KS-1

Novel Fertilizer-Drawn Forward Osmosis to Recover Nutrients from Source-Separated Wastewater and Urine for Urban Farming

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Re-thinking our approach to dealing with waste is one of the major challenges in achieving a more sustainable society. However, it could also generate numerous opportunities. Specifically, in the context of wastewater, nutrients, energy and water could be mined from it. Because of its exceptionally high nitrogen (N) and phosphorous (P) concentration, human urine is particularly suitable to be processed for fertilizer production. In the presentation, forward osmosis (FO) was employed to mine the P and N from human urine (Figure 1). Two Mg^{2+}-fertilisers, i.e. $MgSO_4$ and $Mg(NO_3)_2$ were selected as draw solution (DS) to dewater synthetic non-hydrolysed urine. In this process, the Mg^{2+} reverse salt flux (RSF) were used to recover P as struvite. Simultaneously, the urea was recovered in the DS as it is poorly rejected by the FO membrane. The results showed that, after 60% urine concentration, about 40% of the P and 50% of the N were recovered. XRD and SEM – EDX analysis confirmed that P was precipitated as mineral struvite. If successfully tested on real urine, this process could be applied to treat the urine collected in urban areas e.g., high-rise building. After the filtration, the solid struvite could be sold for inland applications whereas the diluted fertiliser used for direct fertigation of green walls, parks or for urban farming. Finally, reduction in the load of N, P to the downstream wastewater treatment plant would also ensure a more sustainable urban water cycle.

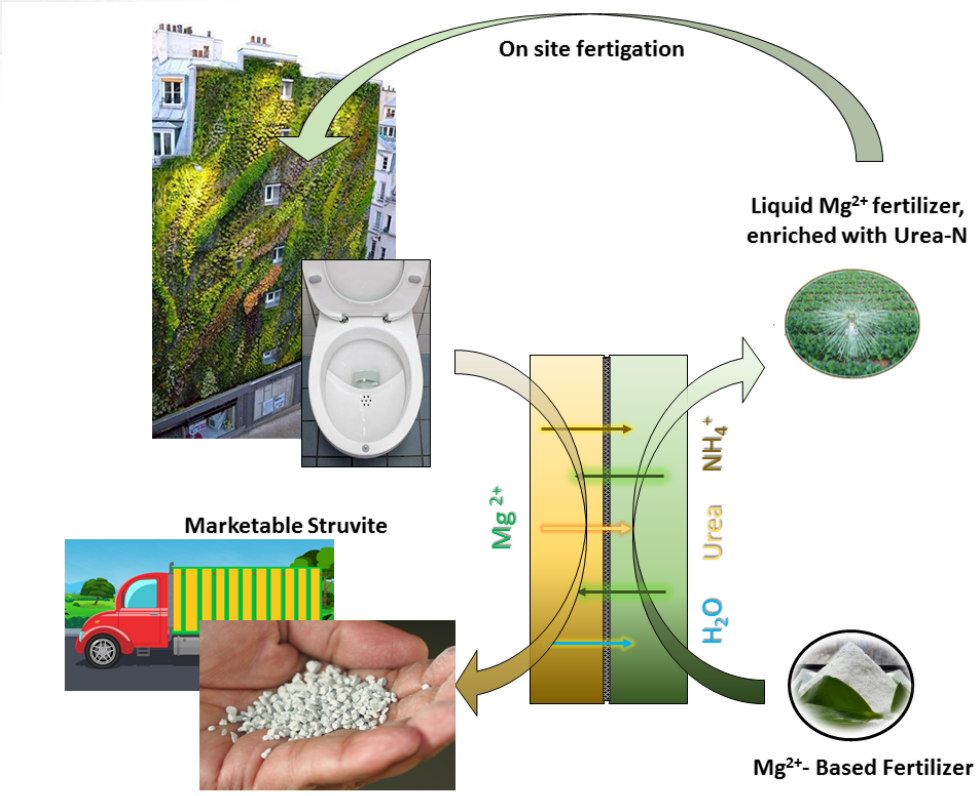

Figure 1 Schematic conceptualisation of the proposed presentation

KS-2

Controlling chloroplast performance: The role of the NTRC/2-Cys peroxiredoxin redox system

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Chloroplast metabolism shows an extraordinary versatility to be adapted to unpredictable changes in light intensity and thiol-dependent redox regulation plays a key role in this process. In addition to the classic redox system formed by photosynthetically reduced ferredoxin (Fd), thioredoxins (Trxs) and a Fd-dependent Trx reductase (FTR), the so-called Fd-FTR-Trxs system, chloroplasts harbor an NADPH-dependent Trx reductase (NTR) with a joint Trx domain, termed NTRC. NTRC is an efficient reductant of 2-Cys peroxiredoxins (2-Cys Prxs), thus having antioxidant function. In this talk I will discuss results showing the genetic interaction of NTRC and 2-Cys Prxs in *Arabidopsis thaliana*. Based on these results, our group has recently proposed a novel model of chloroplast redox regulation according to which the function of the Fd-FTR-Trx and NTRC redox systems is coordinated by the redox balance of 2-Cys Prxs, which thus plays a crucial role in the regulation of chloroplast photosynthetic metabolism and its adaptation to environmental changes in light intensity.

KS-3

Identification of bioactive compounds responsible for therapeutic benefits of traditional Chinese medicine

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Natural products have been a major resource of new drugs due to their highly diverse structures and biological activities. Searching for leads from traditional herbal medicines with long clinical practices in China have drawn more and more attention from scientists. Compared to high throughput screening, an intentional searching through interpreting the accumulative knowledge of traditional medicines is believed to be a shortcut for new lead discovery. A systematic investigation was carried out on a commonly used traditional Chinese medicine Radix Stemonae, from which an array of alkaloids reflecting its therapeutic effects were discovered.

Radix Stemonae (Baibu, 百部) have long been used as an antitussive and insecticidal agent in history. Three species, *Stemona tuberosa*, *S. sessilifolia* and *S. japonica*, are all documented in Chinese pharmacopoeia as plant origin of Baibu. More than 90 *Stemona* alkaloids featuring a pyrrolo[1,2-α]azepine or pyrido[1,2-α]azepine nucleus were characterized. The alkaloidal extracts and major alkaloids were found to exhibit significant antitussive activity in a citric acid-induced cough model. Stemofoline and its analogs demonstrated *in vitro* and *in vivo* insecticidal activity. A comprehensive investigation of three species collected from different habitats revealed that the alkaloidal constituents vary significantly with species and habitats while little with harvesting seasons. A multivariate statistical analysis based on the UPLC-QTof-MS was established to further evaluate chemical diversity of the *Stemona* plants. Chemical marker was revealed for each species through an OPLS-DA model. A PCA model based on the mass fingerprints was then built for identification and quality control of Baibu (Fig. 1).

Out study revealed that both crude alkaloidal extracts and different types of alkaloids exhibited strong antitussive activity while they also showed different side effects. *S. sessilifolia* growing in Chuzhou, recorded in ancient medicinal books as the geo-authentic species, contains a major alkaloid with the most potent antitussive activity and the least side effects. The findings provide scientific evidence for therapeutic applications of the TCM “Baibu” in history and in current clinic, and contribute to the global recognition of traditional Chinese medicines.

Keywords: Natural products; Radix Stemonae; Antitussive; Alkaloid

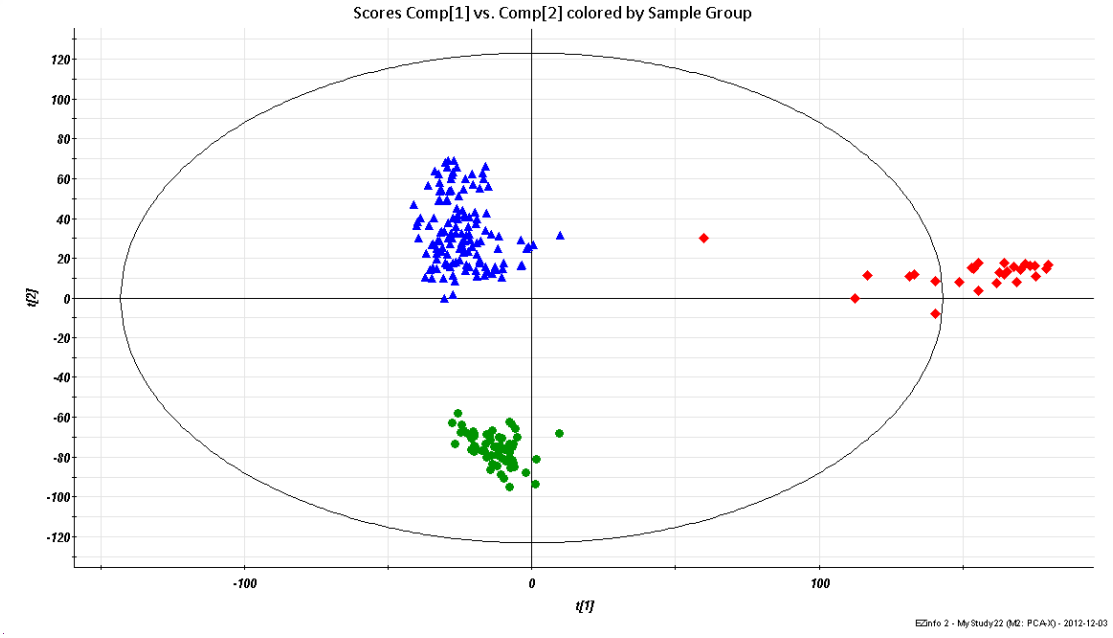

Fig. 1. Score plot of a PCA model to distinguish *S. tuberosa* (red), *S. sessilifolia* (blue), and *S. japonica* (green).

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KS-4

Microbial Ecology of Nitrogen Dynamics in Agricultural Soils: Key Drivers of Reductive N Transformation in Paddy Field and N_2O Generation in Upland Field

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Nitrogen is one of the major essential elements for plant growth. Large amount of nitrogen fertilizer is applied to agricultural fields to support crop production, which sometimes causes environmental and energy problems such as nitrate leaching, nitrous oxide (N_2O) emission, and large petroleum consumption. Understanding of microbial ecology of nitrogen dynamics in agricultural soils is important to establish appropriate soil management system for sustainable and environment-friendly agriculture.

Waterlogged paddy soils are characterized by development of anoxic zone, where reductive nitrogen transformations (RNT), i.e., denitrification, dissimilatory nitrate reduction to ammonium, and nitrogen fixation actively progress by soil microbes, leading to low leaching of nitrogen pollutants (nitrate and nitrous oxide gas) and large retention of nitrogen nutrition. Although many past studies have estimated diversity of RNT microbes based on PCR-based analyses of RNT genes, recent genomic researches have warned an underestimate of the diversity by previous methods.

To avoid such a risk, we performed shotgun sequencing analysis (metatranscriptomics) of soil RNA extracted from paddy soils using MiSeq sequencer. The sequences of RNT genes were retrieved from the metatranscriptomic libraries obtained and taxonomically annotated through a tandem similarity search with the BLAT and BLAST programs.

As a result, most of the RNT gene transcripts in paddy soils were derived from *Deltaproteobacteria*, particularly the genera *Anaeromyxobacter* and *Geobacter*. Despite the frequent detection of the rRNA of these microbes in paddy soils, their RNT-associated genes have rarely been identified in previous PCR-based studies. *Anaeromyxobacter* and *Geobacter*, well-known iron reducers universally dominating in paddy soils, were suggested to associate with RNT, partial reactions in denitrification, ammonium formation via nitrite reduction, and nitrogen fixation. By combination of these reactions, *Anaeromyxobacter* and *Geobacter* could generate ammonium, which contributes to nitrogen fertility of paddy soils.

Our metatranscriptomics provides novel insights into the diversity of RNT microbes in paddy soils and the ecological function of *Deltaproteobacteria* dominating in paddy soils, which may lead to the establishment of low-nitrogen agriculture.

Upland agricultural field is a major source of nitrous oxide (N_2O), one of greenhouse gases and ozone depleting substances. N_2O emission from agricultural fields is mostly derived from microbial transformation of

nitrogen contained in fertilizers applied to soil. Information on the N_2O generating soil microorganisms is essential to mitigate the N_2O emission from agricultural fields.

We observed large emissions of N_2O after basal and supplemental application of organic fertilizer to upland field soil. Soil microcosm study revealed that bacterial (basal application) and fungal (supplemental application) denitrification are largely responsible for the N_2O emissions, whereas the contribution of nitrification is small. By combination of culture-dependent and culture-independent analysis, we successfully identified bacterial and fungal species responsible for the N_2O emissions. Characterization of the N_2O-generating microorganisms will be helpful to establish agricultural practice for N_2O mitigation.

KS-5

Temperature-mediated responses in plants

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Changes in temperature influence developmental programs in plants, including flowering and vegetative growth. Temperature changes influence developmental programs in many species. In order to cope with climate change, it is necessary to understand the molecular regulatory networks that allow organisms to respond to changes in temperature. Our research program is designed to understand the genetic and epigenetic mechanisms that govern temperature-triggered developmental reprogramming in plants. In particular, we use both low (vernalization response) and high (warmth-triggered responses) temperature-triggered developmental changes using Arabidopsis as a model organism. Our current understanding of molecular mechanisms by which plants renders developmental programs under different temperature will be discussed. Both responses are mediated by regulations at the level of chromatin and I will cover the range of mechanistic details of genetic and epigenetic regulation of gene expression by temperature changes.

KS-6

Genomics-Based Enzyme Technology for Targeting the Sweet Spot

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Global food markets for sugars and sweeteners need high-end products for health benefits as well as their nutritional value. Such an ever-lasting demand faces the big challenges over conventional food technology. In this regard, rare sugars, which occur naturally in small amounts, have attracted considerable interest in the food and drug industries because they are potent sugar substitutes as well as important building blocks for pharmaceutical active ingredients. However, there is little information on non-phosphorylated sugar isomerases, which might be potentially applied for the production of rare sugars. Excavating the molecular details of how large metagenomes deposit useful gene signatures remains a daunting task in the high throughput enzyme screening field. Recent advances in sequencing technology provides rapid identification and characterization of novel enzymes with a level of precision not previously possible. Here we present a versatile platform method that greatly accelerates enzyme profiling for highly selective gene capture in metagenomes using next-generation sequencing (NGS). This approach enabled us to generate targeted enzyme profiling datasets in metagenomes, thereby allowing minimal hands-on time and high-throughput screening (HTS). This large-scale HTS provided us with a targeted inventory of the predicted proteins on a metagenomic scale. Overall, our genomics-based enzyme screening and evolution strategies can be an effective and versatile tool for the elucidation of structural and evolutionary features of sugar enzymes, providing important implications for the design and development of non-natural sugar enzymes in the agriculture, food and pharmaceutical industries. Furthermore, we anticipate that the high throughput and sensitivity of this approach will help accelerate the decryption of the diverse protein profiles in metagenomes.