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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 lethargy 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

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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 explosion of respiratory activity, and an enhancement in the senescence- specific protein synthesis [11]. Banana starch disappearance during ripening has been reported to originate at the central portion of the fruit radiating, afterwards, to the surface; the amylose/amylopectin ratio remains constant during this process. The surface of starch granules appear to be smooth, and the unique modification observed during ripening 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 conserved 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 understood 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 arrangement of secondary structures connected by linker polypeptides [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 web server, www.ncbi.nlm.nih.gov. These protein sequences belonging to different families, function in different pattern during the ripening process. These profiles were briefly shown in Table **1**.

Table 1. Domains Identified in Banana (*Musa acuminata*) using the CDART

Protein Domains	Protein Acc. No.	Protein	Protein Acc. No.	Protein
Glyco_hydro	CAE51357.1	putative polygalacturonase	AAF08679.1	beta-1,3-glucanase
	CAE51356.1	putative beta-galactosidase	AAO27531.1	Polygalacturonase
	CAE51355.1	putative beta-galactosidase	AAZ94622.1	beta-amylase
	AAB82772.2	beta-1, 3-glucananse	AAT74603.1	Polygalacturonase
AAT_LIKE	CAD44267.2	putative aminocyclopropane carboxylic acid synthase	CAE53271.1	1-aminocyclopropane-1-carboxylate synthase
	AAR00513.1	1-aminocyclopropane-1-carboxylate synthase	AAR00512.1	1-aminocyclopropane-1-carboxylate synthase
	AAL82597.1	aspartate aminotransferase 2	AAL82596.1	aspartate aminotransferase 1
	CAA75749.1	1-aminocyclopropane-1-carboxylate synthase	AAU09672.1	ACC synthase
	BAA84947.1	ACC synthase		
	AAC31571.1	1-aminocyclopropane-1-carboxylate synthase	BAA84945.1	ACC synthase
20G_FE	AAR00511.1	1-aminocyclopropane-1-carboxylate oxidase	AAB68602.1	1-aminocyclopropane-1-carboxylate oxidase
	CAD44265.2	putative aminocyclopropane carboxylate oxidase	ABW20470.1	ACC oxidase
	CAE53174.1	1-aminocyclopropane-1-carboxylate oxidase	ABV45543.1	1-aminocyclopropane-1-carboxylate oxidase
	AAB00556.1	1-aminocyclopropane-1-carboxylate oxidase	AAC31967.1	1-aminocyclopropane-1-carboxylate oxidase
DPBB,POLLEN	AAN31756.1	expansin1	AAM08930.1	expansin 1
	ABN09942.1	expansin A5	ABN09939.1	expansin A4
GT1	CAD44260.1	putative sucrose-phosphate synthase	CAD44259.1	putative sucrose-phosphate synthase
	AAB82780.1	ripening-associated protein	AAC23914.1	sucrose-phosphate synthase
AMBALL	AAF19196.1	AF206320_1 pectate lyase 2	AAF19195.1	AF206319_1 pectate lyase 1
Polyprenyl	AAL82595.1	AF470318_1 farnesyl pyrophosphare synthase	ABI73983.1	farnesyl pyrophosphate synthase

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 aldimine 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.

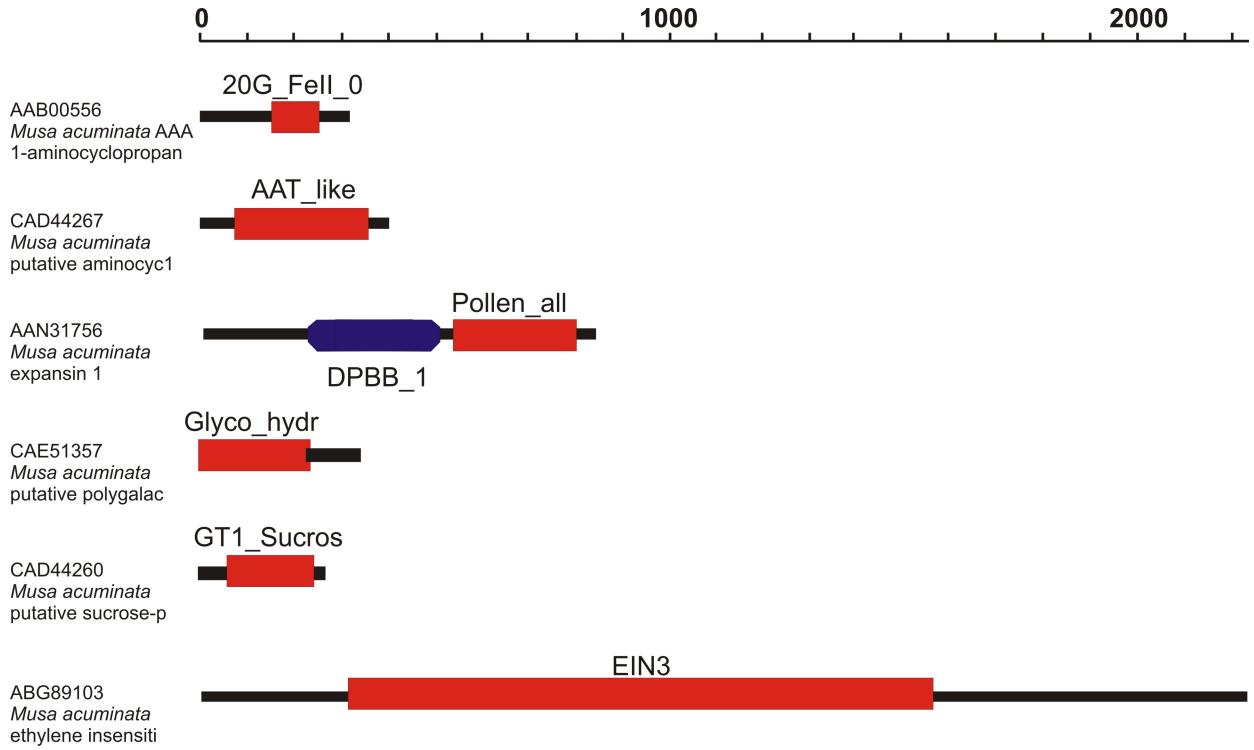

Fig. (1). Conserved domains identified in the banana (*Musa acuminata*) protein.

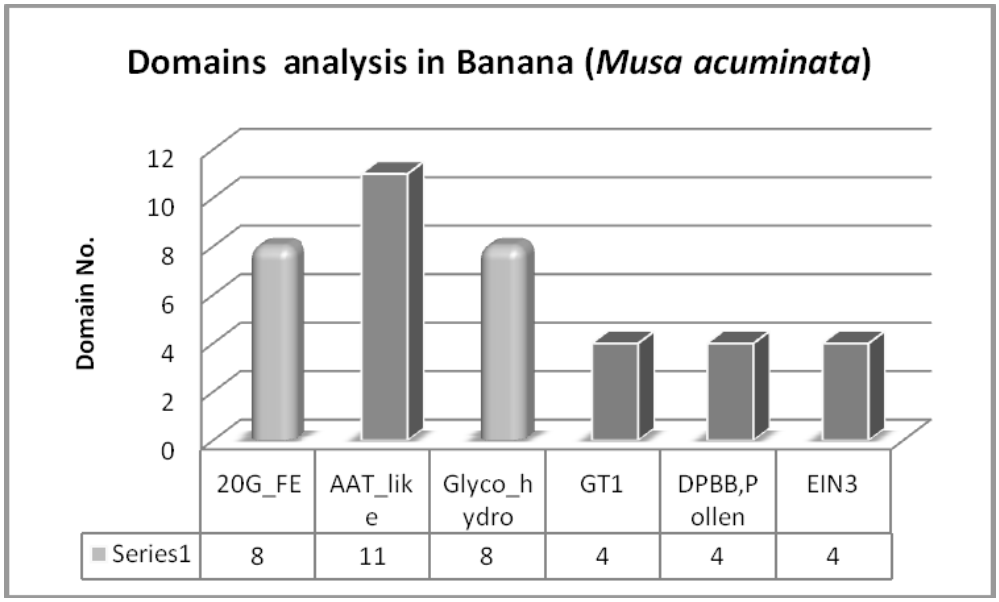

Fig. (2). Sequence wise representation of domains

The major groups corresponds 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:

Procollagen L-proline + 2-oxoglutarate + O2 <=> Procollagen Trans-4-hydroxy-L-proline + Succinate + CO2

The full enzyme consists of an alpha2 beta2 complex with the alpha subunit 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 PI, 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, and are essential for ethylene-mediated responses including the triple response, cell growth inhibition, and accelerated senescence.

Characteristic of domains were analyzed and it was found that ripening process in banana involved a number of protein domains and these domains have some specific functionality *viz*. The domain AAT_like (Aspartate aminotransferase) belongs to pyridoxal phosphate (PLP)-dependent aspartate aminotransferase superfamily (fold I). PLP combines with an alpha-amino acid to form a compound called a Schiff base or aldimine intermediate, which depending on the reaction, is the substrate in four kinds of reactions transamination (movement of amino groups), racemization (redistribution of enantiomers), decarboxylation (removing COOH groups), and various side-chain reactions depending on the enzyme involved. The domain Glyco_hydr (Glycosyl hydrolase) is important in cell wall metabolism. Similarly, the domain EIN3 (Ethylene insensitive 3) found in a family of plant DNA-binding proteins that regulate transcription in response to the gaseous plant hormone ethylene. 20G_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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