.unmc.edu/AP/ accessed December 19, 2012 [12], where peptides have action only on fungi. #1: (N) peptides not accepted by polarity index method. Source: National Center for Biotechnology Information, U.S. National Library of Medicine http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins in database: Swiss-Prot (swissprot), accessed March 20, 2013. Cytotoxicity: the concentration (μg/mL) required for inhibiting the growth of mammalian cells, usually red blood cells or fibroblasts. NF: The toxicity report was not found

20 cancer cells, 0 HIV, 88 fungi, 35 H1N1, 2 insects, 11 mammalian cells, 9 parasites, 1 protists, 0 chemotaxis, 30 SCAAP, 0 sperms, and 21 virus. The classification of single-acting peptides, took place checking all peptide occurrences in all groups.

Results

The computational mathematical method described here was evaluated with the APD2 database [12] ranked by single and multiple action peptides, showing the following results:

It showed over $62/88 = 70\ \%$ efficiency detecting antifungal peptides in all APD2 database sub-classifications (Table 3, column Fungi), and $1/1 = 100\ \%$ detecting anti-protists (Table 3, column Protists). Similarly, it is observed that in the remaining groups of the same database, the method does not identify false positives by more than $26/88 = 30\ \%$ (Table 4, entries with N-letter). The method does not establish a significant difference with regard to their toxicity (Table 4, column Cytotoxicity), as it does not differentiate the most toxic from the least toxic.

Discussion

The re-emergence of pathogens resistant to many antibiotics must be an alarm signal to trigger their combat with multidisciplinary procedures, under this idea we could think that the identification of anti-fungi peptides should be aimed toward the design of algorithms that include diverse factors. The thesis of this work focuses only on the linear sequence of the peptide as the main source of the peptide toxic action. This does not mean that the lipid aqueous medium or cell membrane has no influence, in fact, we consider they are important as our algorithm uses "training peptides" with the desired profile. From this perspective, we can say that this method, in its identification of anti-fungi peptides is inclusive of these and other factors as yet unidentified. So far, we have found that one of the factors relate to the toxic profile of the peptide. Polarity index method informs extensively and in detail about the polar profile and the electro-negativity of the peptide, but in a different way than conventional algorithms do, as the polarity matrix reports every possible polar interaction, providing a dynamic and also a static profile of the peptide.

Furthermore, many algorithms designed for anti-fungi identification generate a single element which represents little information, in comparison with the 16 elements provided by the matrix of our polarity method. The method has already been used for the efficient identification of SCAAP [7] and prebiotic analysis [11] due to its robustness; however, we consider the method is not efficient enough identifying the most toxic anti-fungi peptides, which means we have to improve its search profile. With this in mind, we have sub-classified the groups located in APD2 database [12] from 9 to 14 groups ([7] Table 3), and in this sense we make sure the method remains exclusive.

On the basis of the above, we believe this method is a reliable filter of anti-fungi peptides as it reduces considerably the number of experimental trials; it can also be used to know how nature constructs these peptides and utilize this information to perfect the search profile to find peptides more toxic to pathogens.

Conclusions

In summary, we report an implementation of a polarity index method in the prediction of anti-fungal peptides from APD2 database, with high level of discriminative efficiency ($63/89 = 70$), from single physicochemical feature, polarity.

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Conflict of interest We declare that we do not have any financial and personal interest with other people or organizations that could inappropriately influence (bias) our work.

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