SAVARY, BRETT J.[1,2]*, JIANFENG (JAY) XU[1,2], JOSE C. TOVAR[1], NINGNING ZHANG[1], and HONG FANG[2], [1]Arkansas Biosciences Institute, [2]College of Agriculture and Technology, Arkansas State University, P.O. Box 639, Jonesboro, AR 72467. **Thermostable pectinase technologies for remodeling sugar beet root cell walls and generating functional products.**

ABSTRACT

We are investigating novel biochemical and molecular technologies for applying thermostable pectinases to remodel the cell wall in sugar beet roots to provide economic and environmental benefits for sustainable beet-biomass processing. Of immediate concern for traditional beet processing is the need for more efficient processing technologies to reduce energy inputs and lower the footprint for greenhouse gas emissions. Sugar beets are also targeted for expanded industrial sugar (sucrose) production beyond traditional growing regions to meet national needs for advanced biofuels, renewable chemical feedstocks, and for conversion to value-added biobased products. Thus we are assembling a toolbox with thermostable glycohydrolases to evaluate effective action on beet pulp and expression models to establish their bioproduction. This presentation summarized our progress towards developing a naturally thermally-tolerant plant pectin methylesterase (PME) and a thermostable *Geobacillus* endo-arabinanase (ABN) – two pectinases that are optimally active at the temperature of diffusion water during sucrose extraction from cossettes. We also highlighted a novel "designer" glycopeptide technology that has been shown to stabilize recombinant proteins and improve their targeting to the cell wall. Our working model tests PME action to increase anionic charge of homogalacturonan regions of pectin to promote cooperative calcium crosslinking, while ABN action may "shave" the "hairy" rhamnogalacturonan 1 regions to reduce the pectin network pore size. These modification of pectin structure within the cell wall matrix is hypothesized to generate a denser, more compressible tissue in wet beet pulp, resulting in more efficient mechanical separation of water contents. Experimental results were highlighted showing dramatic reduction in water binding through combined PME-calcium treatments. Other results (not presented) indicated ABN action showed no impact on water binding in treated pulp. Bioproduction of ABN was demonstrated with the *Pichia pastoris* expression system. Application of this enzyme is targeted for recovering ferulated arabino-oligosaccharides, which we are testing for their ability to modulate tight-junction function in a human colonocyte bioassay. Future work in our group is the application of hydroxyproline-rich glycopeptide technology to these thermostable pectinases. This Hyp-glycopeptide technology exploits the *O*-glycosylation code for plant cell wall extensin and arabinogalactan protein glycans, which can dictate the glycan structure and the length of the glycopeptide. Our research program's goal is to establish the suitability for using these thermostable pectinases to rationally manipulate the structural and functional properties of cell walls and to engineer their direct expression in sugar beet tap-roots to ultimately deliver benefits to sugar beet processors.