IDENTIFICATION OF SPECIFIC PROTEINS OF THE RICE PEST "GOLDEN APPLE SNAIL" (*Pomacea canaliculata*) (GASTROPODA: AMPULLARIIDAE) AIMING THE DEVELOPMENT OF A NEW NATURAL MOLLUSCICIDE

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Key words: *Pomacea canaliculata*, biotechnology, bioinformatic, *Oryza sativa*, BLASTp

INTRODUCTION

Rice is one of the world's largest agricultural systems, being the main food for more than half of the population. Brazil is responsible for more than 50% of the rice production in Latin America, occupying the 9th place in the list of the 10 largest producers in the world, behind eight Asian countries. Also, rice plantation is one of the main economic activities of Rio Grande do Sul State, which, according to IBGE (*Governmental Brazilian Institute of Geography and Statistics*), 2021, was responsible for nearly 70% of the national production in 2020. Nonetheless, there are several factors that cause significant damages and economical losses to the irrigated rice cultivation, the most used system in Brazil, such as insects, birds and mollusks. Among the latter, it is important to highlight the snail *Pomacea canaliculata* (Gastropoda: Ampullaridae), popularly known as ampulária or golden apple snail (HICKEL et al., 2012). This species is ranked in the 100 most invasive species in the world, being the main cause of damage to rice production, feeding fiercely on newly emerged plants. These gastropods live in swampy habitats, places where the water is removed for rice irrigation, and therefore the species ends up entering and reproducing in these crops, causing damage that can exceed 90% in some extreme cases. Furthermore, *Pomacea canaliculata* is also responsible for being the host of the *Angiostrogylis cantonesis*, a nematode that causes eosinophilic meningitis (DE BRITO, 2015). The species present high reproductive capacity, making the oviposition in non-submerged places, such as stems and leaves, causing seedlings to fall over. Their eggs contain proteins with storage function, source of energy, defense against predators and abiotic factors. These proteins, called perivitellines, are neurotoxic, anti-digestive, anti-nutritive and aposematic, giving the pink pigment in eggs that serves as a warning to predators (HERAS, et al., 2017). Traditional methods can be used to contain this pest, however, they are not selective and increase the chances of contamination. Hence, the main management practices in all rice crops is the continuous use of pesticides, harmful non-selective and expensive products that bring risks to human health and the environment, causing significant changes in the ecosystem.

Being considered the main pollinating agents in almost all reproductive systems, bees are very susceptible to loss of their habitats, because they are subject to the action of pesticides in agricultural areas. Even low concentrations can alter the functioning of the hive, and, consequently, their services to ecosystems, biodiversity and agricultural crops (NOCELLI, et al., 2012).

Due to the snail's resistance to pesticides, the high price of these chemicals, for causing damage to the environment and other species (MCCULLOUGH et al. 1980), and significant changes in the ecosystem, it becomes necessary the search for a natural alternative, biodegradable and

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with high selectivity for the control of this pest. Thus, the objective of this work was to identify specific proteins of the snail *Pomacea canaliculata* using bioinformatic techniques, aiming the development of a new natural molluscicide, capable of inhibiting the activity of these target proteins.

MATERIAL AND METHODS

The genomes of the snail (*Pomacea canaliculata*), human (*Homo sapiens*), rice (*Oryza sativa*) and bee (*Apis mellifera*) were identified in the NCBI (*National Center for Biotechnology Information*) database. Using the BLASTp program, which evaluates the probability of the alignment of these sequences occurring by chance, it was possible to compare the protein sequences of the snail (40,392) with those of human (268,900), rice (54,425) and bee (557,906). At the end of the alignments, only the specific proteins of the mollusk were obtained, from which uncharacterized proteins were removed, and then separated in 18 groups, according to their number of amino acids.

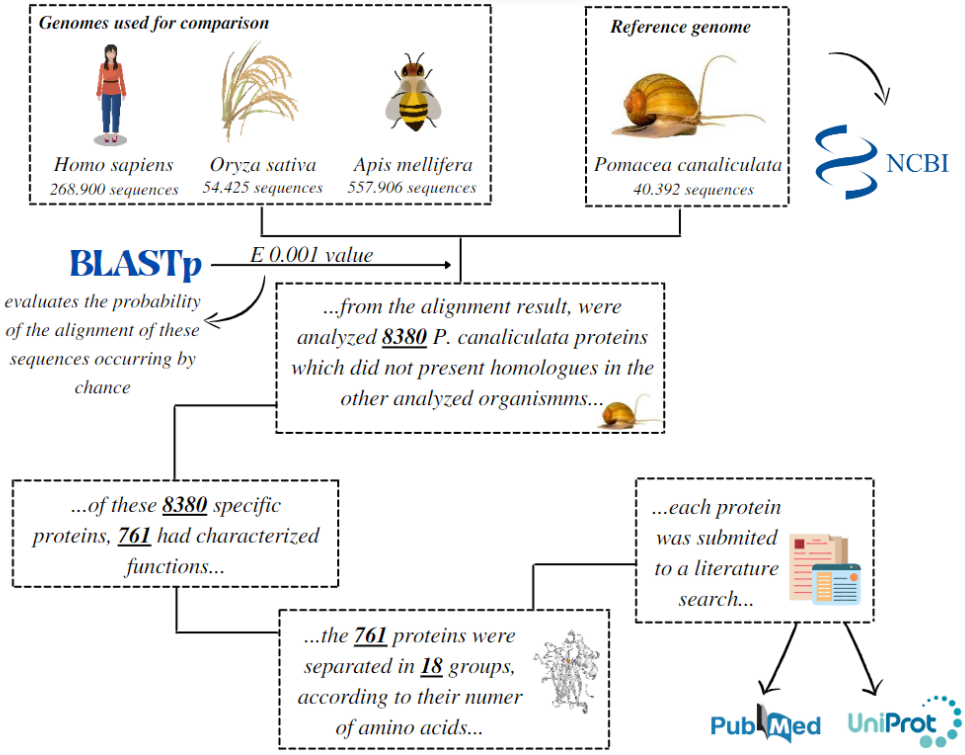

Figure 1. Scheme of the methodology used to identify the *Pomacea canaliculata*-specific proteins.

RESULTS AND DISCUSSION

After aligning the sequences of the four organisms, we were able to identify 8,380 *Pomacea canaliculata*-specific proteins that have no homologs in the other three analyzed organisms. Of these specific proteins, 761 sequences had characterized functions. Now, each of these 761 proteins are being submitted to a literature search, in which 57 potential target proteins have been identified so far. Three of these potential target proteins have already been mentioned in articles related to the snail, and 54 have a similar structure in other organisms, whose functions are being sought. Some of these proteins, as well as its functions, are presented in Table 1.

Table 1. Potential target proteins identified in the literature search.

Protein	Function	Author
Perivitellin-2 and Perivitellin-3	It is a neurotoxin that disrupts the regulation of calcium in the central nervous system, causing neuronal degeneration and cell apoptosis.	HERAS, et al., 2017
Ovorubin (PcOvo)	Multifunctional perivitellin, involved in defense against abiotic stress. It also provides the pigment of the eggs, serving as a warning to predators, and reduces the digestability and nutricional quality of the eggs.	DREON, et al., 2013
Calcium binding protein	One of the main membrane metabolism regulation genes that controls cold resistence. The protein reduces the snail's ability to absorb Ca++, reducing the stability of the snail's cell membrane, which can lead to a decline in cold resistence.	LIU, et al., 2020
Histidine-rich glycoprotein (HRG)	It has a multidomines structure that allows the molecule to interact with several ligands. This suggests that HRG may function as an adapter and regulatory molecule for several important biological process, such as in immunity and vascular biology.	POON, et al., 2011
Calmodulin-like	Primary calcium sensor in eukaryotes. It binds to calcium and regulates the activity of a wide range of effector proteins in response to calcium signals.	PEROCHON, et al., 2011
AP-5 complex subunit beta-1-like	Transports proteins from endossomes to other membranous compartments.	HIRST, et al., 2011
Tropomyosin-like	It plays an important role in contractile activity and regulation of cell morphology and motility. It is reported as the most important allergen in several snail species.	MISNAN, et al., 2016
Elevelin-Vc1-like	One of the peptides that composes the venom of the gastropod *Conus victoriae*.	ROBINSON, et al., 2015
Proline rich transmembrane protein 1B-like	Regulates AMPAR function and synaptic transmission during development. It also plays role in regulation basal phosphorylation levels of glutamate receptor GRIA1 and promotes GRIA1 and GRIA2 cell surface expression.	TROYANO-RODRIGUEZ, et al., 2019

CONCLUSION

Based on these results, predictions from the tree-dimensional models of the 54 proteins identified as potential molecular targets will be performed through comparative modeling. Further, we will search for small molecules derived from natural compounds that can modulate the activity of the most promising proteins through molecular docking methodology, to evaluate the binding capacity of the compounds into the active site of the target proteins. We expect to identify small natural molecules capable of inhibiting the activity of these target protein, acting as a molluscicide, and leading specifically to the death of the snail, which will be a great benefit to rice cultivation, since it would not be needed to use pesticides to control the proliferation of *Pomacea canaliculata*. Moreover, such a strategy would not cause damage to the workers and consumers health, as well as the environment, ecosystem and rice itself.

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