# SINGULAR VALUE DECOMPOSITION (SVD) AND BLAST
## *Quite Different Methods Achieving Similar Results*

Bráulio Roberto Gonçalves Marinho Couto
*Centro Universitário de Belo Horizonte / UNI-BH, Av. Professor Mário Werneck 1685, Belo Horizonte, Brazil*

Macelo Matos Santoro
*Departamento de Bioquímica e Imunologia, UFMG, Av. Antonio Carlos 6627, Belo Horizonte, Brazil*

Marcos Augusto dos Santos
*Departamento de Ciência da Computação, UFMG, Av. Antonio Carlos 6627, Belo Horizonte, Brazil*

Keywords: Genomics, Matrix analysis, BLAST, SVD.

Abstract: The dominant methods to search for relevant patterns in protein sequences are based on character-by-character matching, performed by software known as BLAST. In this paper, sequences are recoded as *p*-peptide frequency matrix that is reduced by singular value decomposition (SVD). The objective is to evaluate the association between statistics used by BLAST and similarity metrics used by SVD (Euclidean distance and cosine). We chose BLAST as a standard because this string-matching program is widely used for nucleotide searching and protein databases. Three datasets were used: mitochondrial-gene sequences, non-identical PDB sequences and a Swiss-Prot protein collection. We built scatter graphs and calculated Spearman correlation (ρ) with metrics produced by BLAST and SVD. Euclidean distance was negatively correlated with bit score (ρ>-0.6) and positively correlated with E value (ρ>+0.7). Cosine had negative correlation with E value (ρ>-0.7) and positive correlation with bit score (ρ>+0.8). Besides, we made agreement tests between SVD and BLAST in classifying protein families. For the mitochondrial gene database, we achieved a kappa coefficient of 1.0. For the Swiss-Prot sample there is an agreement higher than 80%. The fact that SVD has a strong correlation to BLAST results may represent a possible core technique within a broader algorithm.

## 1 INTRODUCTION

Comparison of protein sequences is one of the most fundamental issues in Bioinformatics. The dominant methods of such analysis are based on character- by-character matching, made by rapid but not very sensitive algorithms with heuristics, known as BLAST – the basic local alignment search tool (Altschul et al., 1990). Even with good performance, these methods still have difficulties, due to computational complexity and other issues, as problems with genetic recombination and genetic shuffling (Vinga and Almeida, 2003). BLAST, for example, is inherently subjective and highly sensitive to the substitution matrix used in cut-off points and applied gap penalties, that are difficult to define and when altered, can produce conflicting results (Krawetz and Womble, 2003) and even "BLASTphemy" when users are unable to interpret its results (Pertsemlidis and Fondon III, 2001). Database redundancy, very common in a large protein sequence collection, is another problem for BLAST, slowing down searches and reducing the significance of an alignment because of the linear dependency of BLAST E value and the database size (Holm and Sander, 1998).

Several methods for comparing sequences and complete genomes, which do not explicitly use comparisons of character-by-character, have been proposed and successfully applied as alternative to alignments approaches (Wu et al., 1992; Stuart et al., 2002; Stuart & Berry, 2004; Yuan et al., 2005; Dong

Roberto Gonçalves Marinho Couto B., Matos Santoro M. and Augusto dos Santos M..
SINGULAR VALUE DECOMPOSITION (SVD) AND BLAST - Quite Different Methods Achieving Similar Results.
DOI: 10.5220/0003162301890195
In *Proceedings of the International Conference on Bioinformatics Models, Methods and Algorithms* (BIOINFORMATICS-2011), pages 189-195
ISBN: 978-989-8425-36-2

et al., 2006; Teichert et. al, 2007; Liu et al., 2008; Jun, S.R. et al., 2010). In this paper, proteins are recoded as p-peptide frequency matrix that is reduced by singular value decomposition (SVD), in a latent semantic indexing information retrieval system as described by Stuart (Stuart et al., 2002) and adapted by Couto (Couto et al., 2007). We first represented proteins as vectors and then calculated sequences similarities using linear algebra methods.

Figure 1 shows the simplest case where proteins are represented as three-dimensional vectors (3D): frequencies of Cystein, Alanine and Isoleucine are used to recode mitochondrial genes for four species. It is interesting to notice that protein vectors from the same family (COX3 and COX2) point to the same direction, which can be measured by the cosine among the vector angles (Eldén, 2006).

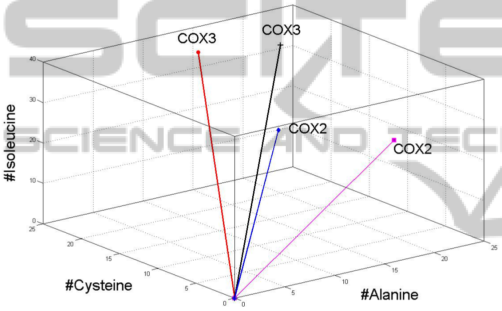


Figure 1: Representation of proteins as three-dimensional vectors.

The first objective here is to assess the relationship among similarity metrics from SVD, cosine and Euclidean distance, bit score and E value, statistics used by BLAST. We applied a scatter graph analysis and Spearman's rank correlations technique to do so (ρ). The second objective is to verify if there is an agreement, when an unknown sequence is classified or identified, among SVD results and the "gold standard", defined by the most similar BLAST hit. This was made by analysis of percent agreement, kappa coefficient, sensitivity, specificity and ROC curve (Altman, 1991). We chose BLAST as a standard because this string-matching program "has become the single most important piece of software in the field of bioinformatics" and it is widely used for nucleotide searching and protein databases (Korf et al., 2003). According to Google, the first paper describing BLAST (Altschul et al., 1990) was cited over 23,000 times (www.scholar.google.com).

# 2 SYSTEM AND METHODS

## 2.1 Programs and Datasets

Programs implemented for this analysis were written in MATLAB (The Mathworks, 1996), using its inbuilt functions (SVD, sparse matrix manipulation subroutines etc). Three datasets were used in this paper. The first evaluated database had 64 vertebrate mitochondrial genomes composed of 832 proteins from 13 known gene families (ATP6, ATP8, COX1, COX2, COX3, CYTB, ND1, ND2, ND3, ND4, ND4L, ND5 and ND6). This curated protein database was downloaded from the online information by Stuart *et al.* paper (Stuart *et al.*, 2002). The file "pdb_seqres.txt.gz", located in http://bioserv.rpbs.jussieu.fr/PDB/, was the second database. This file has 121,556 redundant protein sequences from PDB (Protein Data Bank), which was reduced to 37,561 non-identical sequences. A randomly sample of 40,000 sequences from the Swiss-Prot section of the Universal Protein Resource (UniProt) was the third protein collection (http://www.uniprot.org/downloads).

## 2.2 Vector Representation of Proteins

Before one can apply the linear algebra methods used here, it is necessary to represent proteins as vectors in a high-dimensional Euclidean space.

Firstly, we consider a bio molecular sequence as a complex written language, so its analysis can be very similar to that used by Information Retrieval Systems, where large amounts of textual information are organized, compared and categorized. In this case, individual protein sequences correspond to 'passage' of text, whereas peptides of a given size (p) serve as 'words' (Stuart et al., 2002). Hence, sequences are recoded as p-peptide frequency values using all possible overlapping p-peptides window. With 20 amino-acids it is generated a $20^p$ x n matrix, where p is the word-size and n is the number of proteins to be analyzed. In these matrices, proteins are treated as documents and the p-peptides as terms, which allow the problem to be solved by linear algebra methods (Eldén, 2006).

The amino-acid word-size p that can be used to build the p-peptide frequency matrix varies from one to four. The utility of larger peptides is yet to be explored, but to use 5 or more amino-acids can be result in computational problems. With five amino-acids the frequency matrix will be 3,200,000 rows, most of that with zero. This structure is huge and hard to handle. Besides computational issues, larger peptides will lead to problem during the similarity search step. According to Stuart (Stuart et al., 2002), tripeptides may prove useful with highly diverged sequences and tetrapeptides with highly related proteins. On the other hand, larger peptides will remain real undetected similarity, even between very highly related proteins.

Representing proteins as frequency vectors of p-peptides has the limitation that it does not consider the occurrences order of p-peptides in the sequence. Despite this possible ambiguity, several studies have shown that this approach is surprisingly effective in discriminatory analysis of protein sequences (Vinga and Almeida, 2003). Anyway, before using this protein vector representation, we made an analysis of its ambiguity rate according to the number of amino-acids (p) in the matrix of frequency protein-peptide. We compared 26,675 non-identical proteins longer than 100 amino-acids and selected from the PDB dataset. To identify ambiguities during vector recoding, we compared 355,764,475 sequences-pairs. The percentage of ambiguity felt from about 4%, when used only one amino-acid in the matrix of frequencies (p=1) to less than 0.5% in proteins with two or more amino-acids. The percentage of uncertainty was calculated considering the number of different sequences with the coding for all sequences that were compared pair-to-pair (26,675). It is noteworthy that in all pairs with identical vector coding, even among the 1,267 pairs with p=1, the protein involved was exactly the same, with minor changes of amino-acids in some positions. This happened because, before analysis, we removed from the PDB database only sequences with 100% identity. We can say that the ambiguity is a theoretical possibility in principle but not in practice.

## 2.3 Singular Value Decomposition

After the generation of the p-peptide frequency matrix (M) representing each dataset with n sequences, the matrix itself is subjected to SVD (Deerwester *et al.*, 1990; Berry *et al.*, 1995) and factorized as $M = USV^T$. Where U is the p x p orthogonal matrix having the left singular vectors of M as its columns, V is the n x n orthogonal matrix having the right singular vectors of M as its columns, and S is the p x n diagonal matrix with the singular values $\sigma_1 \geq \sigma_2 \geq \sigma_3 \dots \geq \sigma_r$ of **M** in order along its diagonal (r is the rank of M or the number of linearly independent columns or rows of M). This is performed by many software, including MATLAB (The Mathworks, 1996), used in this work. The matrix (**U**) is related to the p-peptides of the dataset, whilst (**V**) is associated with the proteins studied. The central matrix (**S**) contains the singular values of (**M**) in decreasing order. These singular values are directly related with independent characteristics within the dataset. Actually, the largest values of (**S**) provide meaning of the peptides and proteins in the matrix (**M**). On the other hand, the smaller singular values identify less significant aspects and the noisy inside the dataset (Eldén, 2006). The number of significant singular values from SVD analysis shows how many process or groups can be hidden in database.

For the sequence similarities analysis, instead of using the original matrix **M**, a rank reduction of **M** is done by using the k-largest singular values of **M**, or k-largest singular triplet $\mathbf{U_k}, \mathbf{S_k}, \mathbf{V_k}$, where k < r. The truncated matrix $\mathbf{M_k} = \mathbf{U_k S_k (V_k)^T}$ has two main advantages. Reduced dimensionality makes the problem computationally approachable, which is crucial in whole genome analysis. Besides, and very important, the rank reduction improve accuracy of protein matrix by discarding noise and reducing the variability in p-peptide usage for the same protein family (Couto *et al.*, 2007). The choice of k, the number of singular values that must be used in the reconstruction of the protein matrix after SVD, is critical and normally empirically decided. Ideally, the k factor or matrix dimension must be large enough to fit all the real structure in the data, and small enough not to fit the sampling error or unimportant details. In this work we used the method proposed by Everitt and Dunn, that recommends analyzing the relative variances of each singular values. Singular values which relative variance is less than 0.7/n, where n is the number of proteins in the document-term matrix, must be ignored (Everitt and Dunn, 2001).

# 3 RESULTS

Firstly, we analyzed 620 sequences randomly selected from the first database with mitochondrial gene families. BLAST, actually bl2seq.exe program with default parameters, were used to compare each pair of sequence, which totalling 191,890 comparisons. The same proteins were recoded as vectors in a high-dimensional space that was reduced by SVD and analyzed according to the methods described by Couto (Couto et al., 2007). Scatter plots were built and suggested that Euclidean distance is negatively related with bit score, but positively correlated with E value. For the cosine we found a negative association with E value and a positive relationship with bit score. Those results are consistent because, the higher cosine, the more similar are the two protein vector. The same happens with BLAST bit score. As the E value, the smaller Euclidean distance between the end points of two protein vectors, the more similar are the sequences. Figure 2 and 3 presents respectively scatter graphs between the bit score and cosine and between the bit score and Euclidean distance.

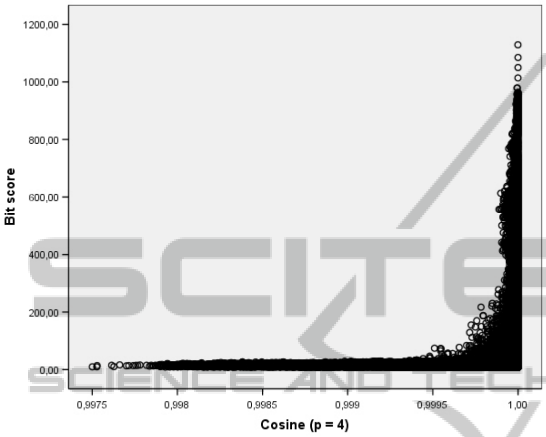


Figure 2: Scatter graph for mitochondrial gene dataset: cosine of angle between protein vectors has a positive correlation with BLAST bit score.

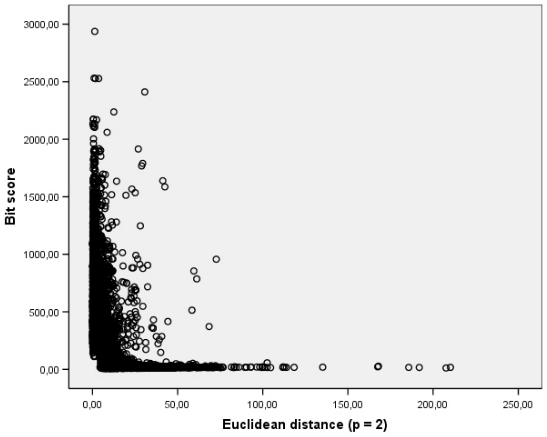
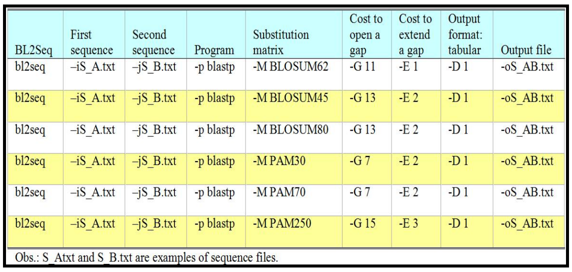


Figure 3: Scatter graph for mitochondrial gene dataset: Euclidean distance between protein vectors has a negative correlation with BLAST bit score.

For the second database, 27,361 non-identical PDB sequences longer than 100 amino-acids were compared with BLAST and SVD. In this analysis, the first protein was compared with the second, than was compared with the third and so on, which totalled 27,360 comparisons. Figure 4 shows the parameters used by bl2seq.exe program analysis.



| BL2Seq | First sequence | Second sequence | Program | Substitution matrix | Cost to open a gap | Cost to extend a gap | Output format: tabular | Output file |
|---|---|---|---|---|---|---|---|---|
| bl2seq | –iS_A.txt | –jS_B.txt | -p blastp | -M BLOSUM62 | -G 11 | -E 1 | -D 1 | -oS_AB.txt |
| bl2seq | –iS_A.txt | –jS_B.txt | -p blastp | -M BLOSUM45 | -G 13 | -E 2 | -D 1 | -oS_AB.txt |
| bl2seq | –iS_A.txt | –jS_B.txt | -p blastp | -M BLOSUM80 | -G 13 | -E 2 | -D 1 | -oS_AB.txt |
| bl2seq | –iS_A.txt | –jS_B.txt | -p blastp | -M PAM30 | -G 7 | -E 2 | -D 1 | -oS_AB.txt |
| bl2seq | –iS_A.txt | –jS_B.txt | -p blastp | -M PAM70 | -G 7 | -E 2 | -D 1 | -oS_AB.txt |
| bl2seq | –iS_A.txt | –jS_B.txt | -p blastp | -M PAM250 | -G 15 | -E 3 | -D 1 | -oS_AB.txt |

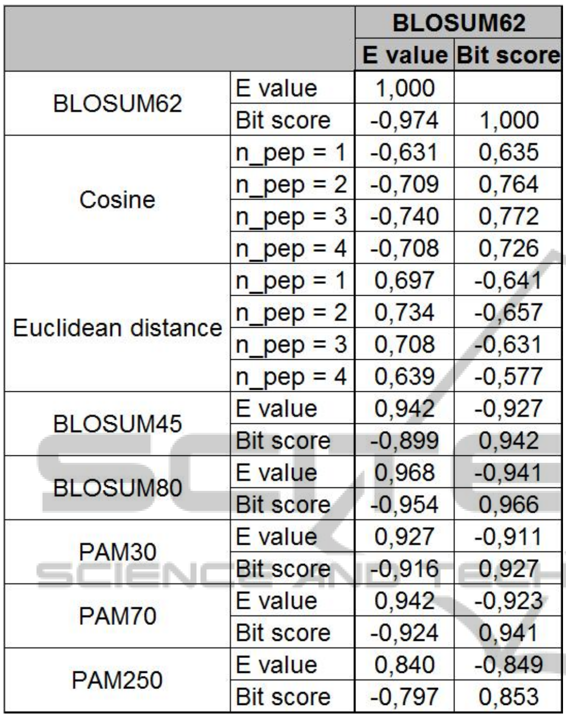
Obs.: S_Atxt and S_B.txt are examples of sequence files.

Figure 4: BLAST parameters used in the PDB database.

We built scatter graphs and calculated Spearman correlations (ρ) among bit score and E value from the most similar BLAST hit, respective cosine and Euclidean distance from SVD (Figure 5). All plots had the same shape that observed for the first database. For BLAST analysis we also compared the results obtained by applying different substitution matrix: BLOSUM62, BLOSUM45, BLOSUM80, PAM30, PAM70 and PAM2050. The Euclidean distance was negatively correlated with bit score (ρ>-0.6) and positively correlated with E value (ρ>+0.7). For the cosine we found a negative correlation with E value (ρ>-0.7) and a positive correlation with bit score (ρ>+0.8). It is interesting that the correlation between E value and bit score was not exactly 1.0 because of rounding errors.

Besides the correlation analysis, we made an agreement test between SVD and BLAST in classifying protein families. For the mitochondrial gene families database, we used a sample of 212 sequences from the 13 gene families as queries (test set), and the other proteins (620) were used to generate the frequency matrix (training set): the kappa coefficient between SVD and BLAST was 1.0 (agreement = 100%). If we use the first three significant singular values from the SVD analysis of the thirteen gene families' database, we can generate a three-dimensional graph showing how these genes can be visualized in space (Figure 6). It is interesting how the families are well separated in space, which facilitates classification.

In another analysis, the 27,360 pair-to-pair comparisons made by BLAST and SVD of the PDB sequences, were evaluated in order to asses the agreement of both techniques in detecting biological significance. The gold standard for a biological significant alignment was defined by an E value less than 0.05 obtained using BLOSUM62 as the substitution matrix (Pertsemlidis and Fondon III, 2001). The area under the ROC curve (AUC) was estimated for both, cosine, Euclidean distance and for the frequency matrix using one, two, three and four peptides. The eight AUCs estimated were higher than 0.80 (Figures 7 and 8), which indicates a good performance of SVD in detecting biological significant similarities (Altman, 1991).



| | | BLOSUM62 | |
|---|---|---|---|
| | | E value | Bit score |
| BLOSUM62 | E value | 1,000 | |
| | Bit score | -0,974 | 1,000 |
| Cosine | n_pep = 1 | -0,631 | 0,635 |
| | n_pep = 2 | -0,709 | 0,764 |
| | n_pep = 3 | -0,740 | 0,772 |
| | n_pep = 4 | -0,708 | 0,726 |
| Euclidean distance | n_pep = 1 | 0,697 | -0,641 |
| | n_pep = 2 | 0,734 | -0,657 |
| | n_pep = 3 | 0,708 | -0,631 |
| | n_pep = 4 | 0,639 | -0,577 |
| BLOSUM45 | E value | 0,942 | -0,927 |
| | Bit score | -0,899 | 0,942 |
| BLOSUM80 | E value | 0,968 | -0,941 |
| | Bit score | -0,954 | 0,966 |
| PAM30 | E value | 0,927 | -0,911 |
| | Bit score | -0,916 | 0,927 |
| PAM70 | E value | 0,942 | -0,923 |
| | Bit score | -0,924 | 0,941 |
| PAM250 | E value | 0,840 | -0,849 |
| | Bit score | -0,797 | 0,853 |

Figure 5: Correlation matrix: BLAST versus SVD.

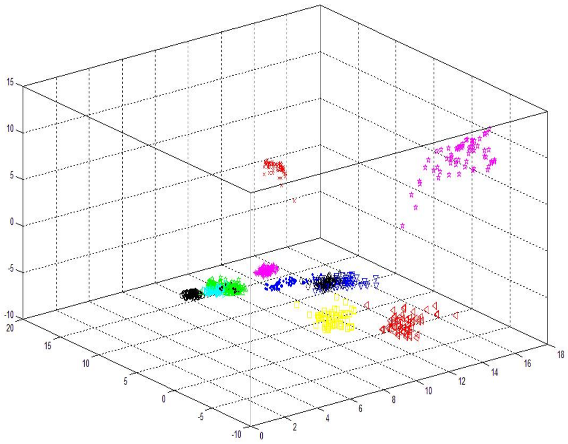


Figure 6: Visualization of mitochondrial genes using the three first singular values from SVD: the 13 gene families are well separated in space, which facilitates classification.

Table 1 summarizes the results when cosine among protein vectors is used to detect a biological significance similarity. When is used a cut-off of 0.90 for the cosine, the sensitivity and specificity for detecting biological significance were, respectively, 72% and 94%.

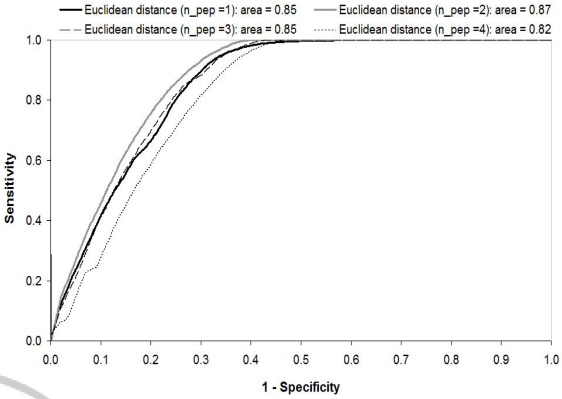


Figure 7: ROC curve built when SVD Euclidean distance is used to detect biological significant similarity.

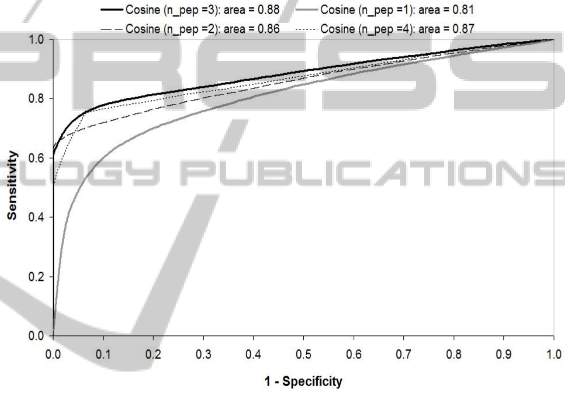


Figure 8: ROC curve built when SVD cosine is used to detect biological significant similarity.

Table 1: Two-way contingency table: cosine higher than 0.90 between protein vectors has 72% sensitivity and 94% specificity to detect biological significant similarities.

| BLOSUM62 biological significance? | Cosine biological significance? | | |
|---|---|---|---|
| | (+) | (−) | Total |
| Yes | 9,678 | 3,843 | 13,521 |
| No | 808 | 13,031 | 13,839 |
| Total | 10,486 | 16,874 | 27,360 |

During the analysis of the third protein collection, a sample of 40,000 Swiss-Prot sequences was randomly divided into two groups: 9,953 proteins were selected as queries (test set), and the other 30,047 sequences (training set) were used to generate the frequencies matrix of SVD and to become the BLAST database for evaluating the queries. All 9,953 unknown proteins were analyzed by SVD and BLAST (actually, *blastall* program with default parameters) and results of both methods were compared in order to detect agreement. If the Swiss-Prot mnemonic protein identification code of the most similar BLAST hit was identical as that obtained by a SVD analysis, so we had an agreement. When this happened, the matched proteins are the same, from the same or different species. Table 2 presents the percent agreement between BLAST and SVD: the results were good, except when the p-peptide matrix is built by using just one amino-acid as the word-size.

Table 2: Agreement between SVD and BLAST for classifying proteins from the Swiss-Prot dataset.

| *p*-peptide matrix | SVD similarity metric | Percent agreement with BLAST |
|---|---|---|
| p=1 | Cosine | 20% |
|  | Euclidean distance | 30% |
| p=2 | Cosine | 79% |
|  | Euclidean distance | 82% |
| p=3 | Cosine | 80% |
|  | Euclidean distance | 82% |
| p=4 | Cosine | 69% |
|  | Euclidean distance | 72% |

## 4 CONCLUSIONS

We worked with quite different techniques and we found important association among their metrics and good agreement between both methods. Despite the fact that is presumably not surprising that e.g. BLAST bit score could be positively correlated to cosine of angle, or negatively correlated to Euclidean distance, the sizes of these correlations are very interesting (Figure 5).

We achieved similar results between BLAST and SVD in several protein analyses. The findings strongly suggest that SVD can be used to protein-protein comparisons with biological significance of the similarities identified both for cosine and Euclidean distance. The fact that SVD has a strong correlation to BLAST results may represent a possible core technique within a broader algorithm.

Besides, SVD has some characteristics that could be an advantage over alignment algorithms. For example, SVD analysis can be very rapid, it does not use any heuristics to asses an unknown sequence, its metrics are exact in a sense of direction and position in a high-dimensional Euclidean space, it is not affected by database redundancy because of rank reduction, its similarity metrics do not depend on the database size, and any analyze does not need a substitution matrix nor gap penalties to produce biological significant results.

An assessment of the singular value spectrum visualized as *scree plots* (Zhu and Ghodsi, 2006) can unreveals the main components, the process that exists hidden in a database. This information can be used in many applications as clustering, gene expression analysis, immune response pattern identification, characterization of protein molecular dynamics and phylogenetic inference.

SVD can be also used to visualize the relationships between sequences and even whole genomes, which can be essential to better analyze complex systems and can be very helpful to categorize genes or species in phylogeny.

All results found in this work and the characteristics described are important because SVD can be a solution for the potential problems with alignment algorithms and can be a substitute for those methods, for example, in whole genome analysis.

## ACKNOWLEDGEMENTS

We are thankful to Professor Gary W. Stuart from Indiana State University, Department of Life Sciences, who sent us helpful data. We also thank Marlon C. Souza from UNI-BH, who revised the manuscript.

## REFERENCES

Altman, D. G., 1991. Practical Statistics for Medical Research. Chapman and Hall, London, UK.

Altschul, S. F. et al., 1990. Basic local alignment search tool. J. Mol. Biol., 215, 403-410.

Berry, M. W. et al., 1995. Using linear algebra for intelligent information retrieval. SIAM Review, 37, 573-595.

Couto, B. R. G. M. et al., 2007. Application of latent semantic indexing to evaluate the similarity of sets of sequences without multiple alignments character-by-character. GMR, 6(4), 983-999.

Deerwester, S. et al., 1990. Indexing by Latent Semantic Analysis. *Journal of the American Society for Information Science*, 41(6), 1-13.

Eldén, L., 2006. Numerical linear algebra in data mining. Acta Numerica, 327-384.

Everitt, B. S. and Dunn, G., 2001. Applied multivariate data analysis. *2nd edn. Arnold*, London, UK.

Holm, L. and Sander, C., 1998. Removing near-neighbour redundancy from large protein sequence collections. Bioinformatics, 14(5), 423-429.

Jun, S. R. et al., 2010. Whole-proteome phylogeny of prokaryotes by feature frequency profiles: An alignment-free method with optimal feature resolution. Proc Natl Acad Sci U.S.A, 107(1):133-8.

Korf, I.; Yandell, M.; Bedell, J., 2003. An essential guide to the Basic Local Align-ment Search Tool – BLAST. O'Reilly & Associates Inc., Sebastopol, U.S.A.

Koski, L. B. and Golding, T. B., 2001. The closest BLAST hit is often not the nearest neighbor. J. Mol. Evol., 52, 540-542.

Krawetz, A. S. and Womble, D. D., 2003. Introduction to Bioinformatics: a theoretical and practical approach. Humana Press, Totowa, USA.

Liu, B. et al., 2008. A discriminative method for protein remote homology detection and fold recognition combining top-n-grams and latent semantic analysis. *BMC Bioinformatics*, 9, 510.

Pertsemlidis, A. and Fondon III, J. W., 2001. Having a BLAST with bioinformatics (and avoiding BLASTphemy). *Genome Biology*, 2(10), 1-10.

Stuart, G. W. et al., 2002. Integrated gene and species phylogenies from unaligned whole genome protein sequences. *Bioinformatics*, 18(1), 100-108.

Stuart, G. W. and Berry, M. W., 2004. An SVD-based comparison of nine whole eukaryotic genomes supports a coelomate rather than ecdysozoan lineage. *BMC Bioinformatics*, 5: 204+.

The Mathworks, 1996. MATLAB: mathematical computation, analysis, visualization, and algorithm development (version 5.0). Natick, Massachusetts, USA.

Teichert, F. et al., 2007. SABERTOOTH: protein structural alignment based on a vectorial structure representation. *BMC Bioinformatics*, 8, 425.

Vinga, S. and Almeida, J., 2003. Alignment-free sequence comparison: a review. *Bioinformatics*, 19(4), 513-523.

Wu, C. et al., 1992. Protein classification artificial neural system. *Protein Science*, I, 667-677.

Yuan, Y. et al., 2005. A Protein Classification Method Based on Latent Semantic Analysis. *Conf Proc IEEE Eng. Med. Biol. Soc.*, 7, 7738-41.

Zhu, M. and Ghodsi, A., 2006. Automatic dimensionality selection from the scree plot via the use of profile likelihood. *Computational Statistics and Data Analysis*, 51, 918-930.