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Analysis of Suitable Signal Peptides for Designing a Secretory Thermostable Cyanide Degrading Nitrilase: An in Silico Approach

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Abstract

Secretory production of recombinant proteins has many benefits such as solubility and ease of purification. The aim of this study was to find suitable signal peptides for secretory production of nitriles in Bacillus subtilis. The signal peptides were chosen from Signalpeptide web server. SignalP server was used to define probability of suitable signal peptides and their secretion pathways. Physico-chemical properties and solubility were predicted by ProtParam and Protein-sol, respectively. Bioinformatics analysis identified SUBF_BACSU, GUB_BACSU, SACB_BACAM and AMY_BACAM that linked to nitriles are appropriate signal peptides. Their fusion proteins could be stable, soluble and non-antigenic proteins that might have suitable secondary and tertiary structures. The recommended signal peptides by this study are appropriate for rational designing of secretory soluble nitriles. Thus, the results of present work can be useful for future experimental production of secretory and soluble nitriles.

Keywords: In silico, Nitrilase, Cyanide, Signal Peptide.

1 Introduction

Polluted water and soil are major environmental problems. Use of pesticides, fossil fuels, fertilizers, mining, municipal wastes, and sewage are the main cause of this pollution (1). Sewage from industrial wastewater and agricultural processing can hold a considerable amount of potential pollutants, including organisms/pathogens, trace organic chemicals and toxic compounds, such as cyanides, sulphates, phenols, and metals, etc.(3 ,2). Cyanide compounds can be highly toxic to many life forms and are available in the form of nitriles, cyanides, and carbonitriles (4). Cyanide can bind to the enzyme cytochrome c oxidase and inhibit the transportation of electrons from cytochrome c to oxygen. As a result, it affects the cellular use of oxygen and oxidative metabolism (5-7). The organs which are mainly affected by cyanide poisoning are the central nervous system and heart (8, 9). Although conventional methods for treatments of cyanide wastewaters such as chemical and physical treatments efficiently can decrease the toxicity from cyanide, they involve high cost of construction and engineering, long-term storage, and require reagents that can be harmfulto the environment (10, 11). Undertaking biological processes for cyanide degradation is the best alternatives and approaches to overcome these problems (12, 13). Cyanides removal by biodegradation process includes utilizing the microorganisms and plants which have the various enzymes in their systems, and able to converting cyanides to non-toxic compounds (13, 14). Using these methods are efficient, cost-effective, and have a faster rate compare to conventional ones (15). Nitrilases are the enzymes related to the group of hydrolases that convert the nitrile (R-CN) compounds into their corresponding carboxylic acids and ammonia using a simple hydrolytic pathway. They are widely found in nature and can be isolated from microbes

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and plants (16, 17). Nitrilases have been acknowledged as valuable biocatalysts in the protection of the environment due to their capability to convert cyanide to non-toxic products (18). Bacteria are broadly used as cell factories with the aim of recombinant proteins production. Its request in biotechnology has increased dramatically with every passing day, as it represents a multi-billion-dollar market through its application such as technical bulk enzymes and biopharmaceutical proteins (19). Most of its applications include complex proteins are challenging to produce thus in production of a successful recombinant protein the important consideration is the choice of a proper expression system and also the host's potential for the secretion of heterologous proteins since it is critical to provide an active and stable protein (20). High-level expression of heterolegus proteins can lead in accumulation of insoluble misfolded proteins in the cytoplasm, which is identified as the inclusion bodies. These aggregated intermediates can not have a suitable biological activity. As a result, one important step in production of recombinant protoein is to refolded the inclusion bodies to to get the applicable soluble proteins (21). The gram-negative *Escherichia coli* (*E. coli*) and the gram-positive *Bacillus subtilis* (*B. subtilis*) are the favourite hosts for producing the vast majority of recombinant proteins (22, 23). *E. coli* has several advantages for heterologous protein expression, such as easy growth on inexpensive media and rapid biomass accumulation in a short fermentation period (24-26). However, there is some limitation in using this organism, as recombinant proteins in *E. coli* can express in the form of inclusion bodies and incorrect protein folding while, *B. subtilis* has many outstanding features. It is non-pathogenic and endotoxin-free organism (27, 28) and has a great protein secretory capability as they can secrete proteins directly and efficiently into the cultural medium via the Sec and Tat pathway (27). Furthermore, their outstanding biochemical and physiological features make them easy for experiment handling and genetic manipulation (29). Another characteristic of *B. subtilis* that makes it a more suitable host for the production of secretory heterologous protein is its lack of outer membrane (30, 31). Therefore, they can be applied for the efficient production of large-scale industrial enzymes, proteins, and antibiotics. In addition to *B. subtilis* potential for the secretion of recombinant proteins, the signal peptides (SPs) that direct the export proteins into the general secretory pathway are important. SPs can improve the protein folding, solubility of recombinant proteins, and reduces the purification process. (32). SPs are normally short (15 to 30 amino acids long) and share a common triplex structure including a positively charged amino-terminal region (n-region), a hydrophobic region (h-region) and a cleavable site (c-region). N and h-region have a role in channelling the recombinant proteins into periplasmic space. C-region as a cleavage site location is important as it can be recognized by signal peptidase enzyme (33, 34). This study aimed to comparedesigning computationally the several signal peptides with respect to their amino acid composition and their physico-chemical properties in order to investigate and select suitable candidates for secretory production of nitrilase in the *B. subtilis* host.

2 Methods

2.1 Dataset retrieval

The amino acid sequences of 30 SPs peptides were retrieved from database resources, "Signal Peptide Website" (http://www.signalpeptide.de). UniProt" (http://www.uniprot.org) were employed to confirmed selected signal peptides data according to the experimental evidence. The key factors of collecting the data for the following steps were the ones belonged to bacterial secretory proteins and mentioned in previous studies. Retrieved sequences are listed in Table 1.

2.2 Prediction of signal peptides presence, their cleavage site location and Secretion pathway

SignalP with an accuracy of 87% (35) is an artificial neural network (ANN) based tool and is categorized among the most accurate and reliable tools in all proposed data mining models for prediction of the SPs sequences and their precise cleavage sites (36). In this study, the SignalP (version 5) server (http://www.cbs.dtu.dk/services/SignalP) was employed to identify the signal peptide probability and their precise cleavage sites together with their secretion pathway. Sequences with probability value greater than 0.5, were introduced as competent signal peptides for next section analysis.

2.3. Evaluation of signal peptides physico-chemical properties and solubility

ProtParam, an online software at (http://web.expasy.org/protparam/) (37), was utilized for in silico prediction of the diverse physico-chemical features of the recorded signal peptides, containing amino acid composition, GRAVY (grand average of hydropathicity), aliphatic index, molecular weight (MW), theoretical isoelectric point (pI), positively and negatively charged residues and instability index on SPs linked to nitrilase (37, 38). Prediction of protein solubility was done by online software, "Protein-sol"(http://protein-sol.manchester.ac.uk). This server provides a solubility score between 0-1 to make interpreting the results simpler(39). For the final analysis, unstable fusion proteins were removed and the SPs representing a solubility higher than 0.45 selected.

2.4. In silico prediction of signal peptides secretion pathway and sub-cellular localization

Searches for Sub-cellular localization of different signal peptide infusion with nitrilase and their final destination were performed using an online tool "ProtCompB" (http://www.softberry.com). The Softberry prediction is based on neural networks and reports between 86–100% correct prediction (40-42).

3 Result

3.1 Selection of signal peptides and sequence definition

A total number of 30 prokaryotic signal peptides was selected from several different Gram-positive and Gram-negative bacteria and their primary structure was retrieved from online server which are listed in Table 1.

Table 1: Amino acid sequences of the signal peptides.

	Accession No.	Signal Peptide	Protein Name	Source	Amino Acid Sequence
1	P06278	AMY_BACLI	Alpha amylase	*Bacillus licheniformis*	**MKQQKRLYAR**LLTLLFALIFLLPHSAAAA
2	P00808	BLAC_BACLI	Beta-lactamase	*Bacillus licheniformis*	**MKLWFSTLKLKK**AAAVLLFSCVALAG
3	P16397	SUBF_BACSU	Bacillopeptidase F	*Bacillus subtilis*	**MRKKTKNRL**ISSVLSTVVISSLLFPGAAGA
4	Q02113	CWBA_BACSU	Amidase enhancer	*Bacillus subtilis*	**MKSCK**QLIVCSLAAILLLIPSVSFA
5	P42111	YXAL_BACSU	Uncharacterized protein yxaL	*Bacillus subtilis*	**MVKSFRMK**ALIAGAAVAAAVSAGAVSDVP AAKVLQPTAAYA
6	O31737	YLQB_BACSU	Uncharacterized protein ylqB	*Bacillus subtilis*	**MKK**IGLLFMLCLAALFTIGFPAQQADA
7	P00691	AMY_BACSU	Alpha-amylase	*Bacillus subtilis*	**MFAKR**FKTSLLPLFAGFLLLFHLVLAG
8	P39116	PEL_BACSU	Pectate lyase	*Bacillus subtilis*	**MKK**VMLATALFLGLTPAGANA
9	O07532	LYTF_BACSU	Endopeptidase lytF	*Bacillus subtilis*	**MKKK**LAAGLTASAIVGTTLVVTPAEA
10	P42251	PPBD_BACSU	Alkaline phosphatase D	*Bacillus subtilis*	**M**AYDSRFDEWVQKLKEESFQNNTFDRRKFIQ GAGKIAGLSLGLTIAQSVGAFEVNA
11	P34957	QOX2_BACSU	Quinol oxidase subunit 2	*Bacillus subtilis*	**M**VIFLFRALKPLLVLALLTVVFVLGG
12	P04957	GUB_BACSU	Beta-glucanase	*Bacillus subtilis*	**MPYLK**RVLLLLVTGLFMSLFAVTATASA
13	P00648	RNBR_BACAM	Ribonuclease	*Bacillus amyloliquefaciens*	**MMKMEGIALKK**RLSWISVCLLVLVSAAGM LFSTA
14	P21130	SACB_BACAM	Levansucrase	*Bacillus amyloliquefaciens*	**MNIKK**IVKQATVLTFTTALLAGGATQAFA
15	P00692	AMY_BACAM	Alpha-amylase	*Bacillus amyloliquefaciens*	**MIQKRKR**TVSFRLVLMCTLLFVSLPITKTSA
16	P07980	GUB_BACAM	Beta-glucanase	*Bacillus amyloliquefaciens*	**MKR**VLLILVTGLFMSLCGITSSVSA
17	Q05523	DACA_BACST	D-alanyl-D-alanine carboxypeptidase dacA	*Bacillus stearothermophilus*	**MRRRK**QNWLFWLLSICLCLTFGPFQQTVKA
18	P31797	CDGT_BACST	Cyclomaltodextrin glucanotransferase	*Bacillus stearothermophilus*	**MRR**WLSLVLSMSFVFSAIFIVSDTQKVTVEA
19	P06874	THER_BACST	Thermolysin	*Bacillus stearothermophilus*	**MNKR**AMLGAIGLAFGLLAAPIGASA
20	Q5ZF24	Q5ZF24_LACSK	n/a	*Lactobacillus sakei*	MQNTKELSVVELQQILGG
21	P23550	GUNB_PAELA	Endoglucanase B	*Paenibacillus lautus*	MKKRRSSKVILSLAIVVALLAAVEPNAALA
22	Q93MG8	Q93MG8_THIFE	n/a	*Thiobacillus ferrodoxin*	MFKRLANAAIPFALVGMLFGLSVSVASA
23	P50500	IRO_THIFE	Iron oxidase	*Thiobacillus ferrodoxin*	MSEKDKMITRRDALRNIAVVVGSVATTTMM GVGVADA
24	P24930	RUS2_THIFE	Rusticyanin	*Thiobacillus ferrodoxin*	MYTQNTMKKNWYVTVGAAAALAATVGMG TAMA
25	P74917	CY552_THIFE	Cytochrome c-552	*Thiobacillus ferrodoxin*	MTTYLSQDRLRNKENDTMTYQHSKMYQSRT FLLFSALLLVAGQASAA
26	P45741	THI1_PANTH	Thiaminase-1	*Paenibacillus thiaminolyticus*	MSKVKGFIYKPLMVMLALLLVVVSPAGAG
27	P21543	AMYB_PAEPO	Beta/alpha-amylase	*Paenibacillus polymyxa*	MTLYRSLWKKGCMLLLSLVLSLTAFIGSPSNT ASA
28	P45796	XYND_PAEPO	Arabinoxylan arabinofuranohydrolase	*Paenibacillus polymyxa*	MIRKCLVLFLSFALLLSVFPMLNVDA
29	P31835	CDGT2_PAEMA	Cyclomaltodextrin glucanotransferase	*Paenibacillus macerans*	MKKQVKWLTSVSMSVGIALGAALPVWA
30	P04830	CDGT1_PAEMA	Cyclomaltodextrin glucanotransferase	*Paenibacillus macerans*	MKSRYKRLTSLALSLSMALGISLPAWA

The amino acids in the n-region are shown in boldface and the underlined amino acids represent the c-region.

3.2 Signal Peptide prediction by SignalP-5.0 Server

Based on the SignalP result, SPs with probability below 0.5 were excluded from this analysis since signal peptidase might not identify their cleavage site. The probability results are shown in Table 2. BLAC_BACLI, YXAL_BACSU, AMY_BACSU, PPBD_BACSU, QOX2_BACSU, RNBR_BACAM and Q5ZF24_LACSK were under the cut-off value.

3.3 Physico-chemical properties and solubility

ProtParam results as shown in Table 3. The length of all signal peptides were in the range of 21 to 47 amino

acids and net positive charge in the n-region was between 1 to 6. The highest aliphatic index belonged to CWBA_BACSU (160.00), XYND_PAEPO (161.15) and GUNB_PAELA (153.00). XYND_PAEPO (1.669), CWBA_BACSU (1.596) and GUB_BACAM (1.508) had the highest GRAVY. Based on instability and solubility results, nitrilase fused with all SPs were predicted to be stable and soluble.

3.4 In silico prediction of signal peptides sub-cellular localization

Based on ProtCompB results, SUBF_BACSU, GUB_BACSU, SACB_BACAM and AMY_BACAM can translocate nitrilase into extracellular space. So other SPs were excluded in this study (Table 4).

4 Discussion

Nitrilase, as a monomeric protein and lacks disulfide bonds, looks to be a suitable candidate for secretory production in prokaryote hosts like *Bacillus subtilis*. Sec, SRP, and TAT are pathways that are recruited by prokaryotes. Considering the problem of creating high amounts of inclusion bodies, aggregation and misfolding in the high expression levels of recombinant proteins, protein secretion can be a solution and for secretion through above mentioned pathways, an appropriate SP selection is an essential step. Therefore, the SPs have a key role in directing the protein into the periplasmic space and out of cell (43).

Computational tools are being used in the large variety of eras in medical and biological fields. Using bioinformatics tools helps to reduce the cost of experiments and provide more accurate and validate outcomes (44, 45). In this study, several different signal peptides were evaluated and a total number of prokaryotic SPs from different organisms with the acceptable efficiency in the previous studies were selected. For a successful secretion, various features should be balanced during the secretory pathway. The physicochemical and structural features of a signal peptide are important properties that should be carefully considered. Therefore, different computational methods have been applied to predict the physicochemical properties of the SPs.

Table 2: Signal Peptide Probability, Cleavage Site and Secretion pathway

No.	Signal peptide	Probability	Cleavage site	Secretion pathway
1	AMY_BACLI	0.58	AAA-AM (28,29)	(Sec/SPI)
2	BLAC_BACLI	0.15	-	-
3	SUBF_BACSU	0.51	AGA-MV (30,31)	(Sec/SPI)
4	CWBA_BACSU	0.70	SFA-MV (25,26)	(Sec/SPI)
5	YXAL_BACSU	0.26	-	-
6	YLQB_BACSU	0.76	ADA-MV (27,28)	(Sec/SPI)
7	AMY_BACSU	-	-	-
8	PEL_BACSU	0.80	ANA-MV (21,22)	(Sec/SPI)
9	LYTF_BACSU	0.91	AEA-MV (26,27)	(Sec/SPI)
10	PPBD_BACSU	0.44	-	-
11	QOX2_BACSU	-	-	-
12	GUB_BACSU	0.69	ASA-MV (28,29)	(Sec/SPI)
13	RNBR_BACAM	0.07	-	-
14	SACB_BACAM	0.78	AFA-MV (29,30)	(Sec/SPI)
15	AMY_BACAM	0.75	TSA-MV (31,32)	(Sec/SPI)
16	GUB_BACAM	0.56	VSA-MV (25,26)	(Sec/SPI)
17	DACA_BACST	0.89	VKA-MV (30,31)	(Sec/SPI)
18	CDGT_BACST	0.64	VEA-MV (31,32)	(Sec/SPI)
19	THER_BACST	0.76	ASA-MV (25,26)	(Sec/SPI)
20	Q5ZF24_LACSK	-	-	-
21	GUNB_PAELA	0.79	ALA-MV (30,31)	(Sec/SPI)
22	Q93MG8_THIFE	0.69	ASA-MV (28,29)	(Sec/SPI)
23	IRO_THIFE	0.73	ADA-MV (37,38)	(Tat/SPI)
24	RUS2_THIFE	0.63	AMA-MV (32,33)	(Sec/SPI)
25	CY552_THIFE	0.55	ASA-AM (46,47)	(Sec/SPI)
26	THI1_PANTH	0.60	AGA-GM (28,29)	(Sec/SPI)
27	AMYB_PAEPO	0.82	ASA-MV (35,36)	(Sec/SPI)
28	XYND_PAEPO	0.86	VDA-MV (26,27)	(Sec/SPI)
29	CDGT2_PAEMA	0.73	VWA-MV (27,28)	(Sec/SPI)
30	CDGT1_PAEMA	0.84	AWA-MV (27,28)	(Sec/SPI)

Table 3: Physico-chemical properties and solubility and Final prediction site of signal peptides

No.	Signal peptide	Amino acids	Net Charge	Aliphatic Index	GRAVY	Instability Index Without protein	Instability index with protein	Solubility (Probability)
1	AMY_BACLI	29	4	141.72	0.752	unstable (50.51)	stable (30.42)	Soluble(0.540)
2	SUBF_BACSU	30	5	117.00	0.497	stable (20.75)	stable (27.43)	Soluble(0.558)
3	CWBA_BACSU	25	2	160.00	1.596	unstable (42.97)	stable (29.48)	Soluble(0.533)
4	YLQB_BACSU	27	1	119.63	1.122	stable (31.87)	stable (28.54)	Soluble(0.548)
5	PEL_BACSU	21	2	111.90	0.948	stable (10.61)	stable (26.89)	Soluble(0.575)
6	LYTF_BACSU	26	2	116.54	0.769	stable (0.70)	stable (25.71)	Soluble(0.582)
7	GUB_BACSU	28	2	142.86	1.443	unstable (40.54)	stable (29.39)	Soluble(0.567)
8	SACB_BACAM	29	3	107.93	0.710	stable (26.18)	stable (28.00)	Soluble(0.564)
9	AMY_BACAM	31	6	119.35	0.606	unstable (47.44)	stable (30.23)	Soluble(0.533)
10	GUB_BACAM	25	2	148.00	1.508	unstable (45.44)	stable (29.70)	Soluble(0.545)
11	DACA_BACST	30	5	91.00	0.117	unstable (86.84)	stable (34.22)	Soluble(0.476)
12	CDGT_BACST	31	1	116.13	0.887	unstable (46.86)	stable (30.17)	Soluble(0.528)
13	THER_BACST	25	2	121.60	1.100	stable (33.02)	stable (28.62)	Soluble(0.576)
14	GUNB_PAELA	30	4	153.00	0.920	unstable (67.87)	stable (32.27)	Soluble(0.585)
15	Q93MG8_THIFE	28	2	122.14	1.379	stable (18.74)	stable (27.28)	Soluble(0.572)
16	IRO_THIFE	37	1	92.16	0.276	unstable (61.56)	stable (32.33)	Soluble(0.549)
17	RUS2_THIFE	32	2	64.38	0.372	stable (-3.23)	stable (24.78)	Soluble(0.541)
18	CY552_THIFE	47	2	74.89	-0.355	unstable (51.76)	stable (31.78)	Soluble(0.488)
19	THI1_PANTH	29	3	141.03	1.359	stable (34.22)	stable (28.80)	Soluble(0.575)
20	AMYB_PAEPO	35	3	117.14	0.751	unstable (45.90)	stable (30.28)	Soluble(0.500)
21	XYND_PAEPO	26	1	161.15	1.669	stable (15.75)	stable (27.07)	Soluble(0.549)
22	CDGT2_PAEMA	27	3	115.56	0.785	stable (34.65)	stable (28.80)	Soluble(0.536)
23	CDGT1_PAEMA	27	4	115.93	0.467	stable (36.17)	stable (28.94)	Soluble(0.541)

A critical step in designing constructs for secretory production of the recombinant proteins is the exact prediction of the cleavage sites located in the signal peptide. For this purpose, the SignalP server was employed and the probability was considered as the most important parameter to identify SPs. According to the default cutoff value of 0.5, SPs with cut-off value > 0.5 was identified as potential SPs for nitrilase (46).

SignalP can distinguish appropriate and non-appropriate sequences for secretory protein production. Based on the results of SignalP analysis, the cleavage sites of BLAC_BACLI, YXAL_BACSU, AMY_BACSU, PPBD_BACSU, QOX2_BACSU, RNBR_BACAM, and Q5ZF24_LACSK signal peptides infusion with nitrilase cannot properly recognize by signal peptidase. Consequently, they are not recognized as potential choices for nitrilase secretion and were disregarded from further analysis. According to SignalP (Table 2), 23 out of 30 collected SPs were identified as proper SPs for nitrilase. However, more characteristics were required to select appropriate SPs.

Data aslo were compared according to the key physicochemical features of the signal peptides including net positive charge, GRAVY, instability and aliphatic indexes by ProtParam server (Table 3). GRAVY and Aliphatic index are the two factors which are directly associated with hydrophobicity (22) and improving the level of this parameter and length of the h-region, leads to

improve the rate of the protein secretion. (47). As shown in Table 3, all 23 listed SPs in this step, had suitable aliphatic indexes and hydrophobicity for secretion.

It is also suggested that the higher net positive charge could be an advantage for increasing the rate of translocation desired protein from the phospholipid membrane (48). According to Table 3, the net positive charge of all evaluated SPs were between 1 and 6 based on ProtParam results and they were suitable for the next step analysis. Protein instability was indicated by Instability index above 40. It means proteins with instability index lower than 40 are stable (49). Some of SPs are unstable alone but after fusing to nitrilase they would be stable. These SPs were chosen for solubility predictions. In silico methods for evaluation of the Protein solubility in the experimental studies is a very crucial parameter because it can cause the formation of inclusion bodies. By implementing the SOLpro server, all 23 stable fusion proteins were soluble. Hence, according to the stability and solubility analysis in Table 3, all signal peptides connected to nitrilase were predicted to be stable and none of them could be omitted based on these analysis.

Secretory proteins can be directed to the subcellular locations by fusing suitable signal peptides to their N-terminus. To more efficient purification and production of the heterologous proteins, the ProtCompB server was implemented to predict the ultimate destination and subcellular localization of SPs infusion with nitliase. It was elucidated that among 23 stable and soluble SPs, 15 of them can translocate nitrilas protein to cytoplasmic compartment. four SPs could guide nitrilase to extracellular space while 11 of them could not translocate nitrilas into growth medium. Therefore, it seems only these four signal sequences (GUB_BACSU, SACB_BACAM, AMY_BACAM, and SUBF_BACSU) can be introduced as applicable SPs. The secretion of heterologous proteins into the extracellular space can considerably reduce the purification process since cell disruption and the subsequent purification would be skipped. Moreovere, intracellular expression of recombinannat protein can lessen the toxic impacte of target proteins on the production of host microorganism and As a result, intracellular expression strategies can significantly reduce the cost of recombinanat protein production (50).

As far as we know, this is the first study with the aim of investigating the suitable SPs connected with nitrilase by evaluating their potential impact on the secretion pathway of this protein. This study evaluated 30 different signal peptides to introduce the most reliable ones for secreting the recombinant nitrilase protein out of Bacillus subtilis host. The results of this study showed that GUB_BACSU, SACB_BACAM, AMY_BACAM, and SUBF_BACSU could be considered as appropriate candidates for the nitrilase secretion. However, additional experimental studies should be carried out to confirm these results.

Table 4: Evaluation of secretion pathways and sub-cellular location of SPs

No.	**Signal peptides**	**Cytoplasmic**	**Cytoplasmic-Membrane**	**Cell wall**	**Extracellular**	**Final prediction site**
1	AMY_BACLI	0.00	8.80	0.22	0.98	Cytoplasmic-Membrane
2	SUBF_BACSU	0.00	0.09	0.18	9.73	Extracellular
3	CWBA_BACSU	0.32	9.55	0.12	0.01	Cytoplasmic-Membrane
4	YLQB_BACSU	02.50	2.50	2.50	2.50	Unknown
5	PEL_BACSU	7.50	1.15	0.62	0.73	Cytoplasmic
6	LYTF_BACSU	0.00	3.33	3.33	3.33	Unknown
7	GUB_BACSU	0.01	0.09	0.18	9.72	Extracellular
8	SACB_BACAM	0.00	0.09	0.18	9.73	Extracellular
9	AMY_BACAM	0.01	0.09	0.18	9.72	Extracellular
10	GUB_BACAM	7.50	1.15	0.62	0.73	Cytoplasmic
11	DACA_BACST	0.00	10.00	0.00	0.00	Cytoplasmic-Membrane
12	CDGT_BACST	0.00	4.60	2.48	2.92	Unknown
13	THER_BACST	2.50	2.50	2.50	2.50	Unknown
14	GUNB_PAELA	0.00	9.87	0.12	0.01	Cytoplasmic-Membrane
15	Q93MG8_THIFE	0.32	9.55	0.12	0.01	Cytoplasmic-Membrane
16	IRO_THIFE	7.50	1.15	0.62	0.73	Cytoplasmic
17	RUS2_THIFE	2.50	2.50	2.50	2.50	Unknown
18	CY552_THIFE	0.32	9.55	0.12	0.01	Cytoplasmic-Membrane
19	THI1_PANTH	2.50	2.50	2.50	2.50	Unknown
20	AMYB_PAEPO	0.00	8.80	0.22	0.98	Cytoplasmic-Membrane
21	XYND_PAEPO	1.78	8.16	0.06	0.01	Cytoplasmic-Membrane
22	CDGT2_PAEMA	0.00	3.33	3.33	3.33	Unknown
23	CDGT1_PAEMA	0.00	4.60	2.48	2.92	Unknown

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