OPTIMIZATION OF CULTURE CONDITIONS FOR SCALE-UP PRODUCTION OF THE PCSK9 AXIS-TARGETING SPIRO-HETEROCYCLIC-γ-LACTAM PSEUROTIN A

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Introduction

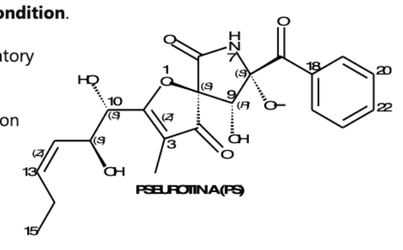
Natural products (NPs) remain the top innovative resources for the discovery of bioactive hits, leads, and drug entities. NPs occupy a unique chemical space, apart from synthetic small molecules and biologics, due to their unprecedented diverse regioselective structural frameworks and novel scaffolds. They are also renowned for their biological relevancy, which stems from their interactions with the biosynthetic machinery of the producing organisms and their role as possible chemical defense and survival tools in the producing organism. In this sense, we have recently identified pseurotin A (PS) as a novel and first-in-class small molecule inhibitor of the human proprotein convertase subtilisin/kexin type 9 (PCSK9) for the control of the aggressive and metastatic castration-recurrent prostate cancer (mCRPC).

Our preclinical studies have shown the significant inhibition of locoregional and distant tumor recurrences after surgical excision of primary tumors in mice xenografted with mCRPC cells upon daily oral administration of PS at 10 mg/kg. Future studies, including safety and toxicological evaluation of PS, are highly warranted for near future PS development as an mCRPC recurrence suppressor. These studies necessitate the fermentation scale-up production of PS to fulfill the high experimental demand. We purified PS from the culture broth of *Aspergillus fumigatus* (ASP) at a yield of 10 mg/L after 28 days of incubation, which is the maximum reported yield in the literature.

Our current goal is to optimize culture conditions for PS large-scale production. Various fermentation parameters were evaluated, including period, degree of aeration, shaking speed, temperature, media composition, and diverse elicitation. Furthermore, we have recently acquired a *Penicillium* (PEN) species that produces PS at a comparable yield. Fermentation of *Penicillium* species is preferable over ASP from the biosafety perspective.

Optimization efforts successfully led to a significant improvement of PS mass production up to 60 mg/L culture broth over seven days of incubation, which is 6-fold higher than the best-reported fermentation condition.

The current achievement is satisfactory for proper laboratory supply of PS at a double-digit g-scale; however, further optimizations are required for PS cost-effective production at advanced stages before clinical trials.



Methods

To maximize PS production by ASP and/or PEN fermentations, different liquid media, including YMG (Yeast extract, Malt extract, and Glucose), Soybean media, rice water, and compound medium-α (20 g glucose, 5 g each of peptone, yeast extract, Na_2PO_4, and NaCl/L) tested. Each fungus spores were taken from fresh PDA solid agar plates aseptically and transferred to the sterile liquid media. The inoculated flasks were sealed and isolated and were kept in an incubator for seven days. Each day one flask was collected and analyzed by HPLC to determine the concentration of PS in the media. For this purpose, 40 mL ethyl acetate was added to a 40 mL collected sample media; after shaking the mixture well in separating funnels, the upper organic layer was collected and evaporated at reduced pressure and temperature to reach a 1 mL high concentrated sample. The final sample was centrifuged and then analyzed by HPLC (isocratic elution, 1 mL/min, λ254 nm, mobile phase: Acetonitrile + Water, Cosmosil C-18RP 30mm x 4.6mm) to determine the concentration of PS using a previously established calibration curve.

Different environmental conditions such as varied pH 3-9, and temperature 25-52 °C ranges have been examined. Finally, ASP and PEN produced the highest PS yield when inoculated in compound medium-α.

The next PS production optimization step was cocultures. In this case, ASP and PEN spores were inoculated into two small flasks separately and kept in the incubator at 40 °C for two days. On day-3, the contents of small flasks, mycelium and media, were transformed into a larger flask containing 500 mL compound medium-α. After seven fermentation days at controlled temperature incubator, the sampling process and HPLC analyses were performed.

Methods

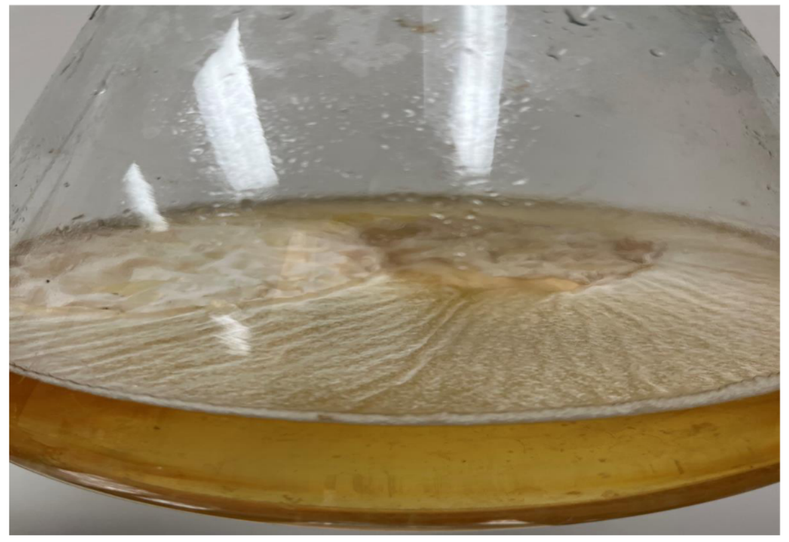


Figure 1. PEN + ASP Coculture fermentation at controlled temperature on Day 1.

Figure 2. Comparison of the PEN + ASP Coculture fermentation (left flask) versus PEN single culture fermentation on day 3 (right flask) at controlled temperature.

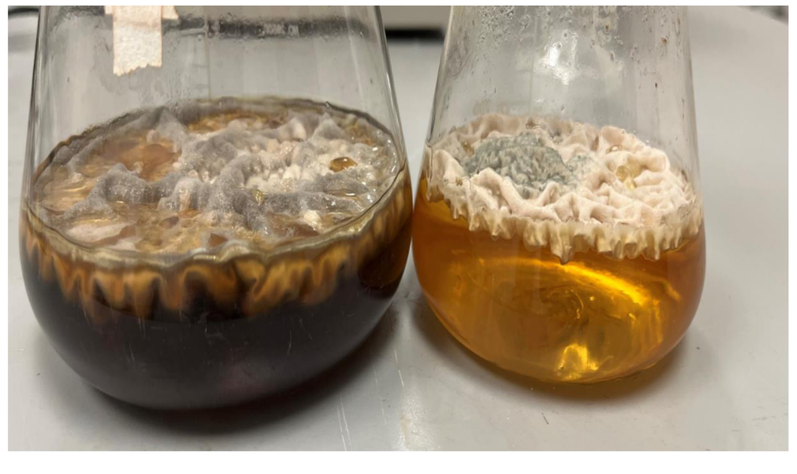


Figure 3. Comparison of the PEN + ASP Coculture fermentation (left flask) versus PEN single culture fermentation on day 7 (right flask) at controlled temperature.

Results

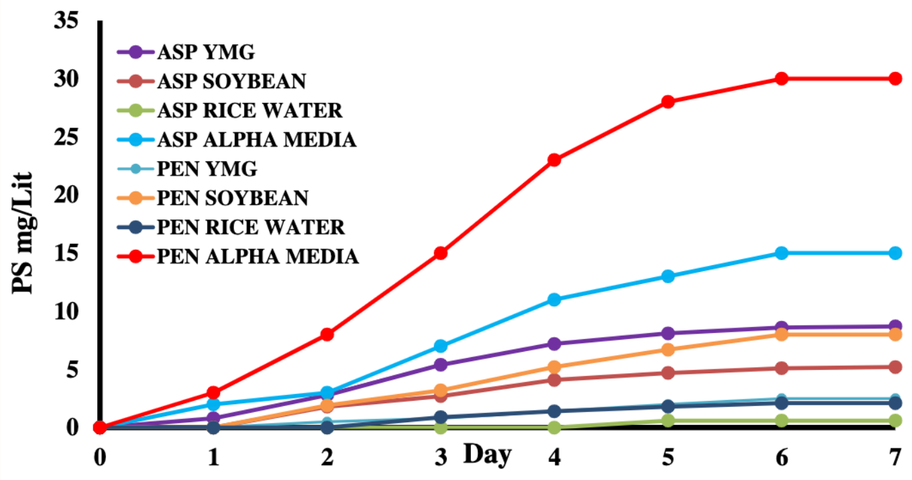


Figure 4. Effect of various fermentation liquid media on PS production.

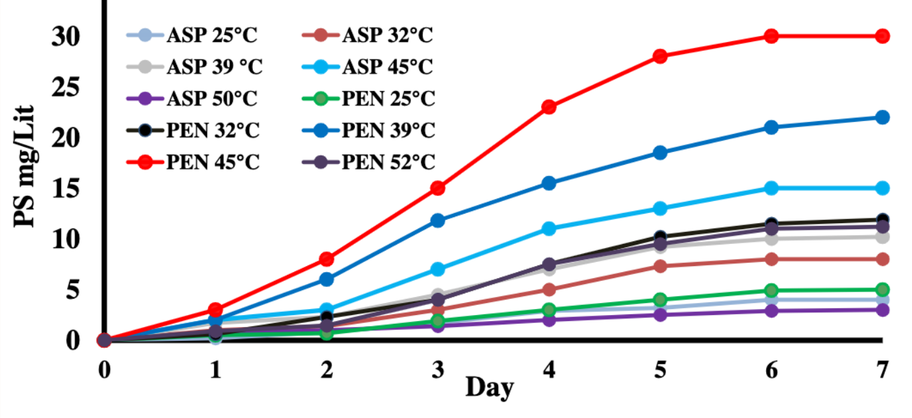


Figure 5. Effect of controlled temperature on PS production by ASP and PEN fermentations.

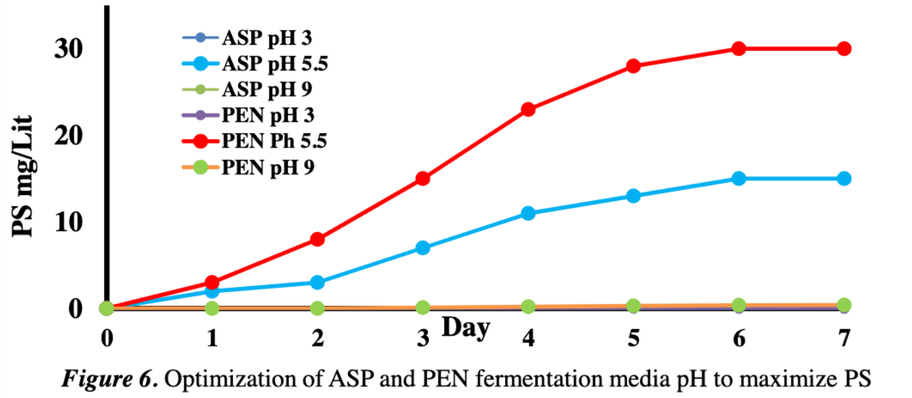


Figure 6. Optimization of ASP and PEN fermentation media pH to maximize PS production.

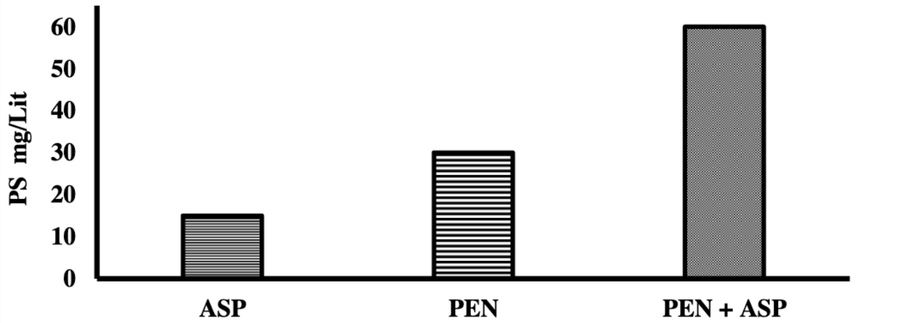


Figure 7. Comparison of ASP and PEN single and co-culture fermentation on PS yield.

Results

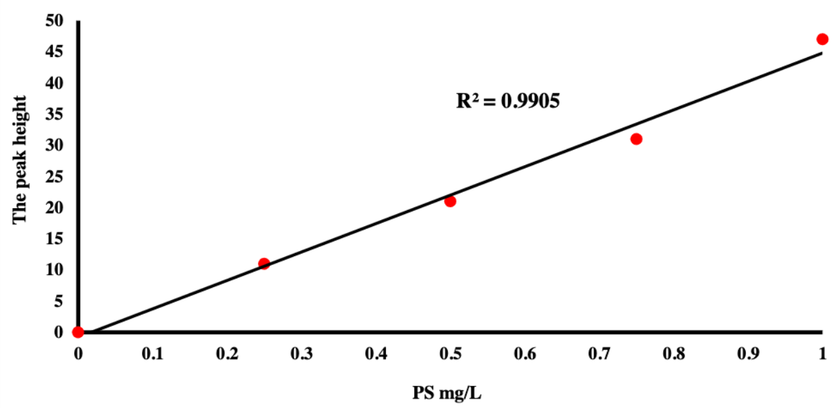


Figure 8. The peak height/ Conc. Relation graph for PS based on HPLC chromatogram

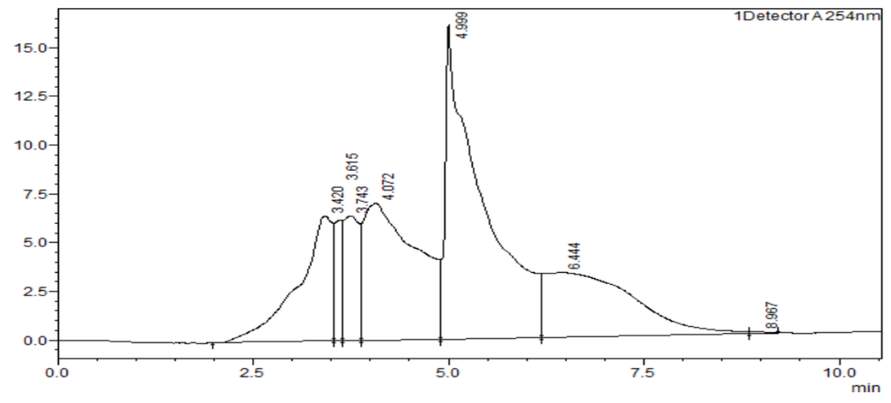


Figure 9. HPLC chromatogram for ASP 7-day fermentation (PS retention time 4.99 min).

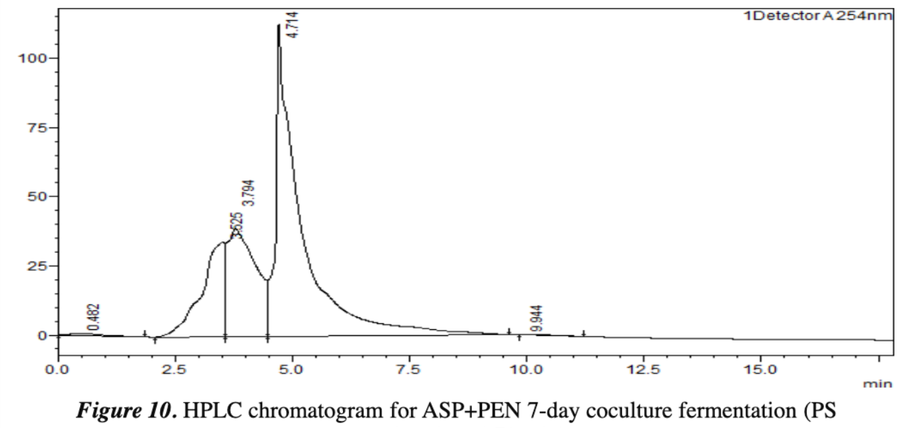


Figure 10. HPLC chromatogram for ASP+PEN 7-day coculture fermentation (PS retention time 4.71 min).

Conclusions

Optimization of culture conditions of ASP and PEN afforded 6-fold increase for PS fermentation production by ASP and PEN mixed cocultures at 40 °C in compound medium-α for 7-14 days. Future directions will aim at precursor-directed feeding studies and chemical and physical elicitation to enhance the PEN/ASP coculture fermentations productivity.

Acknowledgements

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