lectronic Cloning and Bioinformatics Analysis of the Chitinase Gene of Sugarcane

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Abstract: The gene has an important effect on the basic structure and performance of life, and is a DNA fragment with genetic effect. In this paper, the function of the chitinase was introduced, and the encoding protein of the chitinase gene of the sugarcane was analyzed by the electronic cloning technique of the gene. The results showed that the total length of the chitinase gene SCCHI was 1236bp, which was similar to that of the chitinase gene of the plants such as sorghum, corn and rice..

1. Introduction

Bioinformatics covers biology, computer science, information engineering, mathematics and statistics, and uses biological algorithms and related gene sequence analysis software to collect, process, store, analyze and interpret the biological data. The research focuses on the two aspects of genomics and proteomics, and the analysis process is based on the sequence of ribonucleic acid and protein, and the structure function of the organism is studied.

2. Chitinase Overview

The chitinase is an enzyme that catalyzes the hydrolysis of the chitin and generates N-glycinate, which is first found in the intestinal gland secretion of the snail by Keller and Hoffman, and has the function of digesting and decomposing the aged stratum corneum, and is widely believed to play an important role in the defense system in the plant body, It is a very important course-related protein. The chitinase activity in the plant self-protection system is greatly enhanced, and the spores of the pathogens, such as bacteria and the like, are greatly enhanced when the plant is subjected to diseases and insect pests, the growth of the hypha has a very effective inhibition effect, and the plant cells are resistant to the new substances and the plant safety is protected. In recent years, by a lot of experiments, scientists have shown that the chitinase plays an important role in plant protection, especially its antibacterial effect, and has become a breakthrough in the research of the anti-fungal hazards. Chitinase is generally contained in the plant, and the active chitinase has been detected from nearly 100 plants.

3. Brief Analysis of Electronic Cloning Technology of Genes in Bioinformatics

The electronic cloning technology is based on the expression sequence label (ESTs) in the biological database, is an emerging gene rapid cloning mode, and the core content is that the ESTs sequence is assembled and extended by using the bioinformatics-related technology, so that part or all of the cDNA sequence of the gene is obtained, In particular, that sequence homologous ratio pair, the cluster analysis, the region overlap splicing or the assembly can be used for rapidly analyzing the functional gene. The cost of this technology is low, and it is very efficient to analyze the specific function. In the future, the electronic cloning technology will be an important way to explore the new gene of the plant. This article is based on the number of NCBI According to the sugarcane EST sequence in the library, the cDNA sequence in the sugarcane chitinase gene is extracted by the electronic cloning technology, and the gene coding proteins such as amino acid composition, hydrophilicity, secondary and tertiary structure are predicted and analyzed by using the bioinformatics principle.

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4. Electronic Cloning and Bioinformatics Analysis of the Chitinase Gene of Sugarcane

4.1 Experimental Material and Method for Sugarcane Chitinase Gene

And a novel chitinase gene sequence is obtained by using an electronic cloning technology. Optionally, the gene sequence of the sorghum or the corn is used as a query probe (the corn is selected in the paper), and when the sugarcane ESTs database is searched, the Blast tool in the NCBI is adopted, and the searched sugarcane EST sequence is spliced, clustered and extended to obtain a new contig group. The ESTs database is continuously searched for the probe in this group until all of the sugarcane EST sequences have been searched and no spliced content is available. The processed chitinase gene sequence is placed in a non-redundant database and is compared with the existing gene sequence. A full contrast, such as a non-highly similar sequence, can be determined as a new gene sequence.

In that case of bioinformatics analysis of the chitinase gene of the sugar cane, the following software tool can be used for assisting to complete: using the DNA star software to analyze the ribonucleic acid of the chitinase gene of the sugarcane and the sequence of the amino acid sequence, and carrying out translation look-up on the open reading frame by using the open reading frame of the ORF; The physical and chemical properties of the sequence coding protein were analyzed by using Internet on-line tools such as Prot-Param, pI/ Mw, and the sequence signal peptide was predicted by using the SignalP3.0 Server software, and the on-line software of ExPASy-ProScale was used. The analysis is made on the hydrophobicity of the sequence, and when the sub-cell localization in the coding protein of the chitinase gene of the sugarcane is analyzed, the Psport software can be used for prediction, and the research software such as the ESyPred3D and the SOPMA can be used for analyzing the composition of the protein secondary and tertiary structures. After the above analysis is completed, the homology comparison of the gene ribonucleic acid and the amino acid sequence of the sugarcane chitinase gene is carried out by using the Blast Internet on-line analysis tool and the Vector Machine software, and the two software [1] can also be used for the multi-sequence similarity ratio.

4.2 Experimental Results of the Chitinase Gene of Sugarcane

With corn gene sequence as the probe, more than ten sequences with high homology with sugar cane EST were found after the search comparison operation in ESTs database. After splicing with bioinformatics, 1236bp group sequence was obtained. After detailed demonstration, the group sequence had complete open code reading frame, ranging from 80bp1236bp. Because of the initial code sub-ATG and termination codon TAG, it was in line with the definition of full-length cDNA sequence, it was named SCCHI.

The amino acids encoded by SCCHI of sugarcane chitinase gene were predicted by software tools. The results showed that the number of encoded amino acids was 3.29, the isoelectric point (PI) data was 6.83, the molecular weight was 34128Da. The positive and negative charge residues were 21, the instability coefficient was 32, the average hydrophilicity was-0.17, the fat coefficient was 55.4, and the molecular formula was C1493H2258N410O465S23.

The signal peptides of SCCHI encoded protein were predicted and concluded as follows: the signal peptide breaks appeared in the amino acid sequence 20 of SCCHI gene coding protein, and it was inferred that SCCHI contained signal peptide, and the mature peptide chain began to extend backward from sequence 22. It was proved that the N-terminal signal region of chitinase was actually composed of not less than 20 amino acids, and chitinase was transported to endoplasmic reticulum in plants. It plays a bactericidal role, which can be seen to be consistent with the experimental results in this paper.

The hydrophilicity of amino acid sequence encoded by SCCHI gene was tested by ProtScal software, and the hydrophilic / hydrophobic prediction map of SCCHI shown in Fig. 1 was generated. The results showed that 10 showed the highest value and the weakest hydrophilicity in SCCHI polypeptide chain, which was consistent with the study and analysis of low hydrophilicity in chitinase N-terminal signal peptide.

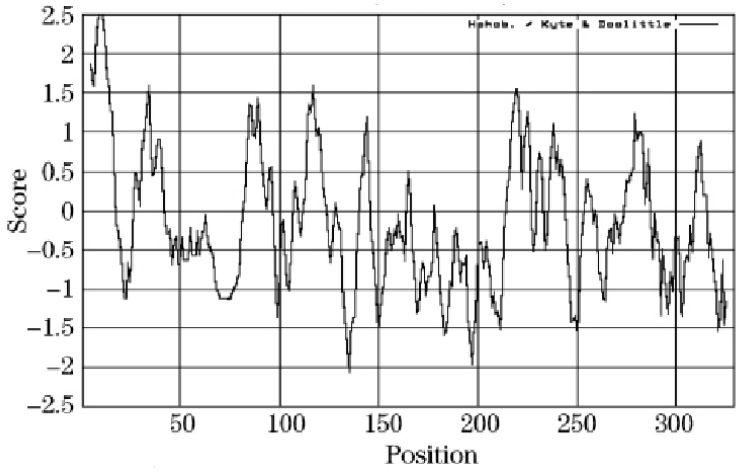

Fig.1 Scchi Amino Acid Affinity / Hydrophobicity Prediction.

The protein function of chitinase gene in sugar cane was studied, and the breakthrough direction was protein subcell. the localization and prediction of SCCHI protein cells were analyzed by psort software. The results showed that the probability of localization outside the cell was 52.8%, the probability of localization in microsomes (peroxisome) was 13.8%, the probability in endoplasmic reticulum was 10%, and the probability in endoplasmic reticulum was 10%. It can be seen that most of SCCHI proteins are located outside the cell and in the cell space, and a small number of them are located near the endoplasmic reticulum.

The secondary structure of SCCHI protein was predicted by SOPMA analysis. The conclusions were as follows: the amino acid residual cardinal number of α helix structure was 83, accounting for 25%, the amino acid residual cardinality of extended chain structure was 36, accounting for 11%, the amino acid residual cardinality of β folding structure was 14, accounting for 4.9%, and the irregular curling amino acid residual cardinality was 19.4%, accounting for 59%. The three-dimensional simulation prediction of SCCHI protein structure was carried out by ExPASy online analysis. It was concluded that the composition of chitinase gene in sugar cane was basically the same as that in corn, sorghum and other plants. Thus, it can be seen that the chitinase present in the existing plant can be regarded as the same substance[2].

4.3 Conclusion Analysis of Chitinase Gene in Sugarcane

The publication of the human genome map has a landmark significance for the study of biological inheritance. To search for new genes, the analysis of the function of different kinds of proteins has become the focus of the research. The electronic cloning technology of the gene replication through the computer equipment and the online software of the Internet can accelerate the discovery speed of the novel gene, and provide powerful data support for the research of the proteomics and the genomics. More than 26,000 sugarcane EST sequences have been released at NCBI and will continue to increase in the future. In this paper, using the technology of electronic cloning, the chitinase gene of sugarcane is obtained by using the scientific principle of bioinformatics. The length of SCCHI, is 80 BP $\leqslant$ 1236 BP, and the coding amino acid sequence is in accordance with the glycoside hydrolase 19 family. Because its tertiary structure contains chitinase N-terminal signal peptide and chitin binding region, it can be confirmed that the chitinase is class I enzyme. SCCHI protein is supposed to be secretory protein and evolutionarily conservative, which is consistent with the amino acid sequence arrangement of many plants and plays an important role in plant protection. Through data comparison, the evolution sequence of chitinase gene RNA extracted from sorghum, corn, wheat and other plants was the same as that of sugar cane SCCHI gene, which was slightly lower than that of rice and slightly higher than that of grape. It belongs to the same category, has subtle differences in composition, and needs to be certified in future research.

5. Conclusion

In summary, SCCHI gene sequence cloning and functionality need to be demonstrated by a large number of experiments. However, through electronic cloning technology, combined with bioinformatics analysis methods, it is confirmed that chitinase gene can be extracted directly from sugar cane, which provides a theoretical basis for further research. Not only that, in the course of the study, it was found that the chitinase content of sugar cane was very similar to that of other plants, so the research process was successful.

References

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