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The "After-Life" Experiment - Mass Spectrometry Imaging Used to Demonstrate the "Cycles of Life“

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Overview

➢MS Imaging featured in 2011 joint BBC/Discovery Channel documentary "After life - The Strange Science of Decay".

➢Imaging Distribution of 15N labeled NO_3^- used to show uptake of species from dead organisms by living ones.

Introduction

In May 2011 the MS Imaging group at Sheffield Hallam University was approached by the BBC to take part in a project to demonstrate the science of decay. Set up in Edinburgh Zoo in summer 2011 a fully equipped kitchen and garden together with all the detritus from a family barbeque was sealed in a glass box. The programme a joint venture between the BBC and the Discovery Channel followed the events as maggots, moulds, bacteria, flies and mushrooms transformed the contents beyond all recognition. As part of the experiment the programme makers wished to demonstrate that decay and decomposition was only part of the story with atoms and molecules from dead plants and animals being incorporated into new life**.**

Methods

Two groups of radish (Raphanus sativus) were grown hydroponically in an artificially controlled environment. The control group utilized a nutrient system containing standard KNO_3, the second group (N15) replaced all of the KNO_3 with15N KNO_3 (98% labelled) as the only source of nitrogen. Plants were cropped, homogenised (Figure 1b) and left to ferment in water for two weeks to create a "tea". The "tea" was used a source of nitrogen for a second hydroponics experiment (2^{nd}G) where radish (Raphanus sativus) were grown in an artificially controlled environment. After five weeks of growth radish plants were harvested. Samples of leaves, bulbs, and stems from each group (Control, N15 and RfR) were cryosectioned and sections imaged by positive ion MALDI imaging and LDI imaging mass spectrometry.

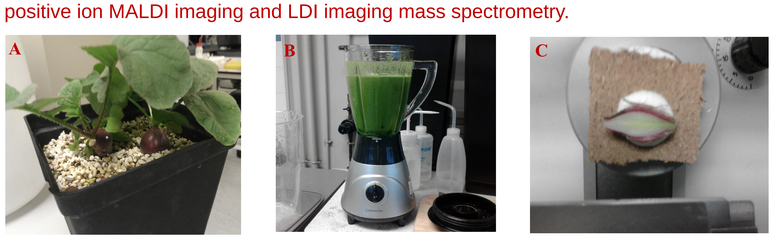

Figure 1 (a) Radish was grown hydroponically using a 15N KNO_3 as the only source of nitrogen. (b)Homogenised radish before fermentation. (c) Cryosection of radish bulb.

Results and Discussion

The original intention of the experiment was to image the distribution of 15N labelled arginine (m/z 185)to demonstrate the incorporation of nitrogen from the now dead radish plants into protein synthesis for the new living plants, however this proved difficult owing to the complex overlapping isotope peaks in this region of the positive ion mass spectrum. However the B-complex vitamin Choline, ($C_5H_{14}NO$), m/z104.1, was found to have incorporated the labelled 15N, producing a m/z 105.1. These data are presented as Figure 3, 4,5 and 6. Further identification of Choline is supported by the MALDI MS/MS data presented in figures 7 and 8.

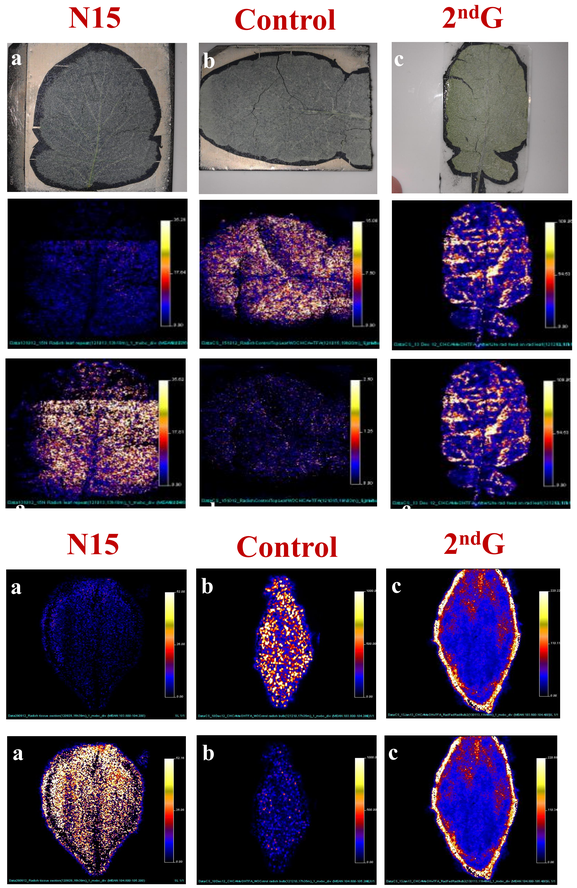

Figure 2: Radish leaves:– Coated with(α-CHCA)(a) N15 group, (b) Control group, (c) 2^{nd}G group grown with radish tea.

Figure 3: Positive Ion MALDI-MSI of the Distribution of Choline (**m/z 104.1**) in Radish Leaves:(a) N15 group, (b) Control group, (c) 2^{nd}G group grown with radish tea.

m/z 104.1

Figure 4: Positive Ion MALDI-MSI of the Distribution of 15N labelled Choline (**m/z 105.1**) in Radish Leaves:-(a) N15 group, (b) Control group, (c) 2^{nd}G group grown with radish tea.

m/z 105.1

Figure 5: Positive Ion MALDI-MSI of the Distribution of Choline (**m/z 104.1**) in Radish Bulb:(a) N15 group, (b) Control group, (c) 2^{nd}G group grown with radish tea.

m/z 104.1

Figure 6: Positive Ion MALDI-MSI of the Distribution of 15N labelled Choline (**m/z 105.1**) in Radish Bulbs:-(a) N15 group, (b) Control group, (c) 2^{nd}G group grown with radish tea.

m/z 105.1

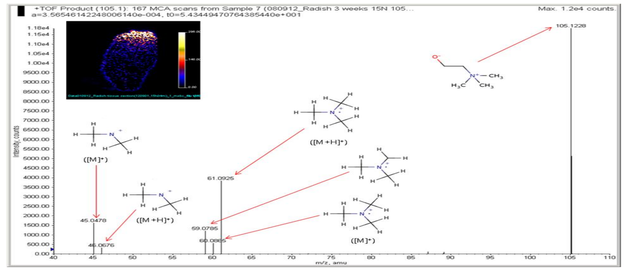

Figure 7: MALDI MS/MS spectrum showing product ions, derived from the precursor ion of ^{15}N labeled choline at m/z 105.1228.

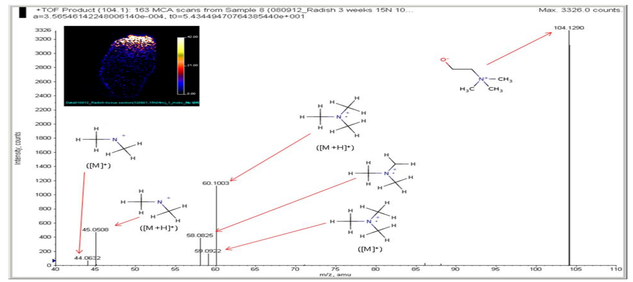

Figure 8: MALDI MS/MS spectrum showing product ions, derived from the precursor ion of choline at m/z 104.1290.

Figure 9: Scene from the Documentary where Prof Clench discusses the MALDI-MSI results with the presenter Dr George McGavin**.**

Conclusions

Mass spectrometry imaging has been used to demonstrate the uptake of labeled nitrogen species from composted radish leaves into growing radish plants. The "cycle" of life was successfully demonstrated. The programme was viewed by just under 1 million people on its first broadcast on BBC4 on 6th December 2011and has been repeated 4 times subsequently.

Reference

The full programme can be viewed at : http://www.youtube.com/watch?v=sNAxrpzc6ws