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a year ago

LBNL Biosciences Area Supports The Next Generation of Scientists

The Biosciences Area celebrated its educational outreach with a campaign on Twitter entitled #BioSciNexGen. Check out below what our interns were up to this summer. For more information contact bioscicomms@lbl.gov.



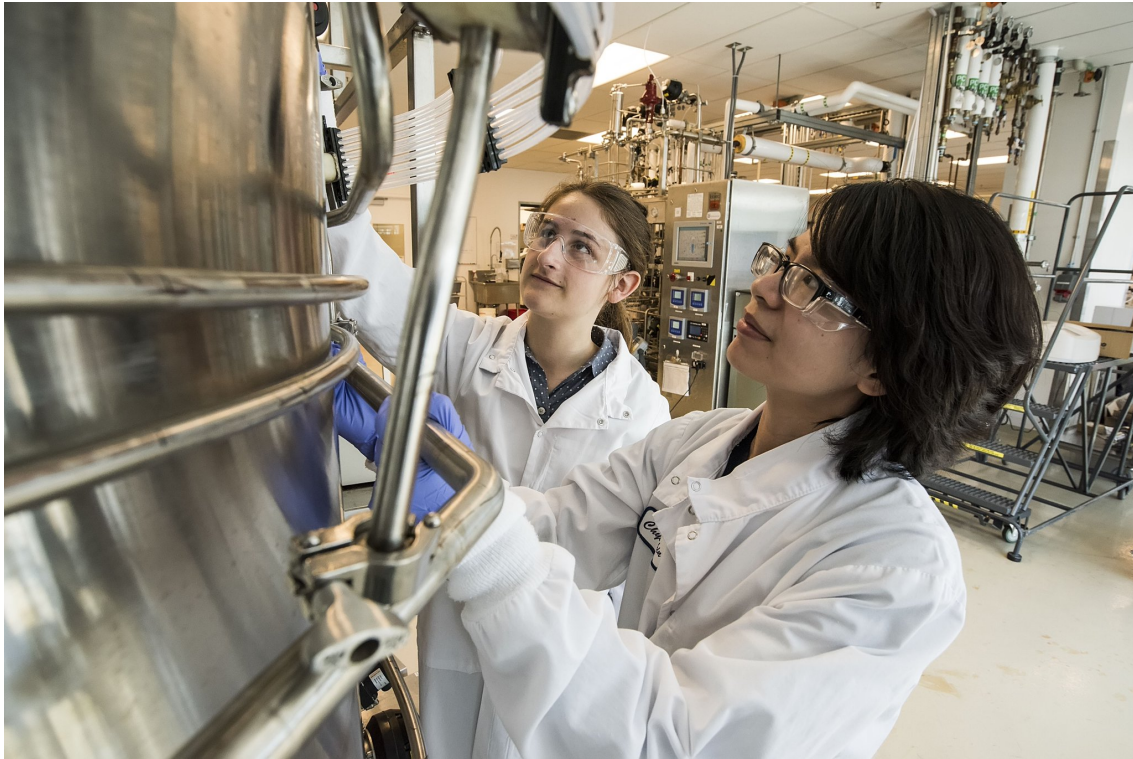
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.@BiotechPartner interns & mentors @doe_jgi compete at #Pipetting #Olympics #BioSciNextGen
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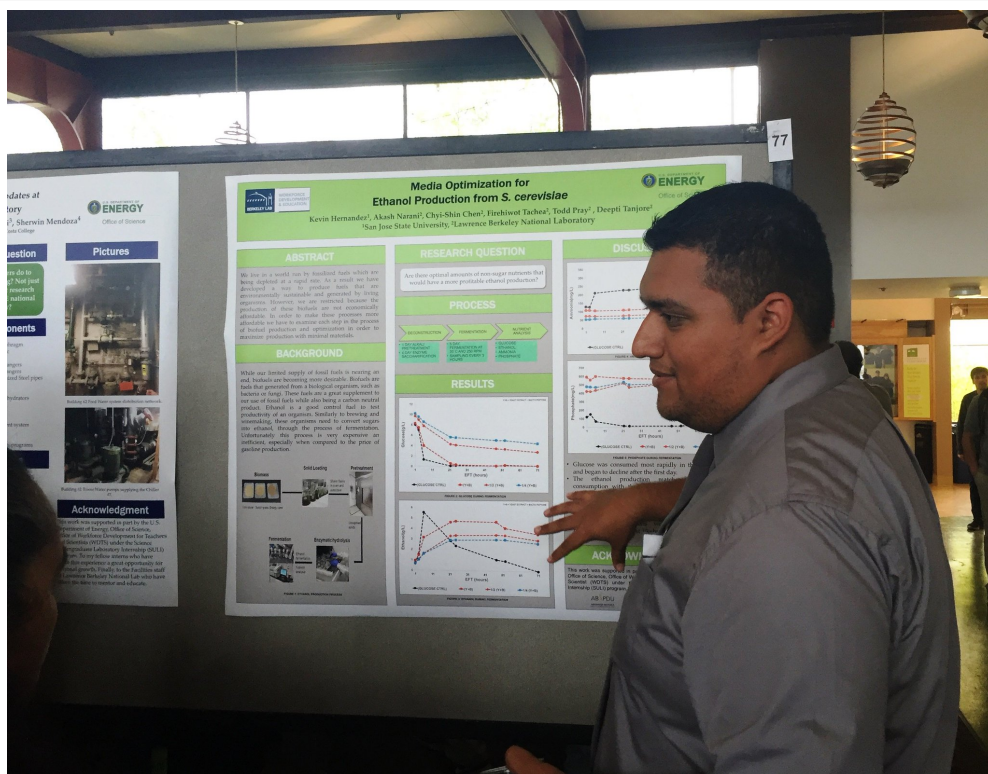
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.@BiotechPartner student gets #HandsonExperience at #ABPDU #BSE #BioSciNextGen
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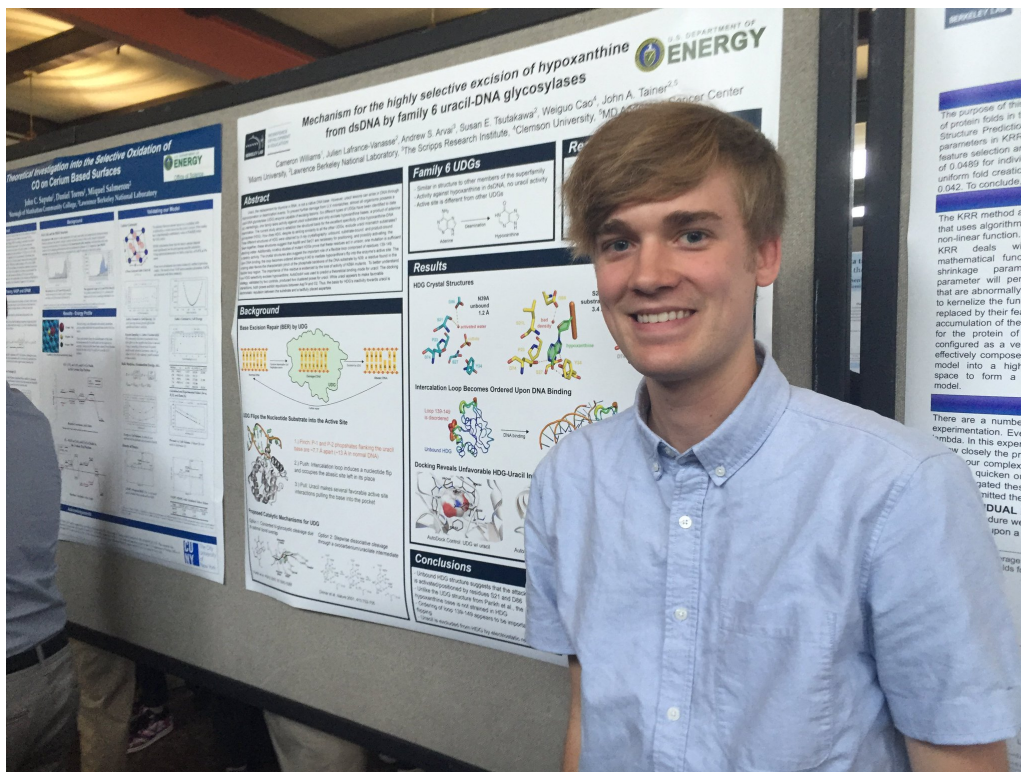
Kevin Hernandez student @SJSU interned at #ABPDU @deeptitanjore #BioSciNextGen #BSE
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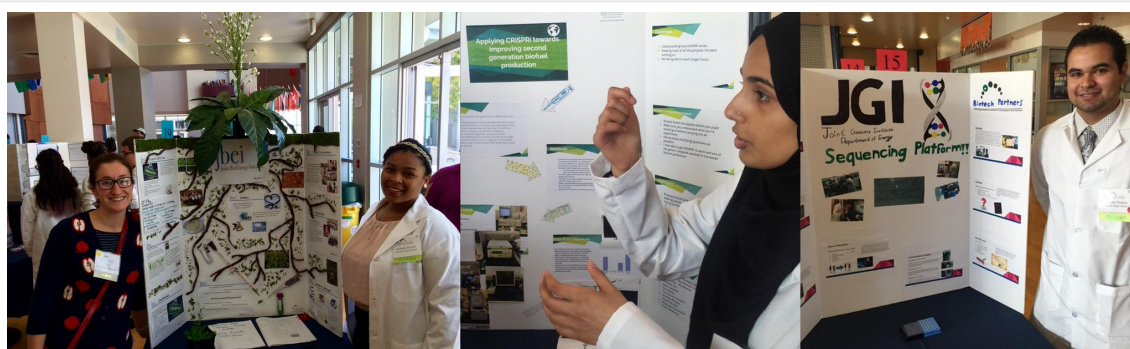
.@ucmerced intern gets hands-on experience in microbial studies @doe_jgi #STEMEducation
#BioSciNextGen pic.twitter.com/3TQrTXXU3K

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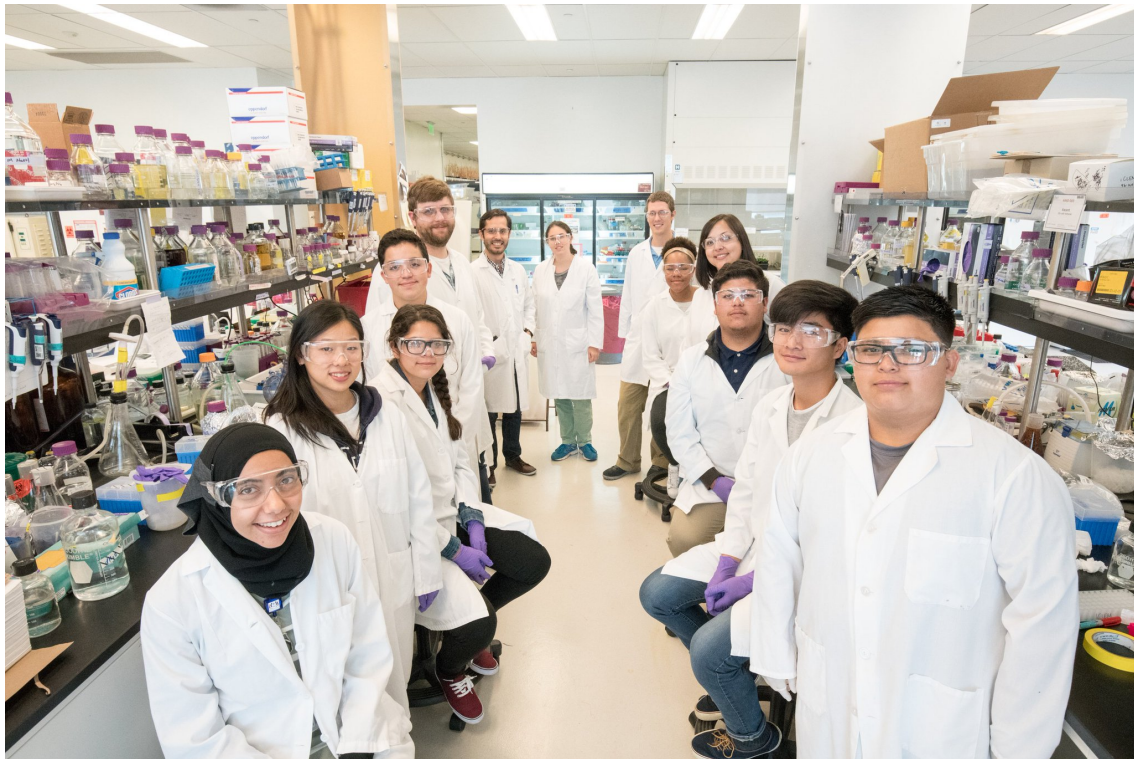
.@miamiuniversity student Cameron Williams intern w/ Tsutakawa @ Tainer lab presents findings
 #MBIB #BioSciNextGen pic.twitter.com/f2Ru8niR8X

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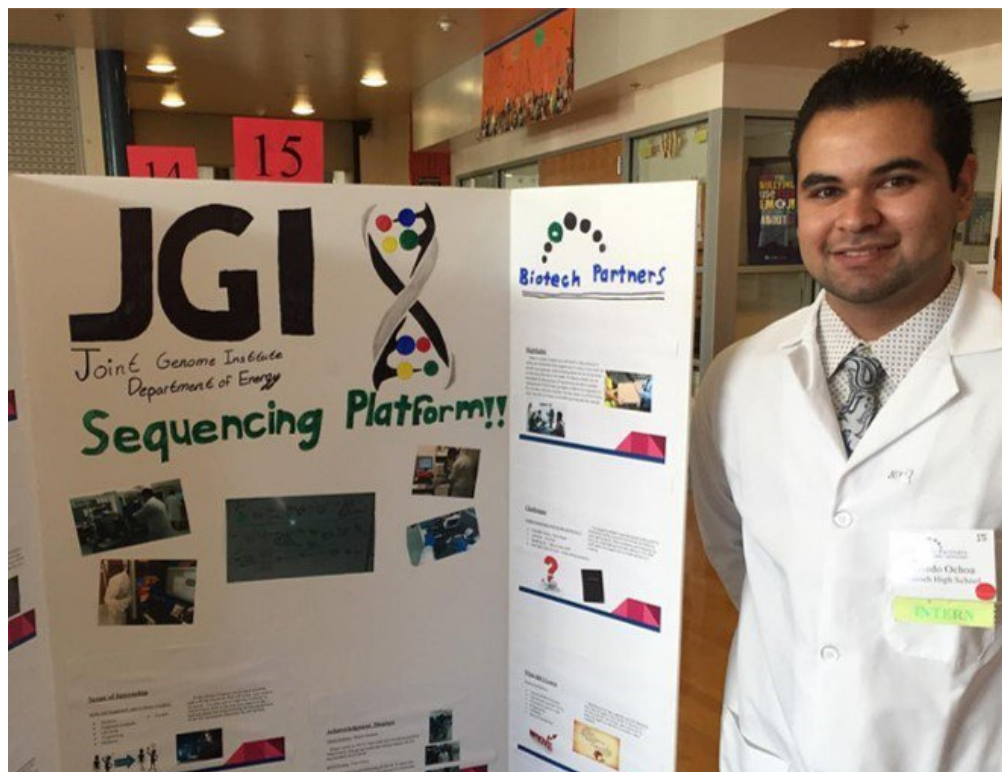
.@LBNLBioSci had 13 HS interns this summer! #STEMEducation @BiotechPartner #BioSciNextGen
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.@BiotechPartner HS intern @doe_jgi Alfredo Ochoa presenting his poster at #2016Bravo
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Investigating the Role of BoA and GrxD in Cellular Lactate Metabolism of *P. stutzeri* and *P. fluorescens*
 Karen Erisse Tolentino¹, Valentine Trotter², Grant Zane³, Judy Wall¹, Gareth Butland²
¹University of California, Los Angeles, ²Lawrence Berkeley National Laboratory, ³University of Missouri

ABSTRACT
 We examined the role of the BoA and GrxD proteins in transcriptional regulation of *Pseudomonas fluorescens* BoA and *Pseudomonas stutzeri* GrxD. The activity of these proteins is thought to influence the expression of lactate dehydrogenase (ldh), impeding the ability of BoA and GrxD mutants to metabolize lactate. We are characterizing BoA and GrxD partner proteins, GrxD, by analyzing the growth of wild type and mutant strains on various carbon sources and performing biochemical assays on protein overexpression and purified from *E. coli*.

BACKGROUND
 Lactate Dehydrogenase Operon
 Lactate → pyruvate → acetyl-CoA → ATP energy
 GrxD → BoA → LdhA
 GrxD → BoA → LdhA → Lactate → Pyruvate → Acetyl-CoA → ATP energy
 The ENIGMA Science Focus Area is dedicated to understanding and characterizing the activities of microbial communities, which play a vital role in regulating the nutrient availability and energy flow of global ecosystems.
 • ENIGMA isolated previously characterized *P. fluorescens* BoA and *P. stutzeri* GrxD from DOE field sites.
 • The BoA protein is evolutionarily conserved across prokaryotes and eukaryotes but was previously described as being a minor factor involved in many cellular pathways.
 • Mutants in both experiments impaired growth when using lactate as the sole carbon source, implying that BoA may play a role in lactate metabolism.

1-Day Growth Curves
 We tracked the growth of wild type and mutant strains on different carbon sources to determine growth.
 Average Growth of OD₆₀₀ vs Time (minutes)
 Average Growth of OD₆₀₀ vs Time (minutes)
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 Average Growth of OD₆₀₀ vs Time (minutes)

RESULTS
Enzymatic Assays
 We isolated and purified BoA and GrxD proteins from *E. coli* and measured their activity using a lactate dehydrogenase assay. We found that BoA and GrxD proteins are active in converting lactate to pyruvate and then to acetyl-CoA.
Heterologous Overexpression in *E. coli*
 We overexpressed BoA and GrxD proteins in *E. coli* and measured their activity using a lactate dehydrogenase assay. We found that BoA and GrxD proteins are active in converting lactate to pyruvate and then to acetyl-CoA.
CONCLUSIONS
 We found that BoA and GrxD proteins are active in converting lactate to pyruvate and then to acetyl-CoA. We also found that BoA and GrxD proteins are involved in lactate metabolism.

RESEARCH QUESTION
 What is the role of BoA and GrxD proteins in lactate metabolism?

ACKNOWLEDGEMENT
 This work was supported by the ENIGMA Science Focus Area, which is funded by the U.S. Department of Energy.

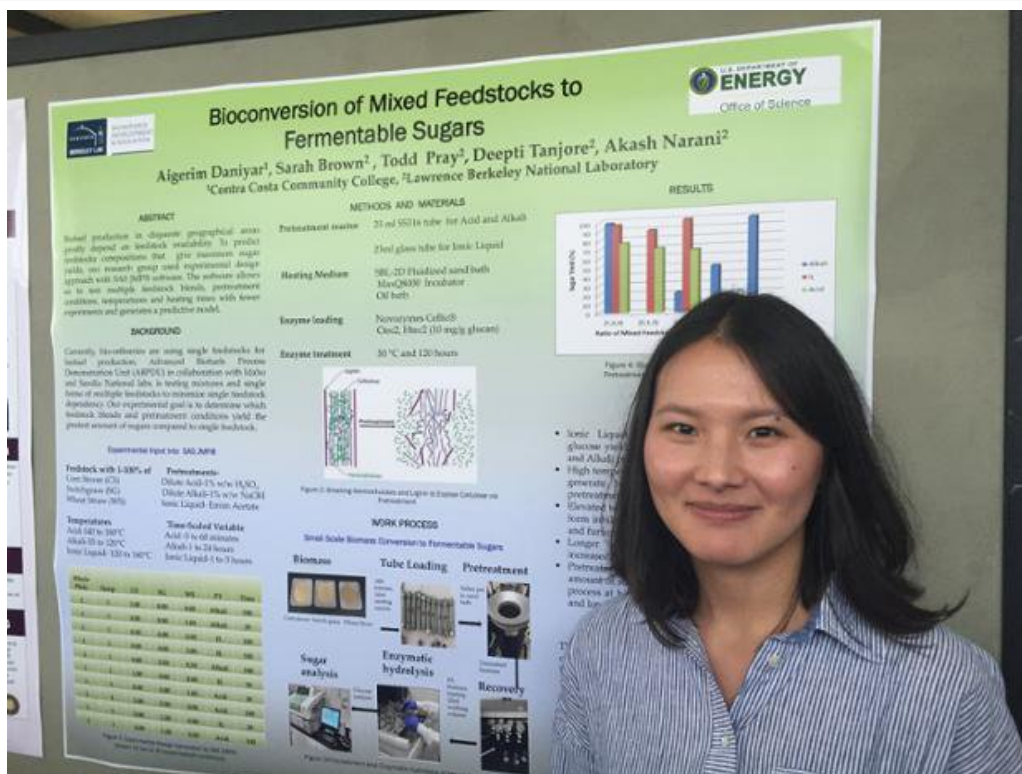
Student Karen Tolentino (r) and Rida Ali conducted [#ProteinResearch](#) this summer! [@UCLA](#) [#EGSB](#) [#BioSciNextGen](#) [pic.twitter.com/X0e25B1Okc](#)

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.@EliDraizen former HS intern @BUBioinfo pre-doc fellow @NCBI @NIAIDBioIT & world traveler!
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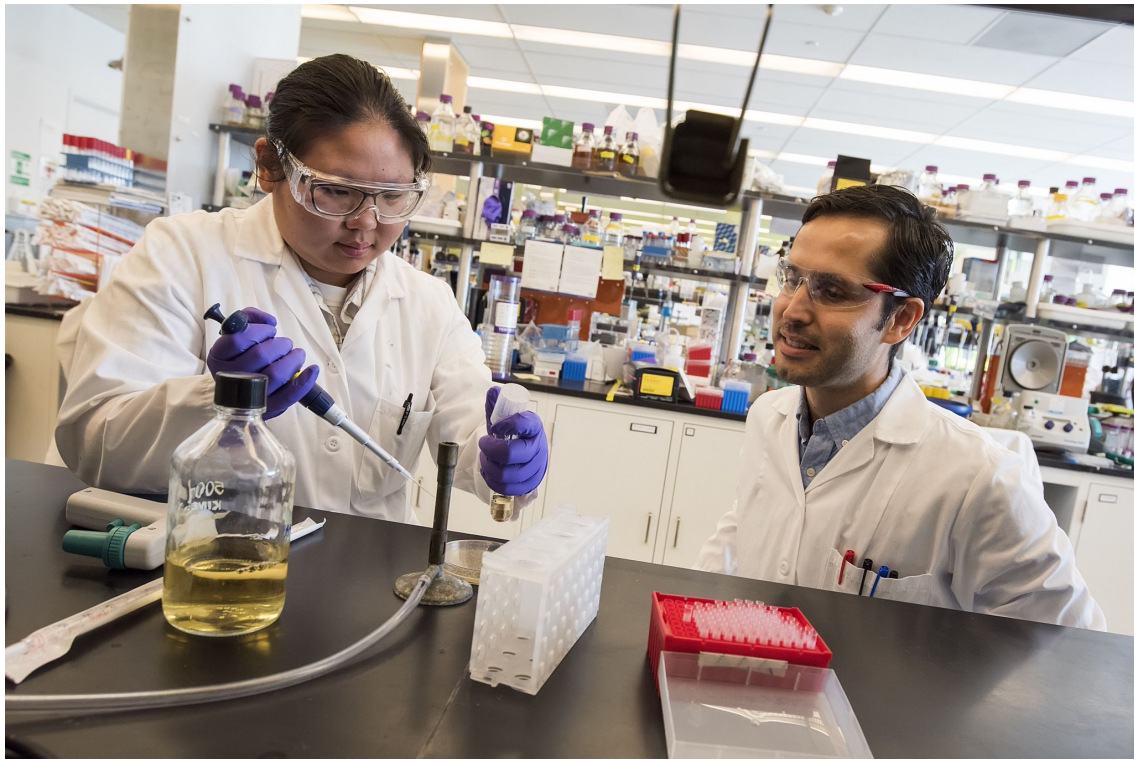
Contra Costa Comm College student Aigerim Daniyar worked on [#feedstocks](#) [#biocoverion](#) at [#ABPDU](#) [#BioSciNextGen](#) [#BSE](#) [pic.twitter.com/VY9ns8GOay](#)

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.@BiotechPartner HS intern @doe_jgi David Romero presenting his poster at #2016Bravo #STEMEducation #BioSciNextGen [pic.twitter.com/C6WTPvcQ90](https://twitter.com/C6WTPvcQ90)

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Improving Plant Biomass Composition by Regulating Cell Wall Glucosaminan Biosynthesis

Vy Ngo¹, Thea Pick², Jenny Mortimer²
¹University of California Berkeley, ²Lawrence Berkeley National Laboratory

ABSTRACT

Glucosaminans are important plant polysaccharides involved in cell wall structure and defense. To date, the biosynthesis of glucosaminans in plants is poorly understood. In this study, we used a combination of genetic and biochemical approaches to identify and characterize glucosaminan biosynthetic pathways in Arabidopsis. We identified a novel glucosaminan biosynthetic pathway and characterized its components. This work will improve our understanding of plant cell wall structure and defense.

BACKGROUND INFO

Glucosaminans are important plant polysaccharides involved in cell wall structure and defense. To date, the biosynthesis of glucosaminans in plants is poorly understood. In this study, we used a combination of genetic and biochemical approaches to identify and characterize glucosaminan biosynthetic pathways in Arabidopsis. We identified a novel glucosaminan biosynthetic pathway and characterized its components. This work will improve our understanding of plant cell wall structure and defense.

RESEARCH QUESTION

What protein factors are involved in regulating plant secondary cell wall glucosaminan biosynthesis?

MUTANT PLANT INFO

13 candidate genes that might modify glucosaminan biosynthesis were disrupted in mutant *A. thaliana* lines.

POLYSACCHARIDE ANALYSIS TECHNIQUES

Human Structure

Polysaccharide Analysis Carbohydrate Gel Electrophoresis (PACE)

High-Pressure Anion Exchange Chromatography - Pulsed Amperometric Detection (HPAEC-PAD)

Trifluoroacetic acid (TFA)

Hydrolysis of specific glycosidic bonds

Hydrolysis of most glycosidic bonds

Polysaccharide composition differences between wild type and mutant plants

The shown structure obtained from A23 is consistent with an example of human structure. The following composition might be similar. The flow chart illustrates individual steps of PACE and HPAEC-PAD. The results of two techniques are compared to determine polysaccharide composition of mutant plants with genes of altered structure compared to wild type plants.

RESULTS

Polysaccharide analysis by carbohydrate gel electrophoresis (PACE) of mutant Arabidopsis lines. The top row shows the results of PACE analysis for mutant lines. The bottom row shows the results of PACE analysis for wild-type plants. The mole percentages of various sugars are shown. The right chart shows the mole percentages of specific sugars (Xylose, Mannose, Galactose, Glucose, and Arabinose) in mutant and wild-type plants.

CONCLUSIONS

PACE

Mutant lines of candidates 5, 6, 11, and 13, show lower intensity detection compared to the WT.

HPAEC-PAD

There were no significant differences in monosaccharide composition between the WT and the candidate lines.

FUTURE WORK

PACE

Characterize remaining candidate glucosaminans.

Immunoblotting

Reproduce biosynthesis.

Transcriptomics

ACKNOWLEDGMENTS

There are no conflicts of interest declared. This work was supported by the Lawrence Berkeley National Laboratory, Office of Science, Office of Biological and Environmental Research, Biological Sciences Division, Plant Sciences Group.

BACKGROUND

The monogalactosyl species *Chlamydomonas reinhardtii* is a model organism that is of interest in the generation of value biofuels and biorefining of carbon. The core histones of *Chlamydomonas* have previously been identified, and reported to be post-translational modifications, such as acetylation, which is hypothesized to be transcription of chromatin, by modification interaction with DNA, and methyl which is generally associated with transcription silencing. Histone protein function to provide structural support to eukaryotic chromatin DNA as well as binding platform for proteins involved in chromatin packaging and expression. Chemical modification of proteins at specific amino acids largely influences DNA interactions, gene expression, chromosomal structure. While C. n. histone H4 post-translational modification (PTM) have been studied using biochemical techniques, there has not been complete characterization of the protein at intact level by mass spectrometry.

METHODS

Cell Culture A cell wall-less strain of *Chlamydomonas reinhardtii* (pH1) was used, and was grown in a minimal medium (DM) with 1% yeast extract (YE) as a nitrogen source. Cells were grown in DM + YE at a cell density of 6.5x10⁶ cells/ml.

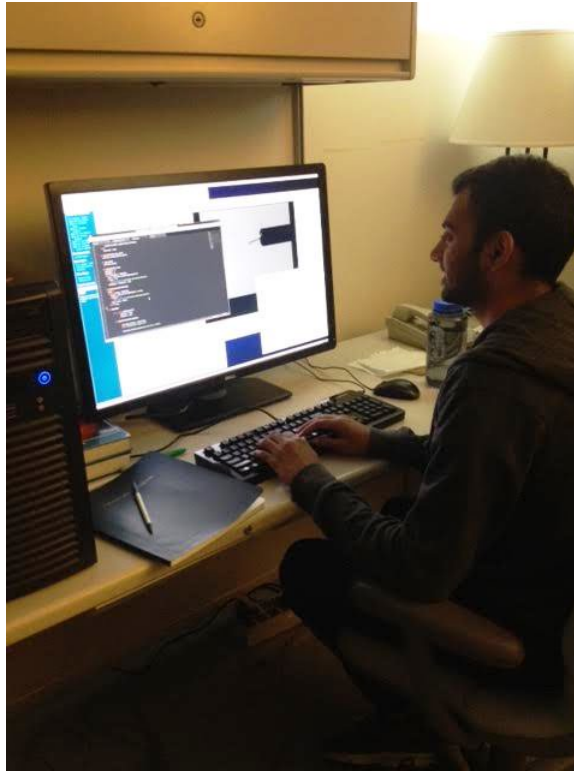
Protein Extraction Protoplasts isolated from cells were homogenized and extracted.

Histone Methylation Assay Nuclear acid (DNA) was isolated, and H4M (methyl C2) was detected.

Protein Blot **Mass Spectrometric Analysis** **LC-MS/MS** were performed on the protein samples. The results were analyzed using the Proteome Discoverer 1.4.0 software.

We are proud of Vy Ngo intern @Jenny_Mortimer1 lab @TheaPick researching
 #PlantSystemsBiology #BioSciNextGen #BSE pic.twitter.com/diMiWfzvrM

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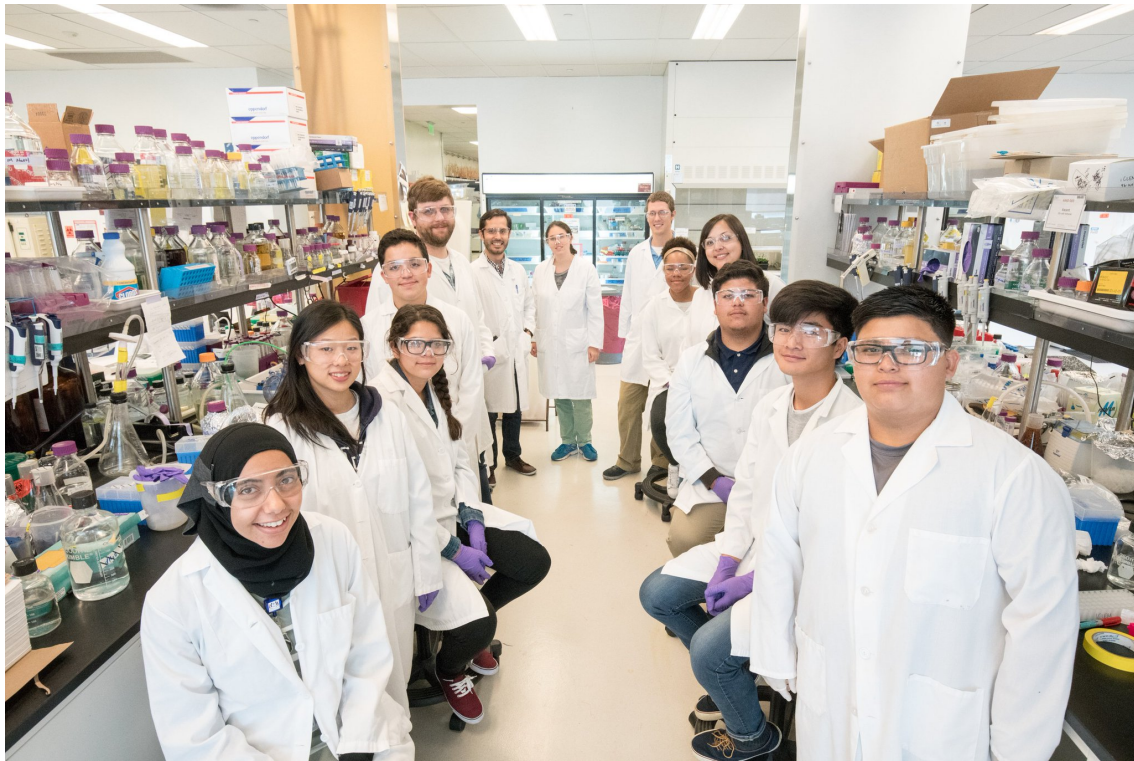
Intern Sayan Roychowdhury writes code to process visual protein crystal images with CYRalston at [#BCSB](#) [#MBIB](#) [#BioSciNextGen](#) [@advlightsource](#) [pic.twitter.com/L61bJtB9rw](#)

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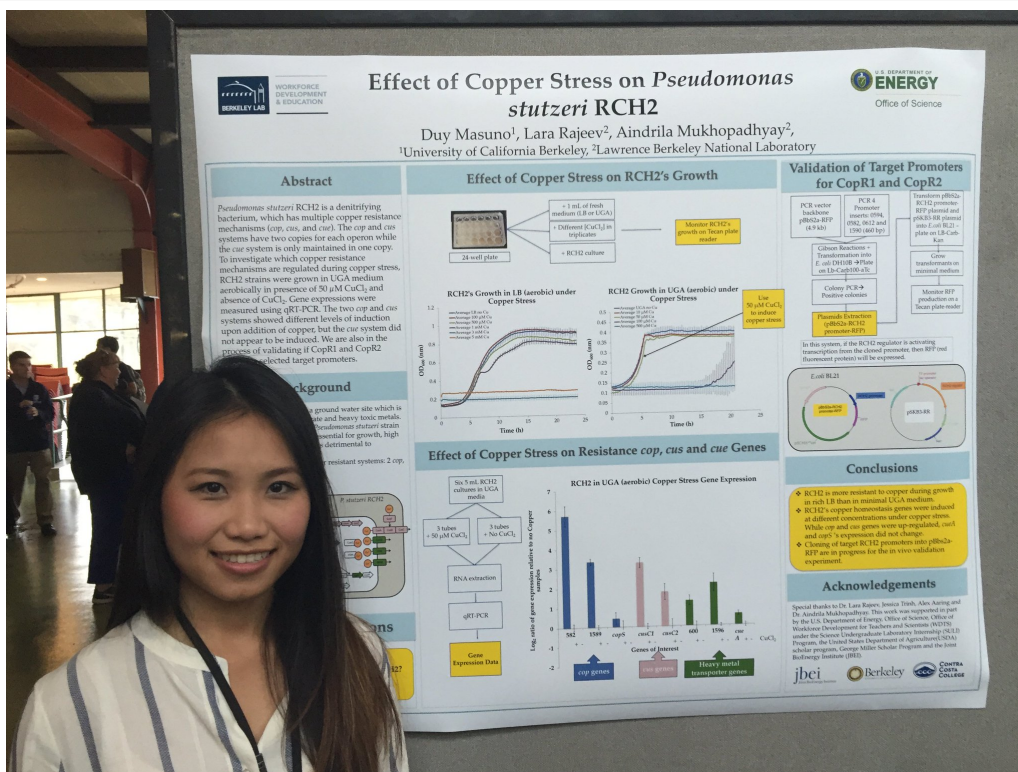
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Duy Masuno @UCBerkeley student engineered #*Pseudomonas stutzeri* @aindrilam lab #BSE #ENIGMA #BioSciNextGen pic.twitter.com/mLjxYE1X6h

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