Incilius alvarius cell-based synthesis of 5-MeO-DMT

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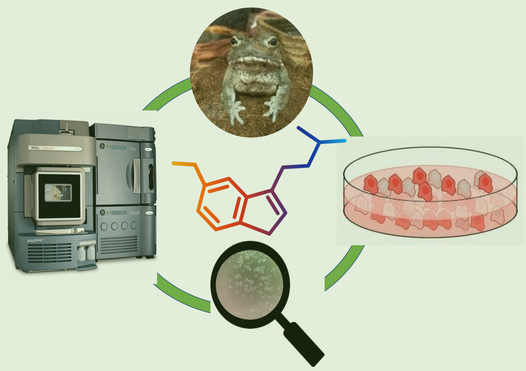
BACKGROUND

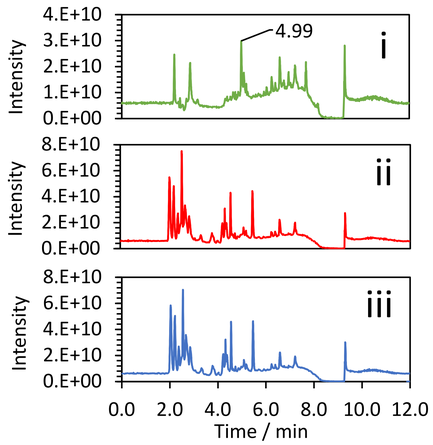
- There is growing interest in the therapeutic potential of 5-MeO-DMT (5-methoxy-N,N-dimethyltryptamine) [1].
- An optimized chemical synthetic pathway exists to produce 5-MeO-DMT [2].
- The parotoid gland secretions of *Incilius alvarius* (also known as the Colorado River or Sonoran Desert toad) are a source of a range of psychoactive tryptamines in addition to 5-MeO-DMT and bufotenine [3][4].
- These molecules include bufagenins, bufotoxins, and indole alkylamines, which may have individual clinical utility or act as "*entourage molecules*" to enhance the activity of 5-MeO-DMT.
- *Incilius alvarius* is currently under severe ecological pressure due to consumer demand for natural 5-MeO-DMT and habitat loss [5].
- We aimed to establish a cell line from tissue collected by wedge biopsy from the parotoid gland of *Incilius alvarius* and demonstrate cell-based synthesis of 5-MeO-DMT.

METHODS

- We anesthetized 2 *Incilius alvarius* toads and conducted a wedge biopsy under aseptic conditions in conformity with current best practice in the care of laboratory animals.
- Glandular tissue was identified in the explants, prepared, dissociated, and seeded using standard amphibian cell culture protocols in basal media supplemented with 10% FBS in 24-well cell culture plates.
- Cell immortalization was achieved using the SV40 T Antigen Cell Immortalization kit (Alstem, Richmond, CA, USA).
- Cell culture was undertaken in an incubator at 25℃ and 5% CO_2.
- Upon confluence, the cells were disassociated and passaged with media exchange every 3-4 days.
- The culture was maintained for 45 days.
- Prior to analysis cell media was filtered and purified with an Amicon Ultra-0.5 Centrifugal Filter Unit (cut-off 1 x 10^3 Da, Millipore Sigma, Burlington, MA, USA).
- Media was analyzed after 36 days for the presence of 5-MeO-DMT using a Xevo TQ-S LC-MS/MS system (Waters Corporation, Milford MA, USA) [6].



LC-MS/MS CHROMATOGRAPHY

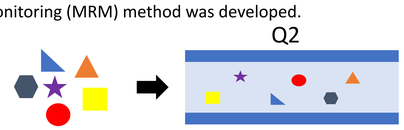


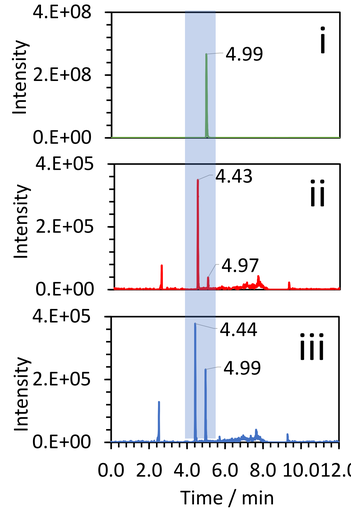
i. Parotoid secretion
ii. Parotoid cell-free culture media
iii. Blank media + 1 ng/mL 5-MeO-DMT

1. Full Scan MS - total ion chromatogram (TIC)

- These chromatograms depict **(i)** the natural parotoid secretion of *Incilius alvarius*, **(ii)** cell-free culture media of the immortalized explanted cells after 36 days, and **(iii)** blank media spiked with 1 ng/mL 5-MeO-DMT.
- 5-MeO-DMT was detected at t = 4.99 min in the parotoid secretion.
- Due to the high volume in the parotoid cell-free culture media and the low concentration of 5-MeO-DMT produced by the cells, peaks are hidden in the TIC from the parotoid cell-free media.
- For detection of 5-MeO-DMT in the parotoid cell-free culture medium, a multiple reaction monitoring (MRM) method was developed.

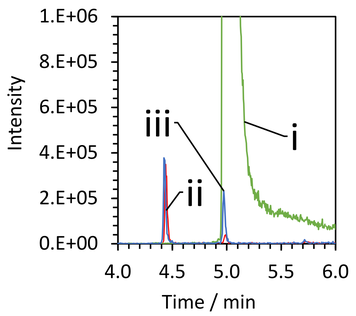
LS MS/MS Mode: Full Scan

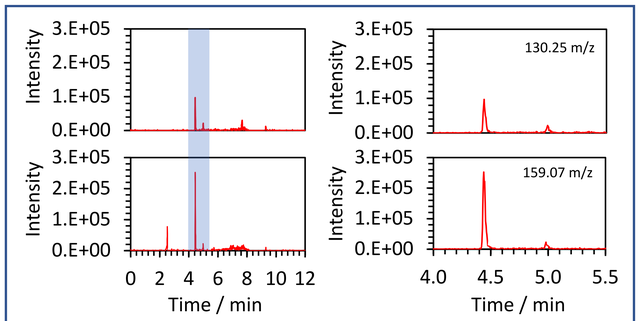




2. MRM TIC

- The parotoid cell-free culture media showed a similar retention time to the blank media spiked with 1 ng/mL standard.
- In the parotoid cell-free culture media, a peak can be observed at t = 4.97 min.
- This to our knowledge is the first time that 5-MeO-DMT is detected in parotoid cell culture media.
- This peak was further confirmed using detected ion fragments.

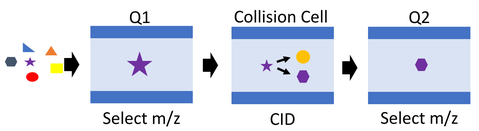




3. FRAGMENTED ION TIC

- TIC of ions of the 5-MEO-DMT, quantification scan - 130.25 m/z (top), and confirmation scan - 159.07 m/z (bottom).
- On the left, the chromatograms of the ions used to quantify and confirm the presence of 5-MeO-DMT are shown.
- On the right, a magnified version of the peaks detected in the parotoid cell-free culture media is shown.

LS MS/MS Mode: MRM



RESULTS & DISCUSSION

- Both toads recovered after the biopsy and are alive and well.
- Dried media from the parotoid cell culture had a light tan appearance that was similar to the known appearance of dried *Incilius alvarius* parotoid secretion.
- MRM LC-MS/MS validated the presence of 5-MeO-DMT in the parotoid cell culture media.
- MS/MS analysis (130.25 m/z and 159.07 m/z) showed conformity in structure with 5-MeO-DMT (observed neutral mass: 218.234 g/mol).
- To our knowledge, this is the first report of the successful production of 5-MeO-DMT from an *Incilius alvarius* parotoid cell line.
- These findings constitute preliminary evidence of the feasibility of cell-based 5-MeO-DMT production as a potential scalable source of research and clinical material.
- The potential availability of "*natural*" 5-MeO-DMT produced through cellular agriculture, as opposed to the cruel and destructive practice of "milking" *Incilius alvarius*, also supports efforts to ensure the protection of our planet's entheogen heritage.

LIMITATIONS

- In the light of the small quantities produced and the limitations of the detection methods, it was not possible to determine concentration or yield.
- Further research is required to optimize the cell lines for upscaled production.

REFERENCES

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DISCLOSURES

LL, ER, GO were employees of BYAS at the time of this research. BL has nothing to disclose. All authors met ICMJE authorship criteria. Neither honoraria nor payments were made for authorship.