Geraniol production via novel protein expression tools in *Methanococcus maripaludis*

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**Abstract**

Geraniol is an intriguing 10 carbon compound with diverse applications including use as an agent for cancer prevention, fragrance, insect repellent, proposed biofuel etc. We explored and engineered a novel gene expression tool (Bba_K1138000) for Methanococcus with the capability of expressing geraniol synthase from Ocimum basilicum (Bba_K1138000). We report the biosynthesis of geraniol at over 0.2% of DW by transforming the vector into Methanococcus thereby expanding its native isoprenoid pathway. Furthermore we engineered new vectors (Bba_K1138001 & Bba_K1138002) with the potential capability of regulating and quantifying the expression of desired proteins via red fluorescence. This work demonstrates the use of Methanococcus as a cell factory for chemical production and highlights synthetic biology advancement by engineering new systems over traditional biological systems such as E. coli.

**Methanococcus maripaludis**

- Methanogenic archaea
- Obligate Anaerobe
- Mesophilic
- Autotrophic
- Creates isoprenoid lipids
- Doubling time of 2.3hrs

*Geraniol*:

- An acyclic monoterpen- alcohol
- Has been shown to inhibit the growth of pancreatic, prostate and colon cancers
- Has a higher energy density than ethanol and a heat of combustion similar to diesel
- Can be used as a botanical insect repellent
- Has many uses in fragrances and flavorings

**Design of Novel Protein Expression/ Quantification Tool in Archaea**

These are the two tools that we created that will allow you to regulate and quantify gene expression. One has an RBS and the other one does not. The one with the RBS can be used as a control and the one without can be used to insert your gene of interest to quantify expression. Both tools have EcoRI, NotI, XbaI, and NsiI restriction sites for insertion of a gene.

This graph shows the fluorescence of Bba_k1138002 in comparison to the Methanococcus wild type strain s0001. We report the fluorescence in our BioBrick with roughly as a 3 fold increase. Note that the wild type gives a small fluorescence reading due to the fact that Methanococcus is naturally bio-luminescent.

**Geraniol Production**

The pAW42-GS (Bba_k1138000) vector was transformed into Methanococcus, thereby extending its natural isoprenoid pathway. The introduction of the Geraniol Synthase gene in Methanococcus will allow it the catalysis of the reaction from Geranyl pyrophosphate into Geraniol.

The extraction of Geraniol from Methanococcus cultures was accomplished using hexanes as an organic solvent. The extractions were concentrated via evaporation under a stream of N₂ gas, and the samples were evaluated by GC/MS.

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**Future Work**

- Manipulation of gene expression using the tools that we created
- Improve efficiency for geraniol production
- Further extension of pathway for geraniol acetate production

**Conclusion**

- We have verified geraniol production as over 0.2% of DW in Methanococcus using the pAW42-GS (Bba_k1138000) model.
- We have shown that our tool fluoresces around 3 times more than normal Methanococcus making it a valuable tool for quantifying and regulating gene expression. We hope that future researchers and iGEM teams will use this tool in the future to use methanogens as an organism for synthetic biology.

**Outreach**

We presented our research to an engineering seminar class to introduce students to synthetic biology research and educate them of opportunities at the undergraduate/graduate program levels at UGA. By engaging students from various departments, we are fostering an inter-disciplinary approach to research.

We taught two classes at Clark Middle School that gave the students an introduction to bacteria. We taught them about the role of bacteria in the environment and how they impact our daily lives. We even let the students swab various items in the classroom and plate their own bacteria on agar plates.

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**References**


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