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RESEARCH ARTICLE

COMPARATIVE STUDY OF AMYLASE ENZYME IN CARNIVORES AND HERBIVORES USING BIOINFORMATICS

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ABSTRACT

To analyze the activities of amylase enzyme in herbivorous and carnivorous family. Currently, there is limited comparative genomics approach into the evolution of dietary habits are reported in literature. With the recent advances in sequencing era, we were able to perform functional genome analyses that represents phylogenetic relationship between different species and their amylase activities. Collect all the sequence information from biological database like NCBI and retrieve nucleotide sequence. Further, perform *BLOSUM-62 algorithm with the Clustal-W for multiple sequence alignment and construct the phylogenetic tree using Clustal Omega*. We observed that evolutionary changes in herbivorous and carnivores species with their respective mutation and conserved domain. This study used phylogenetic and statistical approaches to explore general patterns of amylases evolution, amylases from seven different species in eukaryota family with fully documented taxonomic lineage. First, the phylogenetic tree was created to analyze the evolution of amylases with focus on individual amylases. Second, the average pairwise p-distance was computed for each species, and its diversity implies multi time and multi-clan evolution. In addition to a clear contraction in gene families for carbohydrate metabolism, the carnivore genomes showed evolutionary adaptations in genes associated with dietary habits and other traits responsible for successful stalking and core consumption under strong selective pressure related to diet. However, unlike herbivores showed fewer shared adaptive signatures. The genetic diversity associated with decreased population sizes of carnivores, which may be due to the inflexible nature of their strict diet, emphasizing their susceptibility and critical conservation. The present study provides a comparative genomic analysis represent genomic changes associated with dietary habits. Our genomic analyses also provide useful resources for diet-related personalized healthcare research.

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INTRODUCTION

Amylase is a digestive enzyme predominantly secreted by the pancreas and salivary glands but also found in other tissues in very small levels. Amylase was first described in the early 1800s and is considered one of the first enzymes in history to be scientifically investigated. It was initially termed as *distaste* but was later renamed as amylase in the early 20th century (1). Amylase catalyzes the hydrolysis of 1, 4-glycosidic linkages of starch to sugar (2). So far, several groups of amylases have been defined, and α , β & γ amylases have many applications in clinical and biotechnological settings. *Alpha-amylase is a digestive enzyme that catalyzes starch, glycogen and related polysaccharides hydrolysis and is*

commonly present in plants, animals and micro-organisms. Beta-amylase is found in microbes and plants. Gamma-amylase is found in animals and plants (1, 3) Diet habits are the most necessary selection in all living organism on Earth. In particular, carnivores are interesting due to their evolved constantly in a number of mammalian family (4). In the fossil record, in carnivores are often associated with relatively short life expansion, a likely concern to small population sizes associated with a diet at top of the trophic pyramid (5). Similarly, the other side of the nutritional scale in the cases of herbivores, consuming primarily plant material. This plant material differs enormously in its starch content, depending on the parts of plants consumed. The rodent consumed the whole of a plant whereas a starch digestive enzyme might be advantageous and had longer digestive tracts and higher activity of the amylase in their guts (6). The rationale behind

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this study is to compare the amylase enzyme activities carnivores and herbivorous with natural feeding habits and diet composition. Further, to establish the relationship between carnivorous and herbivorous mammals on the basis of digestive enzymes. The main aim of the present study is to explore the diversity enzymatic activities in different species with different feeding habits. However, the evolutionary differences may be analyzed based on biological databases with other computational techniques supplemented by the experimental data. The present study may also be helpful to explore the additional knowledge about species environmental changes. Till date, scanty information regarding in the scientific literature and might have many applications in clinical and biotechnological Settings.

MATERIAL AND METHODS

Sequence Collection: The nucleotide sequences of various species coded amylase enzyme were obtained from the NCBI databases (<http://www.ncbi.nlm.nih.gov/>). Nucleotide sequences of the following carnivores and herbivores Amylases were retrieved: *Felis catus*, *Clarias batrachus*, *Mus musculus*, *Ochotona princeps*, *Pangasianodon hypophthalmus*, *Oryctolagus cuniculus* and *rattus norvegicus* as shown in Table 1.

Table 1. List of Carnivores and Herbivores with their accession no. and base length

S.no	Organisms	Feeding Habbits	Amylase accession no.	Base Pairs
1	Felis catus	carnivorous	ACO24947.1	275
2	Mus musculus	carnivorous	CAA26417.1	232
3	Clarias batrachus	carnivorous	KY235166.1	267
4	Pangasianodon hypophthalmus	carnivorous	XM_026911354.2	1615
5	Rattus norvegicus	herbivorous	AAA40725.2	508
6	Oryctolagus cuniculus	herbivorous	XP_017201532.1	511
7	Ochotona princeps	herbivorous	XP_004582054.1	511

Multiple Sequence Alignment : The alignments of the following species were performed using publicly available database at EBI. The sequences were aligned using the BLOSUM-62 algorithm with the Clustal-W alignment tool. The full protein sequences, the C-terminal (domain) sequences only and N-terminal(domain) sequences only in some instances were aligned to determine the homology and relationships between the specified carnivores and herbivores species on the basis of amylase enzymes.

Phylogenetic Analysis: Phylogenetic trees of amylase enzyme were contrasted by using clustal omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) for evolutionary relationship between herbivores and carnivores species were constructed using nucleotide sequences obtained from NCBI (<http://www.ncbi.nlm.nih.gov/>). The obtained sequence data were aligned using the Clustal-W alignment tool. The aligned sequences were assembled into a phylogenetic tree using the boot-strapped neighbor-joining algorithm (7).

RESULTS

During this work, we identified 7 different species on their feeding habits i.e. carnivores and herbivores illustrated in table 1. Whereas, multiple sequence alignment showed conserve region as well as changed nucleotide bases during evolution shown in Figure 1.

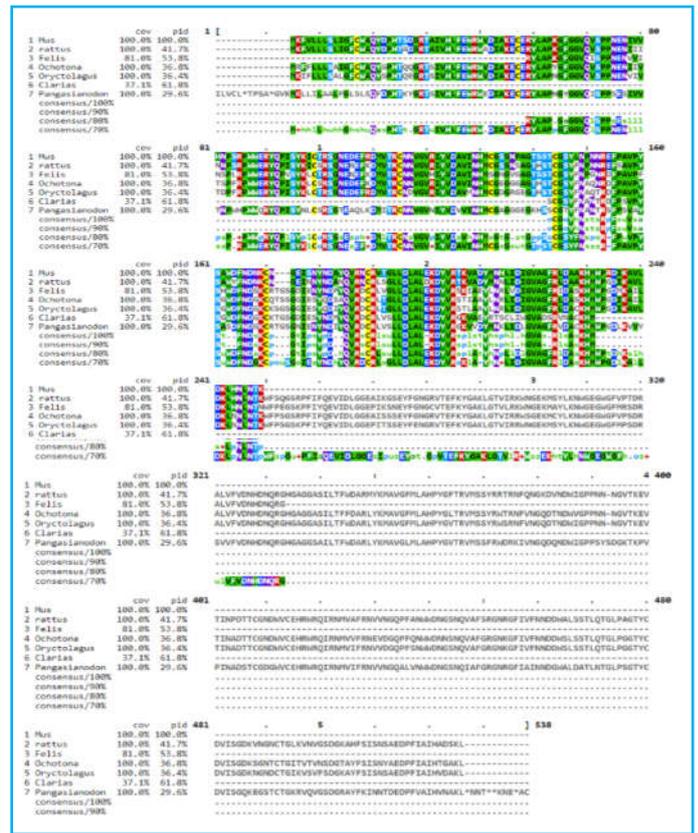


Fig. 1. Multiple sequence alignment showing conserve sequence with percentage of similarity

Then, we used the branch-site model to test for positive selection in individual codons for the lineage leading to divergence of amylase enzymes with camivorous and herbivorous animal. Further, the study based on cladistics analysis performed with a result of seven equally parsimonious cladograms shown in Figure 2, associated evolutionary relationship consensus tree is presented, together with the NJ-tree. The topologies of trees are in good agreement, even though some discrepancies exist. Phylogenetic tree of 7 amylases sequences of different species. The numbers along each branch and scale bar represent the branch length, and their closely association with each other.

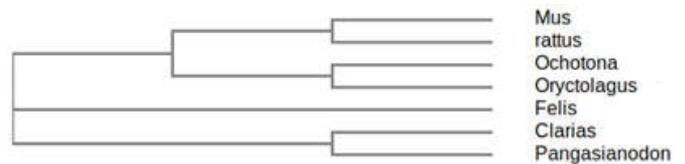


Fig. 2. Neighbor-joining cladogram tree showing distance relationship between species

DISCUSSION

In this study, both multiple sequence alignment and phylogenetic approaches were applied to analyze the evolution of amylases, which are based on diet habits and evolutionary changes. An average different p-distances of similarity give a clear picture on the evolutionary changes of amylases. Whereas, if the larger is the average p-distance value in a taxonomic group, that represent the probability of species gain or loss genetic materials. In the study on eukaryotic amylases, the assumption that takes into concern is an ontogenetic dietary shift from carnivorous to herbivorous.

The lineage-specific expansions were partially driven by directional selection, and gains and losses of genes were lineage dependent (8, 9). In reality, the intestine environment also plays a significant role in positive selections as perceived in indigenous diet (10). The broader aspect of present study on the evolution of amylases is equal to the study on the evolution of conserved regions in amylases. Such as, (α/β) 8 barrel is a conserved region (11). Similarly, α -amylase is a calcium-dependent metalloenzyme responsible for calcium ion binding regions are subject to evolutionary insertions and deletions (12). However, the conserved regions (amylase) might have been impacted by the effort in development of inhibitory substances on α -amylase like SbAI is potato amylase inhibitor gene (13). Still, the level of high and low starch diets in supplementary which effects the evolution of amylase as validated by the number of amylase gene copies in different species (14, 15). In the present study, we explore the general pattern of amylase evolution with the hope to potentially helpful to enhance the scientific view for cloning and expression of amylase coding gene in different species by employing phylogenetic and statistical approaches in a large scale dataset. The phylogenetic tree and evolutionary relationship with diversified species. Whereas, the average pairwise p-distance and this approach would be helpful for the developing the new amylases for pharmaceutical and healthcare sector.

Conclusion

Therefore, from the entire study, it suggests that, in contrast to carnivores and herbivores have considerable amounts of amylase in their saliva. It has to be verified, whether α -amylase is genetically or epigenetically determined. Besides, amylase can be recognized as a nutritional and evolutionary marker. The complete physiological function of amylase in saliva has not yet been explored completely; the enzyme seems to play an important role futuristic enzymes which play a significant role on personalized medicine and evolutionary approaches might offer new perspectives of research horizon.

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REFERENCES

- Akinfemiwa O, Muniraj T. Amylase. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; August 3, 2020.
- Boehlke C, Zierau O, Hannig C. Salivary amylase - The enzyme of unspecialized euryphagous animals. *Arch Oral Biol*. 2015;60(8):1162-1176. doi:10.1016/j.archoralbio.2015.05.008
- Da Lage JL, Binder M, Hua-Van A, Janeček S, Casane D. Gene make-up: rapid and massive intron gains after horizontal transfer of a bacterial α -amylase gene to Basidiomycetes. *BMC Evol Biol*. 2013;13:40. doi:10.1186/1471-2148-13-40
- German DP, Gawlicka AK, Hom MH. Evolution of ontogenetic dietary shifts and associated gut features in prickleback fishes (Teleostei: Stichaeidae). *Comp Biochem Physiol B Biochem Mol Biol*. 2014;168:12-18. doi:10.1016/j.cbpb.2013.11.006
- Hleap JS, Susko E, Blouin C. Defining structural and evolutionary modules in proteins: a community detection approach to explore sub-domain architecture. *BMC Struct Biol*. 2013;13:20. doi:10.1186/1472-6807-13-20
- Linton SM, Saborowski R, Shirley AJ, Penny JA. Digestive enzymes of two brachyuran and two anomuran land crabs from Christmas Island, Indian Ocean. *J Comp Physiol B*. 2014;184(4):449-468. doi:10.1007/s00360-014-0815-2
- Mizutani K, Toyoda M, Otake Y, Yoshioka S, Takahashi N, Mikami B. Structural and functional characterization of recombinant medaka fish alpha-amylase expressed in yeast *Pichia pastoris*. *Biochim Biophys Acta*. 2012;1824(8):954-962. doi:10.1016/j.bbapap.2012.05.005
- Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*. 1987;4(4):406-425. doi:10.1093/oxfordjournals.molbev.a040454
- Santos JL, Saus E, Smalley SV, et al Copy number polymorphism of the salivary amylase gene: implications in human nutrition research. *J Nutrigenet Nutrigenomics*. 2012;5(3):117-131. doi:10.1159/000339951
- Schomburg I, Chang A, Placzek S, et al. BRENDA in 2013: integrated reactions, kinetic data, enzyme function data, improved disease classification: new options and contents in BRENDA. *Nucleic Acids Res*. 2013;41(Database issue):D764-D772. doi:10.1093/nar/gks1049
- Singh S, Guruprasad L. Structure and sequence based analysis of alpha-amylase evolution. *Protein Pept Lett*. 2014;21(9):948-956. doi:10.2174/092986652109140715124139
- Valkenburgh B Van. Major patterns in the history of carnivorous mammals. *Annu Rev Earth Planet Sci*. 1999;27:463-93. <https://doi.org/10.1146/annurev.earth.27.1.463>
- Van Valkenburgh B, Wang X, Damuth J. Cope's rule, hypercarnivory, and extinction in North American canids. *Science*. 2004;306(5693):101-104. doi:10.1126/science.1s102417
- Zhang H, Liu J, Hou J, et al. The potato amylase inhibitor gene SbAI regulates cold-induced sweetening in potato tubers by modulating amylase activity. *Plant Biotechnol J*. 2014;12(7):984-993. doi:10.1111/pbi.12221
- Aakanchha ,Aniket K, R.K. Gupta, et al. Genetic Divergence and Phylogenetic Analysis of Fish Digestive Enzymes in Carnivores, Herbivores and Omnivores. *Biospectra*. 202015(1); 21-28.