Insights on predominant edible bamboo shoot proteins

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Juvenile bamboo shoots have become a US$ 18 billion industry and have lured interest worldwide for its nutritive value and health enhancing properties; making it a suitable candidate for food security. Quantitative analysis revealed that juvenile bamboo shoots are a good source of proteins. However, there is no qualitative analysis describing unique proteins present in the edible bamboo shoots. In order to provide the identity of predominant proteins present in edible bamboo shoots, 13 edible bamboo species were analysed. SDS-polyacrylamide gel electrophoresis revealed that high level of peptides polymorphism among 13 bamboo species was within the range of 20.10-15.50 and 66.50-29 KDa. Gel analysis shows that Bambusa oliveriana expressed the maximum number of diverse peptides while Bambusa nutans expressed the minimum number of peptides. Importantly, MS/MS data revealed that abundant peptides in bamboo shoots are histone-like related (H2A, H3 and H4) which generally form the nucleosome core and can participate in defense, stress and development. This study is the first qualitative data on protein components of bamboo shoots which harmonize the existing quantitative data that edible bamboo species as healthy food and a rich source of protein.

Key words: Bamboo shoot proteins, histone-like proteins, peptide polymorphism, SDS-PAGE-MS/MS, Dendrocalamus hamiltonii.

INTRODUCTION

Over two million tonnes of juvenile bamboo shoots are consumed in the world annually (Yang et al., 2008). The USA alone imports over 14.5% of the world bamboo shoots mostly from Asia, making an estimated US$ 18 billion trade industry (Daphne, 1996; Lobovikov, 2003). In the North Eastern States of India, 1979 tonnes of fermented bamboo shoots are consumed annually (Bhatt et al., 2004) with a price tag of US$ (0.66 - 0.88) per kg (Singh et al., 2010). Due to the high demand for bamboo shoots, efficient protocols for cultivation of edible bamboo species have been developed to balance future demand (Brar et al., 2013; Devi et al., 2012; Singh et al., 2012; Waikhom and Louis, 2014).

Essentially, juvenile bamboo shoots are consumed as vegetable or pickled, but can be processed by fermentation or deep frying, as shredded chips and canned into more palatable forms (Choudhury et al., 2011; Waikhom et al., 2013). Also, bamboo shoots contain high...
level of phytosterols, playing a key role in lowering blood cholesterol and high levels of cellulotic content, an important appetite (Nirmala et al., 2011), anti-fatigue activity (Akao et al., 2004), high levels of antioxidant activity, microminerals, macrominerals and high protein levels per gram of dry weight (Waikhom et al., 2013).

Notwithstanding the rich dietary and therapeutic traits reported for bamboo shoots of several bamboo species (Akao et al., 2004; Waikhom et al., 2013), some species are rich in toxic cyanogenic-like taxiphyllin, significantly associated with neurological disorder called Konzo (Nzwalo and Cliff, 2011; Schwarzmaier, 1997; Waikhom et al., 2013).

Furthermore, only quantitative data on bamboo shoots proteins have been generated thus far (Nirmala et al., 2008; Waikhom et al., 2013). It is unclear which of the reported species of bamboo shoots is endowed with diverse proteins which can only be determined qualitatively. Furthermore, the identity of abundant bamboo shoot proteins has not been determined. Therefore, we set as objective to profile the crude proteins of edible bamboo shoots of 13 bamboo species and identify prominent proteins.

MATERIALS AND METHODS

Plant material

We followed the collection procedures as defined in Waikhom et al. (2013) from the field to the laboratory for edible bamboo species located at different altitudes of Manipur, India (23°47´-25°41´NL; 92°58´ to 94°47´EL), during July to August of 2012 to 2013. The 13 bamboo species studied were authenticated morphologically by the Botanical Survey of India (BSI). Kolkata and voucher specimens are deposited at the Central National Herbarium in BSI (Table 1). These edible bamboo species have been identified by Waikhom et al. (2013) on the basis of the trn-L-F intergenic spacer and the sequences are available at NCBI DNA nucleotide sequences database. These juvenile bamboo shoots of the 13 bamboo species were selected for the study because they are available in the local markets throughout the year in the North Eastern States of India.

Protein extraction

The sheaths of the bamboo shoots were removed (Figure 1A). The inner edible shoot stem was measured with a caliper and cut into three equal portions, that is, the tip, middle and bottom (Figure 1B). Based on data from Waikhom et al. (2013) which showed that consumer prefer the crispy taste of the middle portion of bamboo shoots, only the middle portion was used in this study. The fresh bamboo shoots were crushed in 10 mM CaCl2 solution containing 0.25% Triton-X-114 (Sigma-Aldrich®, Missouri, USA) and 1% of dithiothreitol (DTT, Sigma-Aldrich®, Missouri, USA) as described in Louis et al. (2014). Protein was washed with ReadyPrep™ 2-D cleanup Kit® (Bio-Rad, Hercules, CA, USA) following the manufacturer instructions. Proteins were dissolved in ReadyPrep™ rehydration buffer consisting of 8 M urea, 2% CHAPS, 50 mM DTT, 0.2% (w/v), Bio-Lyte® 3/10 ampholytes, and traces of Bromophenol Blue (Bio-Rad®, Hercules, CA, USA). Additionally, the protein content of 10 μl aliquots was quantified spectrophotometrically at 595 nm by the dye-binding method (Bradford, 1976) using bovine serum albumin as standard.

SDS-polyacrylamide gel electrophoresis and analysis

Using standard one dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE), 15 μg protein for each species was profiled on a 15% SDS-polyacrylamide gel. SDS-PAGE was performed at 170 V in a 1X Tris-glycine-SDS, pH 8.3 (25 mMTris-HCl, 200 mM glycine, 0.1% SDS) running buffer in PowerPac™Basic 300 V system (Bio-Rad®, Hercules, CA, USA). A

Table 1. Morphological authentication of 13 species of bamboo shoots by the Botanical Survey of India (BSI), Kolkata with voucher specimens at BSI Central National Herbarium

<table>
<thead>
<tr>
<th>GenBank accession.bamboo species</th>
<th>BSI Voucher accession</th>
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</thead>
<tbody>
<tr>
<td>KC013282/Chimonobambusa callosa</td>
<td>IBSD/WS/019</td>
</tr>
<tr>
<td>KC013285/Bambusa cacharensis</td>
<td>IBSD/WS/020</td>
</tr>
<tr>
<td>JX564900/Bambusa manipureana</td>
<td>IBSD/WS/008</td>
</tr>
<tr>
<td>JX564901/Bambusa nutans</td>
<td>IBSD/WS/023</td>
</tr>
<tr>
<td>JX507132/Bambusa tulda</td>
<td>IBSD/WS/022</td>
</tr>
<tr>
<td>JX507131/Bambusa oliveriana</td>
<td>IBSD/WS/010</td>
</tr>
<tr>
<td>JX564902/Dendrocalamus giganteus</td>
<td>IBSD/WS/001</td>
</tr>
<tr>
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<tr>
<td>JX564904/Dendrocalamus hookeri</td>
<td>IBSD/WS/005</td>
</tr>
<tr>
<td>JX564905/Dendrocalamus manipureanus</td>
<td>IBSD/WS/002</td>
</tr>
<tr>
<td>JX507133/Melocanna baccifera</td>
<td>IBSD/WS/018</td>
</tr>
<tr>
<td>JX507134/Schizostachyum dulloa</td>
<td>IBSD/WS/003</td>
</tr>
<tr>
<td>JX564906/Bambusa sp.</td>
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</tr>
<tr>
<td>JX564907/Bambusa sp.</td>
<td>IBSD/WS/007</td>
</tr>
<tr>
<td>KC013288/Bambusa tuloides</td>
<td>IBSD/WS/006</td>
</tr>
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</table>

Figure 1. Juvenile edible bamboo shoot of Bambusa oliveriana (GenBank accession JX564901). (A) A sheathed bamboo shoot. (B) A bamboo shoots without sheath.
solution of 0.35% Coomassie brilliant blue R250 (SRL, Mumbai, India) was used for staining overnight. Destaining was performed in a solution containing 50% methanol and 10% acetic acid until visible bands were observed. Triplicate gels and two biological repeats (for July-August 2012 - 2013) were scanned and subjected for analysis in Phoretix 1D v.10.4.4 algorithm (TotalLab Ltd, Newcastle, UK). Following background subtraction, bands were automatically detected based on normalized pixel-to-pixel intensity threshold. Peptides banding polymorphism among the bamboo shoots of 13 species was established using the neighbor-joining method (Saitou and Nei, 1987), based on the relative mobility of bands.

**Peptide fingerprinting and database searching**

Prominent bands were manually excised and subjected to trypsin digestion and elution as earlier described (Shevchenko et al., 2006). The digested protein solution (0.45 µl) was sandwiched in 5 mg/ml α-cyano-4-hydroxy-cinnamic acid (diluted in 0.1% trifluoroacetic acid, 50% acetonitrile) on a matrix assisted laser desorption/ionization (MALDI) target plate (Applied Biosystems, Vernon Hills, IL, USA). MALDI-TOF/TOF MS/MS was performed in SCIEX4800 MALDI TOF-TOF proteomics at an accelerating voltage of 20 kV, and mass resolution was maximized at 1600 Da. All the acquired spectra were processed with the 4700 Explore™ software (Applied Biosystems, Vernon Hills, IL, USA) at default settings. NCBI nr and green plant MSDB sequence databases were searched against all updated entries via the in-house MASCOT server (v.2.3 MatrixScience, London, UK). Search parameters were set as follows: enzyme, trypsin; fixed modifications, carbamidomethyl (C); variable modification, oxidation (M); peptide mass tolerance, 40-100 ppm; maximum missed cleavages, 2. The accepted MOWSE score threshold was inferred at P < 0.05. A false-discovery rate (FDR) (Elias et al., 2005) for the peptide search match was calculated using a decoy database at a cut-off FDR ≤ 1%. To determine the biological signatures and putative domains for the peptides, KEGG Orthology Based Annotation System (KOBAS) 2.0 (Xie et al., 2011) and NCBI Conserved Domains Database (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) servers were used.

**RESULTS AND DISCUSSION**

Bamboo shoots are usually pigmented with secondary metabolites (Figure 1B); as a result the proteins are pigmented. Bamboo shoots are also hard to fine-crush and pose difficulties in protein extraction. We observed that bamboo shoot proteins extracted in buffers containing SDS and urea often produced poor banding on polyacrylamide gel, marked by vertical streaking, smearing and colouring of the gels due to pigmentation (data not shown). Pigmentation was reduced by washing the extracted protein repeated with a clean-up kit (Bio-Rad, Hercules, CA, USA). By using 10 mM CaCl₂ solution containing 0.25% Triton-X-114 extraction buffer as previously described (Louise et al., 2014), smearing and streaking problems were eliminated.

Compelling quantitative data on freshly harvested, fermented and canned bamboo shoots of *Dendrocalamus giganteus* (Nirmala et al., 2008) revealed that bamboo shoots are good source of proteins. Furthermore, Nirmala et al. (2008) found that 100 g of fermented and fresh bamboo shoot of *D. giganteus* contained 2.17 and 3.11 g of proteins, respectively. Based on the analysis of 15 edible bamboo species, Waikhom et al. (2013) reported that bamboo shoots are a rich source of nitrogen. For instance, the juvenile shoot of *C. callosa* was found to contain 1153 mg of nitrogen per 100 g dry weight (Waikhom et al., 2013). However, on the basis of these pioneering quantitative studies (Nirmala et al., 2008; Waikhom et al., 2013), it is difficult to tell which species of bamboo produce shoots rich in diverse proteins given that all species contain TTC and other valuable nutritional attributes. Qualitative proteomics analysis can help stakeholders in the bamboo industry to select bamboo shoots in a quest for diverse protein source.

SDS-PAGE profile of bamboo shoots crude proteins for 13 species revealed high level peptide banding polymorphism within the range of 66.5 to 29.10 KDa and 20.10 to 15.50 KDa (Figure 2). It is worth noting that above 29.10 KDa, polymorphism was less because only banding intensities varied among species (Figure 2). On the contrary, below 20.10 KDa, high level polymorphism was observed, hallmarked by new prominent bands reflected by variations in relative mobility in the dendrogram (Figure 3). On this basis, it is concluded that the major proteins of bamboo shoots have a low molecular weight ranging between 20.10 to 15.50 KDa. Irrespective of the lane used for rooting the dendrogram, two main clades (I and II) were generated, viz. Clade I (*D. manipureanus*, *D. giganteus*, *B. oliveriana*, and *Bambusa sp.*, *S. dullooa*) and Clade II (*B. caharensis*, *Bambusa sp.*, *B. tuldodes*, *B. manipureana*, *B. tulda*, *C. callosa*, *D. hamiltonii* and *M. baccifera*) (Figure 3). Only internal branch length for *B. tuldodes* (lane 11, length = 0.128) and *D. hamiltonii* (lane 7, length = 0.128) matched at 100% revealing a high level of polymorphism among the peptides of other species.

Taxonomic placement of bamboo species has suffered a great deal in the last decade (Baldwin et al., 1995). Previous report based on morphological descriptors discerned 15 edible bamboo species into two clades (Waikhom et al., 2013). Using the same set of bamboo specimens, Random Amplified Polymorphic DNA (RAPD) and TrnL-F intergenic spacer analyses generated three and two clades, respectively (Waikhom et al., 2013). Based on previous taxonomical placement (Waikhom et al., 2013) and the present finding based on peptide polymorphism, it is tempting to suggest that the placement of bamboo species is a function of the experimental approach. Dominant morphological characters in bamboo shoots such as colour, shape, presence of hair in the culm sheath significantly compromises taxonomic placement (Waikhom et al., 2013). Furthermore, rapid concerted evolution because of high level transition-transversion at the rDNA and trnL-F loci impedes accurate phylogenetic inference of bamboo species (Baldwin et al., 1995; Nieto-Feliner and Rosssello, 2007). Although abundant peptides could
Figure 2. Juvenile edible bamboo shoots crude proteins (15 µg per lane) polymorphism profiled on a 15% SDS-PAGE gel. Lane 1, Standard medium range molecular mass markers; lane 2, *Bambusa* sp. JX564906; lane 3, *B. oliveriana* JX564901; lane 4, *B. tulda* JX507132; lane 5, *Bambusa* sp. accession JX564907; lane 6, *B. manipureana* accession JX564900; lane 7, *D. giganteus* accession JX564902; lane 8, *D. hamiltonii* accession JX564903; lane 9, *D. manipureanus* accession JX564905; lane 10, *C. callosa* accession KC013282; lane 11, *S. dullooa* accession JX507134; lane 12, *B. tuldoides* accession KC013288; lane 13, *B. cacharensis* accession KC013285; lane 14, *M. baccifera* accession JX507133; lane 15, bovine serum albumin, respectively.

Figure 3. Dendrogram depicting diversity in proteins of species of edible bamboo shoots based on Neighbour joining generated in Phoretix 1D v.10.4 algorithm (Totallab Ltd, Newcastle, UK). Lane 1- Standard medium range molecular mass markers, lane 2 - *Bambusa* sp. JX564906, lane 3 - *B. oliveriana* JX564901, lane 4 - *B. tulda* JX507132, lane 5 - *Bambusa* sp. accession JX564907, lane 6 - *B. manipureana* accession JX564900, lane 7 - *D. giganteus* accession JX564902, lane 8 - *D. hamiltonii* accession JX564903, lane 9 - *D. manipureanus* accession JX564905, lane 10 - *C. callosa* accession KC013282, lane 11 - *S. dullooa* accession JX507134, lane 12 - *B. tuldoides* accession KC013288, lane 13 - *B. cacharensis* accession KC013285, and lane 14 - *M. baccifera* accession JX507133, respectively.
Figure 4. Mean number of bands observed among edible bamboo species over two seasons of July to August of 2011 - 2013 reveal B. oliveriana encodes diverse proteins and bars represent standard deviations.

mask others and compromise resolution in SDS-PAGE, the approach allows for many samples on the same gel and subsequent accurate analysis of band patterns, an advantage over two-dimensional SDS-PAGE.

Using normalized threshold values for triplicates and two biological repeats, the mean of bands detected varied among species but consistent for each given species (Figure 4). The data revealed that B. oliveriana expressed diverse peptides with the highest mean band number of 28, while the lowest banding of 18 was observed in B. tulda (Figure 4). From previous data on toxicity content in bamboo shoots of different edible bamboo species (Waikhom et al., 2013) and other nutritional components of D. giganteus (Nirmala et al., 2008) and the present findings, it is clear that bamboo shoots are a rich source of protein and could help in areas with poor protein diet. Based on MALDI-TOF/TOF MS/MS results (Table 2, Figure 2), the predominant bands were identified as histone-like related proteins. Although other peptides were identified at MOWSE score ($P < 0.05$) they failed the FDR test at cut-off value ≤ 1% and are not reported. Histones are highly alkaline proteins, located in the nucleus and associated with DNA to form chromatin, highly organized into nucleosome cores. Five core histones are H1, H2A, H2B, H3, and H4 and are extremely conserved throughout evolution and are modified under stress conditions (Pawlak and Deckert, 2007). So far, it has been shown that histones undergoes numerous covalent modification such as acetylation, methylation, phosphorylation and ubiquination, and these modifications controls chromatin functions mediated by histones (Kouzarides, 2007). An important line of evidence showed that trimethylation of histone H3 at lysine 27 (H3K27me3) is involved in cold adaptation in planta (Kwon et al., 2009; Zhu et al., 2012). Furthermore, vigorous temperature fluctuations during the day and night are suggested to influence nucleosome assembly/disassembly and provide a gateway for rapid chromatin configuration to adapt to ambient temperatures (Zhu et al., 2012).

In Arabidopsis, cold stress also triggered rapid and transient upregulation of histone H3 Ser-10 phosphorylation, H3 phosphoacetylation, and H4 acetylation followed by stress-type-specific gene expression (Sokol et al., 2007). In the present study H4, H3 and H2A were predominant histones-like proteins identified (Figure 5, Table 2). Since the bamboo shoots studied herein were collected in month of July and August which falls within the cold monsoon season in the North Eastern States of India, fluctuations in temperature...
Table 2. Identified predominant peptide bands (of figure 2) from 13 species of edible bamboo shoots.

<table>
<thead>
<tr>
<th>Name of species/lane</th>
<th>Band</th>
<th>Accession</th>
<th>Organism</th>
<th>Name of protein</th>
<th>Putative function</th>
<th>N M</th>
<th>Exp. Mr (KDa)</th>
<th>PS</th>
<th>Co.</th>
<th>N-terminal amino acids</th>
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<tr>
<td>M. baccifera / 14</td>
<td>1</td>
<td>gi</td>
<td>41387680</td>
<td><em>Chlamydomonas reinhardtii</em></td>
<td>Phosphoenol pyruvate carboxylase</td>
<td>0</td>
<td>28.81</td>
<td>63</td>
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<td></td>
<td>2</td>
<td>gi</td>
<td>70772</td>
<td><em>Triticum aestivum</em></td>
<td>Histone H4</td>
<td>4</td>
<td>16.31</td>
<td>180</td>
<td>206</td>
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<tr>
<td>D. hamiltonii / 9</td>
<td>3</td>
<td>gi</td>
<td>122084</td>
<td><em>Triticum aestivum</em></td>
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<td>81906</td>
<td>Garden pea</td>
<td>Histone H2A</td>
<td>6</td>
<td>15.23</td>
<td>75</td>
<td>291</td>
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<td>B. oliveriana / 2</td>
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<td>34902360</td>
<td><em>Oryza sativa</em> L.</td>
<td>Hypothetical protein</td>
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<td>14.92</td>
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<td>995</td>
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NM - Number of matches, Exp. Mr - experimental molecular weight kilo Dalton, PS - protein score reported by MASCOT at P ≤ 0.05, Cov. - Amino acid coverage.

Conclusion

Extraction of quality proteins is crucial for downstream proteomics analysis. The protocol for protein extraction in the present study hallmarks the introduction of bamboo into the proteomics era. Based on the nutritive value and health enhancing properties, bamboo shoots are becoming popular food worldwide. While considering the nutraceutical values of edible bamboo shoots and the toxicity implications, dietary intake of proteins can be a deciding factor in region of low protein diet. Hence, an appropriate selection of species of juvenile edible bamboo shoots is the solution for food security as well as a potential source for proteins. In the present study, juvenile edible bamboo shoots expressed diverse peptides, predominantly of low molecular weight (20.10 to 15.50 KDa) histone-like proteins. This first qualitative proteomics analysis on species of bamboo shoots tells a consumer could benefit differently from bamboo...
protein intake as a function of a chosen edible bamboo species.

Conflict of interests

The authors did not declare any conflict of interest.

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REFERENCES


Nieto-Feliner G, Rosssello JA (2007). Better the devil you know?


