Agile BioFoundry

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

BIOENERGY TECHNOLOGIES OFFICE

DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Re-view

WBS: 2.5.3.713; ABF DFO with Invaio Engineering Anti-Microbial Peptide (AMP) Production in Fungi and Bioprocess Development

April 5, 2023
Technology Area Session: Agile BioFoundry

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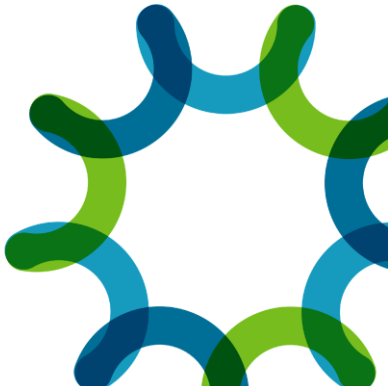


Project Overview

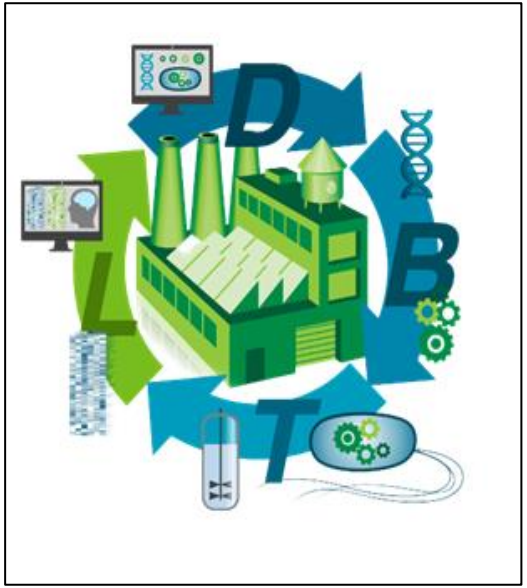
Objective: Develop a high-yield, cost-effective large-scale fermentation bioprocess to produce an Anti-Microbial Peptide (AMP) with Invaio

Mission of Project:

Develop an **innovative biological peptide** at a **scale** that has not previously been achieved and could have **impact on US and global agriculture**. In addition, knowledge and tools generated through this collaboration could be useful in application with other novel peptides, proteins and small molecules.

Approach for project

- R&D collaboration with the team at Invaio on development of expression/secretion host for peptides with antimicrobial action
- Utilize ABF knowledge of fungal genetic engineering for heterologous protein expression
- Utilize ABF targeted proteomics to confirm, quantitate and identify AMP peptide and potentially identify issues with the production of off-target proteins/peptides
- Utilize ABF bioprocess scale-up bioprocess optimization capabilities



Approach for project

Must haves:

- GRAS (Generally Recognized As Safe) host, or a host widely used in the production of food products
- AMP fermentation product is identical to what is found in nature
- Bioprocess can be scaled up for eventual commercial production

Goal 1: Develop a high-yield and cost-efficient bioprocess to produce AMP in a current ABF host

Goal 2: Scale up the process in stirred tank reactors and produce at least 100 grams of AMP at 1-5g/L

Risks

- Expression of an Anti-Microbial Peptide (AMP) in a microbe could prove difficult
- Ambitious scope and schedule with modest funding
- The AMP peptide is difficult to work with (toxicity/solubility/purification)

Mitigations

- Multiple fungal protein-expression hosts with genetic engineering tools were available in the ABF. Early down-select to the strain least affected by the AMP
- Milestones to drive progress with clear roles and responsibilities. Communication with BETO about progress and schedule.
- Monthly communication with Invaio about research directions and their experience working with the AMP: knowledge of its properties and handling procedures

Milestones

Milestone 1: Host Sensitivity to AMP
Complete

- All strains showed some extracellular sensitivity at low levels of AMP but were inhibited at higher AMP levels
- Actual production of the AMP *in situ* is expected to have a different and lesser effect
- **Outcome: *Aspergillus niger* is the best choice.**

Milestone 2: Design & Build of Multiple AMP Expression strains
Complete

- Multiple copies of AMP transgene expression cassettes should integrate into chromosomes randomly and Tag-Purification will be utilized to determine production

Outcome: 8 different transgenic strains for *A.niger* and 1 for *Trichoderma reesei* were built for AMP Expression

Milestone 3: Test Strains for AMP Production and Quantitate
80% Complete

- Many strains were tested for AMP Production
- **Outcome: Two *A.niger* transgenic strains expressed AMP peptide, which was detected and confirmed**

Milestone 4: Culture Optimization and Scale-Up
50% Complete

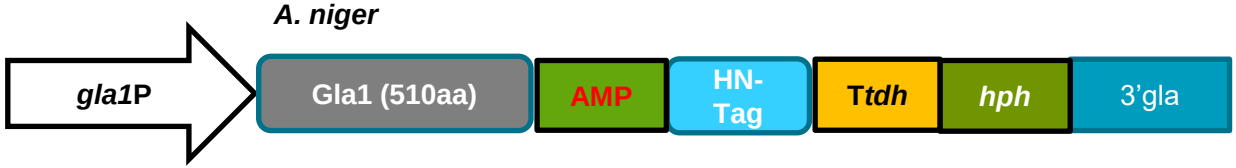
- Buffering, nitrogen source and other media component optimization, and scale-up in progress
- **Outcome: Maintaining neutral pH and adding soy protein improved titer, scaling to 2L further improved titer**

Progress and Outcomes of Milestone 2

Milestone 2: Design and Build of Multiple AMP Expression Strains in *A.niger*

- Multiple AMP transgene expression strains were built
- The two constructs shown here have **expressed AMP**, as verified by Western blots and proteomics

Positive AMP Construct 1 (4159AMP):



Positive AMP Construct 2 (4201AMP):

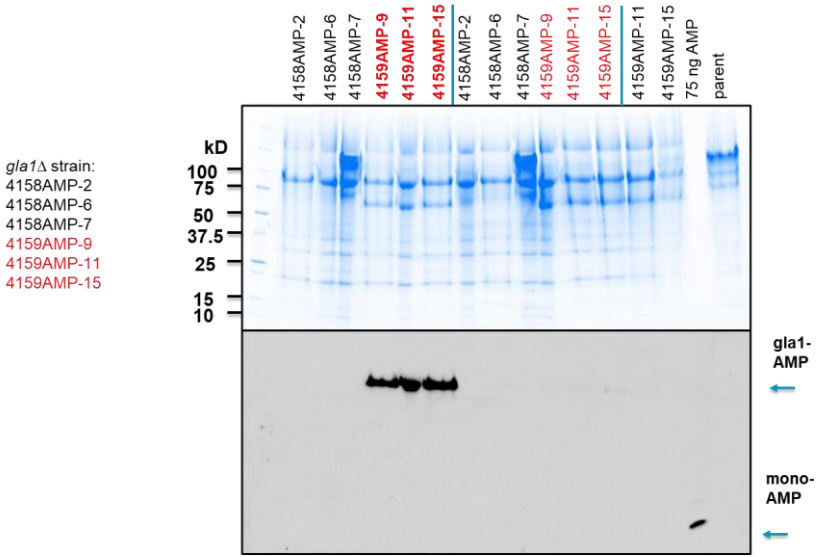
Key

gla1P: native glucoamylase promoter; **gla1**: glucoamylase; **AMP**: Anti-Microbial Peptide; **HN-tag**: $(His\text{-}Asn)_6$ purification tag; **Ttdh**: native terminator; **hph**: hygromycin phosphotransferase; **SP**: secretion signal peptide

Progress and Outcomes of Milestone 3

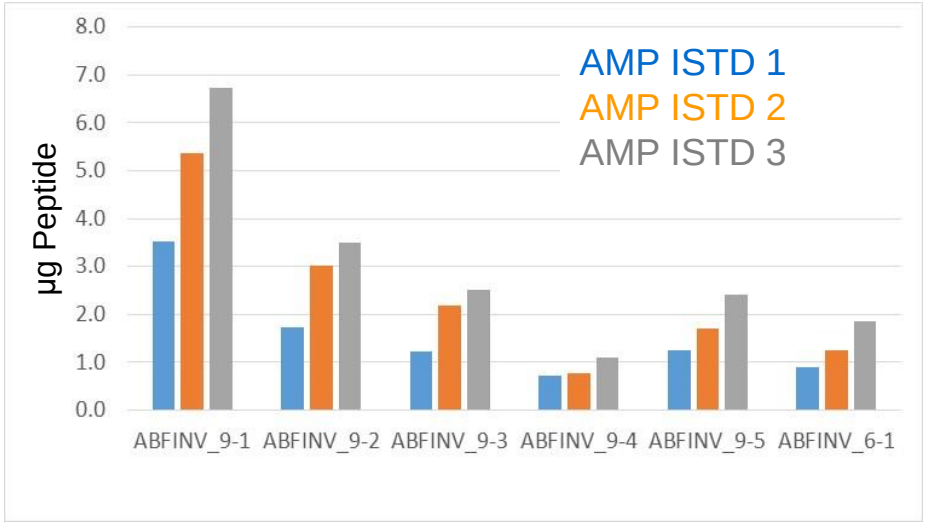
Milestone 3: Test for AMP Production, **AMP Confirmation & Quantitation**

Western Blot for AMP Detection



Targeted Proteomics for AMP Confirmation

^{13}C-labeled peptides as internal standards (ISTD)



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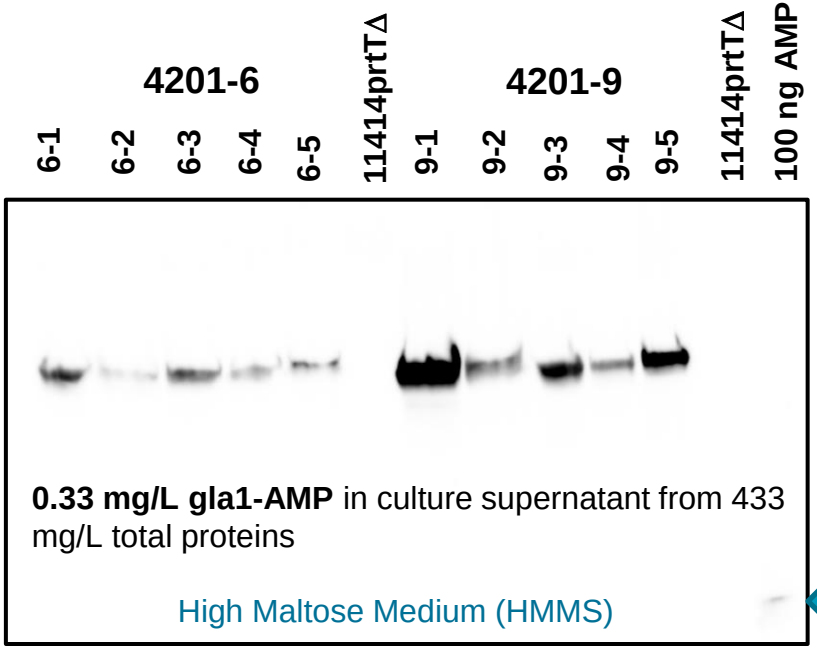
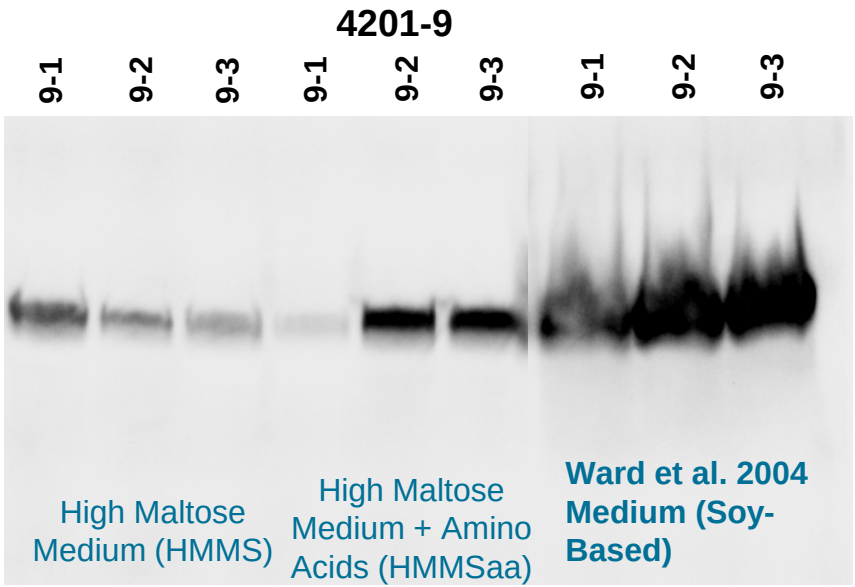
Progress and Outcomes for Milestone 4

Milestone 4: Culture Optimization and Scale-Up

Western Blots: AMP production increased significantly with the first round of media optimization





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Impact

Scientific

- Potential for manuscripts and patent applications to manufacture protein/peptide products, biochemically identical to the natural product
- Genetic engineering of industrial strains of protein-secreting fungi that could be generally applicable to producing functional proteins and peptides

Industrial

- Optimization and scale-up to move toward a price point enabling broad agricultural use
- Working in partnership with Invaio Biosciences to impact US and global agriculture with active biological peptides

Summary

Approach:

- Funding Opportunity project: industrial-ABF partnership leveraging our collective strengths (Invaio, PNNL, NREL) to address a research challenge on heterologous secreted peptide production
- Ambitious milestone-driven project plan with monthly communication to openly discuss research progress; identify and address challenges that arise

Progress:

- Suitable GRAS host selected, AMP peptide produced and confirmed by proteomics, initial media optimization performed
- Scale-up and further media/culture condition optimization is underway

Next Steps

- PNNL will move to our small-scale bioreactors to screen the optimized media and strain, sending product to Invaio for testing biological efficacy and quantitation.

Quad chart overview

Timeline

- Project Start: 4/28/2021
- Project End: 4/27/2023 (NCE)

	FY22 costed	Total Award (FY21-FY23)
DOE Funding	PNNL - $152,000 NREL – $0	$ 410,000 PNNL - $380,000 NREL – $30,000
Cost Share	$0	Invaio - $102,500

Project Partners

ABF Labs: PNNL, NREL

Industry Partner: Invaio BioSciences

Project Goal

Develop a high-yield, cost-effective large-scale fermentation bioprocess to produce an Anti-Microbial Peptide with Invaio and partners

End of Project Milestone

Conduct 100-L bioreactor cultivations to produce at least 100 grams of AMP at 1-5g/L to deliver to Invaio for quantitation and efficacy testing.

Funding Mechanism

FY21 ABF Directed Funding Opportunity

TRL at Project Start: 3

TRL at Project End: 4

This presentation does not contain any proprietary, confidential, or otherwise restricted information

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Acknowledgments

DOE Bioenergy Technology Manager: Gayle Bentley

ABF Project Contributors:

- PNNL: Beth Hofstad (PM), Ziyu Dai (Genetic Engineering lead), Ana Robles,
- NREL: Davinia Salvachua

Industrial Partner

Invaio Bhanu Harrison, Behnam Nazari, Pip Reeder and Connie Caron