Computational Characterization of Proteins Involved in Banana (Musa acuminata) Ripening

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Abstract: In fact, Banana contains a high level of easily digestible natural sugars in both their fresh and dried forms, which it releases quickly in the blood stream. It has an immense nutritional value and medicinal properties apart from being an effective face pack. Any edible part of the plant, be it fruit, flower or stem, provides energy, vitamins and minerals. Cholesterol and fat content is minimal. Banana has the ability to correct acidity, gastritis and peptic ulcer. It is claimed that banana stimulates mucus production by stomach lining. Research shows that bananas contain certain enzyme inhibitors which are reported to be effective in controlling blood pressure in hypertensive people. Bananas are harvested when still green in order to better withstand transportation and to slow down the natural ripening process because of several weeks travel from the production areas to the end markets. There are a number of enzymes involved in the ripening process. The present work is principally aimed to computational study of these enzymes in view of understanding the ripening mechanism. The domains (conserved, 3- dimension region) of the specific enzymes, are identified using a computational tool, CDART (Conserved Domain Architecture Retrieval Tool). As a consequence, Aspartate aminotransferase (AAT_Like), Glycosyl hydrolase (Glyco_Hydr) and oxoglutarate (20G_Fe) were found to be the most conserved followed by Ethylene insensitive (EIN3), GT1, DPBB & Pollen in banana ripening proteins. The data, thus, obtained provide new insights in order to understand the stage specific role of the enzyme-proteins in the ripening process.

Keywords: Musa accuminata, Banana ripening, Domains, CDART, Polygalacturonase, pyridoxal phosphate, Aspartate aminotransferase.

INTRODUCTION

Banana (Musa acuminata) is a tree-like plant (though strictly herb) they are generally yellow when ripe and when ripe and have a soft yellow flesh with a sweet taste. Banana contains about 74% water, 23% carbohydrates, 1% proteins, 0.5% fat, and 2.6% fiber (these values vary between different banana cultivars, degree of ripeness and growing conditions) [1]. In an unripe banana the carbohydrates are mostly starches. In the process of ripening the starches are converted to sugars; a fully ripe banana has only 1-2% starch [2]. Besides being a good source of energy, banana is a rich source of potassium, and hence is often recommended for patients suffering from high blood pressure [3]. It is claimed that bananas have beneficial effect in the treatment of intestinal disorders, including diarrhoea [4]. They contain mucilaginous bulking substances and are easy to digest. Low levels of iron cause lethergy and weakness. Iron deficiency is common among women and vegetarians. Being iron rich, bananas can easily combat iron deficiency. The use of bananas has been found beneficial in the treatments of several medical conditions such as intestinal disorders, constipation, arthritis, gout, anemia, kidney stones, tuberculosis and urinary disorders.

Ripe fruit demonstrates a wide range of diversity in form, texture, pigmentation, aroma, flavour, and biochemical as well as nutrient composition [5]. Fruits of various species undergo modification of cell wall ultra structure and texture, conversion of starch to sugars, increased susceptibility to post-harvest pathogens, alterations in pigment biosynthesis/accumulation, and increased levels of flavour and aromatic volatiles during the course of maturation and ripening. Like other fruits, there are certain factors which influence the rate at which they ripen. Major among them is the presence of ethylene, it is an abundant hormone produced by most fruits.

Ethylene plays a crucial role in the ripening process. It is produced by bananas in natural conditions, however in much smaller quantities that are supplied in the chambers. When a fruit is exposed to ethylene, it ripens at the rate faster as compared to that without ethylene [6]. Oxygen is mandatory for this reaction as well [7]. The affinity and reactivity towards ethylene varies from fruit to fruit. An increased dose of ethylene accelerates the ripening process, which is very important for trading. Ripening process consists not only in changing the color of the peel, but also in breaking the starch into plain sugars which in turn influences the taste of the fruit. In green bananas starch and plain sugars are in the ratio...
of 20 to 1 whereas in yellow fruits the proportion is reversed
and is 1:20. An average process lasts 4-8 days depending on
the program chosen by the reopener.

There are also certain other factors affecting the level at
which all fruit ripen, viz, temperature, light exposure [6-9].
During banana fruit ripening ethylene production triggers a
developmental cascade that is accompanied by an enormous
conversion of starch to simpler sugars [10], a coupled explo-
sion of respiratory activity, and an enhancement in the senes-
cence- specific protein synthesis [11]. Banana starch disap-
pearance during ripening has been reported to originate at the
central portion of the fruit radiating, afterwards, to the sur-
face; the amylose/amylopectin ratio remains constant during
this process. The surface of starch granules appear to be
smooth, and the unique modification observed during ripen-
ing is the reduction of the granule dimensions; at advanced
ripening stages, some striations are detected on the surface
of both small and large granules. Several amylolytic enzymes
are followed during banana ripening [12]. These enzymes
can be studied by computational tool in order to understand
their 3-dimensional conserved region (domains). These con-
served regions (domains) are the 3-dimensional packing of
amino acids, which play an important role in the activity of
proteins. The functions and evolution of proteins can be un-
derstood using genomic, structural and proteomic data [13-
17] upon projections of 3-D structure of the specific protein.
It has been documented that a domain is a compact arrange-
ment of secondary structures connected by linker polypep-
tides [18, 19]. It usually folds independently and possesses a
relatively hydrophobic core. The peculiarities of domains are:
(a) the domains are divided into smaller units (b) they
represent a fundamental building block that can be used
to understand the evolution and function of proteins [20].
Detecting domains from sequence can be a laborious process
with many pitfalls. Thus, present study has been undertaken
with an objective to identify the domains present in different
proteins accumulated in ripening process of banana.

MATERIALS & METHODS

In the present study, a total of 111 protein sequences
were traced out for banana (Musa acuminata) from NCBI
belonging to different families, function in different pattern
during the ripening process. These profiles were briefly
shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Domains Identified in Banana (Musa acuminata) using the CDART</th>
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<td><strong>Protein Domains</strong></td>
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<td>DPBB,POLLEN</td>
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<td>GT1</td>
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<td>AMBALL</td>
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<td>Polyprenyl</td>
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The sequences were filtered on the basis of their length/molecular weight and submitted to the CDART in FASTA format. The e-value was set 0.01. CDART performed similarity searches of the NCBI Entrez Protein Database based on domain architecture, defined as the sequential order of conserved domains in proteins [21]. The algorithm led to projection of protein similarities across significant evolutionary distances using sensitive protein domain profiles rather than by direct sequence similarity. Proteins similar to a query protein were grouped and scored by architecture. Relying on domain profiles allowed CDART to be fast and informative. Domain profiles were derived from several collections of domain definitions inclusive of the functional annotation [22-24]. Searches can be further refined by taxonomy and by selecting domains of interest. CDART is available at http://www.ncbi.nlm.nih.gov/Structure/lexington/lexington.cgi.

RESULTS AND DISCUSSION

In present studies, out of 111 sequences found for the fruit ripening in banana, 67 sequences were filtered (considering the sequences having length >100 aa) for the domain analysis using CDART.

Domain Organization of the Banana Ripening Protein

These 67 sequences (*Musa acuminata*) were submitted to CDART in FASTA format, the e-value is set 0.01 and the domain analysis is performed. The CDART reported a number of domains (Table 1). Analyzing these domains, it was found that only few of them like AAT_like, 20G_FE, Glyco_hydr, GT1, DPBB, Pollen & EIN3 were most conserved (Fig. 1).

The domains AAT_like, 20G_FE, Glyco_hydr, GT1, DPBB, Pollen & EIN3 identified in 67 sequences were further examined, it shown that AAT_like domains were present in 11 sequences, 20G_FE & Glyco_hydro domains were present in 8 sequences and DPBB, Pollen, GT1 & EIN3 were present in 4 sequences each (Fig. 2). Analyzing the functionality of the domains AAT_like, 20G_FE, Glyco_hydr, GT1, DPBB, Pollen & EIN3, it was found that each domains has some specific activity and they play an important role to decide the functionality of the ripening proteins. The functionality of these domains are summarised as:

**Glycosyl hydrolases family 28** family 28 includes polygalacturonase EC: 3.2.1.15 as well as rhamnogalacturonase A (RGase A), EC: 3.2.1.-. These enzymes are important in cell wall metabolism [25]. **Aspartate aminotransferase** family belongs to pyridoxal phosphate (PLP)-dependent aspartate aminotransferase superfamily (fold I). Pyridoxal phosphate combines with an alpha-amino acid to form a compound called a Schiff base or aldime intermediate, which depending on the reaction, is the substrate in four kinds of reactions viz Transamination (movement of amino groups), Racemization (redistribution of enantiomers), Decarboxylation (removing COOH groups), and Various side-chain reactions depending on the enzyme involved.

Pyridoxal phosphate (PLP) dependent enzymes were previously classified into alpha, beta and gamma classes, based on the chemical characteristics (carbon atom involved) of the reaction they catalyzed. The availability of several structures allowed a comprehensive analysis of the evolutionary classification of PLP dependent enzymes, and it was found that the functional classification did not always agree with the evolutionary history of these enzymes.

![Fig. (1). Conserved domains identified in the banana (*Musa acuminata*) protein.](image-url)
The major groups correspond to Aspartate aminotransferase a, b and c, Tyrosine, Alanine, Aromatic-amino-acid, Glutamine phenylpyruvate, 1-Aminocyclopropane-1-carboxylate synthase, Histidinol-phosphate, gene products of malY and cobC, Valine-pyruvate aminotransferase and Rhizopine catabolism regulatory protein. 2OG-Fe (II) oxygenase superfamily contains members of the 2-oxoglutarate (2OG) and Fe (II)-dependent oxygenase superfamily. This family includes the C-terminal of prolyl 4-hydroxylase alpha subunit. The holoenzyme has the activity EC: 1.14.11.2 catalysing the reaction:

\[
\text{Procollagen L-proline + 2-oxoglutarate + O}_2 \leftrightarrow \text{Procollagen Trans-4-hydroxy-L-proline + Succinate + CO}_2
\]

The full enzyme consists of an alpha2 beta2 complex with the enzyme subunits contributing most of the parts of the active site. The family also includes lysyl hydrolases, isopenicillin synthases and AlkB [26]. Rare lipoprotein A (RlpA)-like double-psi beta-barrel contains a conserved region that has the double-psi beta-barrel (DPBB) fold. The function of RlpA is not well understood, but it has been shown to act as a prc mutant suppressor in Escherichia coli. The DPBB fold is often an enzymatic domain. The members of this family are quite diverse, and if catalytic this family may contain several different functions [27]. Another example of this domain is found in the N terminus of pollen allergen. Pollen allergen, this family contains allergens lOl P1, PII and PIII from Lolium perenne. Ethylene insensitive 3 (EIN3) proteins are a family of plant DNA-binding proteins that regulate transcription in response to the gaseous plant hormone ethylene. 2OG_FE-dependent oxygenase superfamily includes the C-terminal of prolyl 4-hydroxylase alpha subunit. The holoenzyme has the activity EC: 1.14.11.2 catalysing the reaction.

Our next objective is to focus on interlinking the above mentioned domains with different stages of ripening process in banana. The work along these notions is in progress through bioinformatics tools along with these domains.

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