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Microbial Metabolic Reprogramming in Waste Decomposition Susinjan Bhattacharya¹ and Soma Mukherjee²

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Abstract Metabolic reprogramming refers to the adoption of altered metabolism depending on the changed environment. Waste matter with its varied composition harbours different microbial flora and fauna which changes with the progress of decomposition of waste material. This change, or evolution also depends on the different intermediate, tertiary and secretory products that are formed with the process of decomposition. Certain molecules, like application of compost, or biochar drives microbial metabolic reprogramming in decomposing environment, but finally leads to the initiation of a manifold beneficial activities. The work here aims to study at the effect of inducer, or enhancer material, called here as 'effector material' in microbial metabolic reprogramming and waste decomposition. Metabolic reprogramming will also help in bioprospecting of microorganisms in terms of diversified or specific functions, apart from working with a greener agriculture, and prediction of global terrestrial carbon models and ecosystem responses to climate changes.

Keywords: Decomposition, Metabolic reprogramming, Soil health, Metabolites, Metabolite engineering

Introduction

Decomposition refers to a step filled process, wherein organic matter is transformed to different state of components that might be suitable for transport or consumption by water. The process and rates of decomposition are important to decide upon the end product formation. Rate of decomposition fluctuates and is dependent on intrinsic and extrinsic factors. Lignin rich materials decompose slowly in most of the environments (Findlay, 2021). Formation of organic acids upon decomposition of forest litter changes pH and helps in reshaping of soil profile and soil formation (Morris, 2004). Sequential decomposition of dead matter and organic matter decomposition results in the formation of humus, that decomposes slowly, enhances cation exchange capacity of soil (CEC), as well as soil aggregate formation ability. (FAO, 2005). Sequential change in state of organic matter decomposition also leads to a change in microbial flora, or microbial dynamics. Addition of antibiotics, synthetic or natural antibiotics accelerate the change in microbial dynamics (Bhattacharya, 2023a, Biswas et al., 2023). Presence of organic matter has been reported to bring up a change in chemical pollutant dissipation in mineral soil. The process has been observed to associated with adsorption and microbial degradation. (Harju et al., 2021). Microbial anaerobic decomposition of organic matter leads also to the formation of methane that can be used as an energy source (Dlugokencky and Houweling, 2003). The process of decomposition proceeds with enzymatic activities, including microbial enzymes. Addition of biochar influences substantially this decomposition process. Biochar also acts as adsorption site for microbial signal molecules. The concentration feedback inhibition of signal molecule in decomposing microbial community changes microbial physiological features, which re-establishes coordination amongst micro-organisms (Hill et al., 2019). Biochar influences upon the metabolite production and can be detected by metabolomic studies (Ren et al., 2023, Wu et al., 2020). Biochar not only influences upon microbial communities, but also enhances maturity and fertility of compost products (Wu et al., 2020, Qu et al., 2022). Reports has observed carbon use efficiency (CUE) to improve upon with application of biochar (Giagnoni and Renella, 2022). The present work here is aimed at to study in brief the influence of metabolic reprogramming, and influence of an inducer, or enhancer material, called here as 'effector material' in the process of decomposition and change of microbial dynamics in the process. The final outcome is the formation of a product, or a process that acts as a beneficial material to manifold systems.

Metabolic reprogramming in microbes

Metabolic reprogramming defines the adaptation of altered metabolism by cells (Bhattacharya, 2023b, Bhattacharya 2023c). Microorganisms, like bacteria in their facultative mode of growth undergoes metabolic reprogramming to switch their metabolism from aerobic to anaerobic mode (Shan et al., 2012). Metabolic reprogramming also leads to the development of cellular phenotypes, and a metabolic pathway analysis tool, 'Elementary mode analysis' has been developed to understand cellular metabolism and phenotypes, analyze metabolic

network properties, phenotypic behavior discovery and strain design (Trinh and Thompson, 2012). Metabolic reprogramming also leads to the development of altered cell envelope characteristics, that leads to the development of antibiotic resistance (Zeden et al., 2023).

Metabolite engineering

Metabolic engineering targets to improve the production of economically valuable molecules by genetic manipulation techniques in microbial metabolism. Metabolic engineering not only helps in industrial biotechnology, but also in agriculture and commodity products (Volk et al., 2023). The omics era has led to the integration of metabolic engineering with synthetic biology wherein model systems are created to predict behavior of biological systems (Garcia-Granados et al., 2019). Microorganisms, like *E. coli, Actinomycetes* has been developed as biocatalysts to aid in production of economically viable molecules (Kumar and Prasad, 2011). Genome scale models of *Pseudomonas veronii* was developed to demonstrate bioaugmentation of mono-aromatic compounds in soil microbiome system (Hadadi et al., 2020). Microbes has been engineered to produce astaxanthin by metabolic reprogramming (Wan et al., 2021).

Intracellular cellobiose metabolism occurs by two pathways, however, with engineered fungal malic acid production, Li et al., 2019 showed that hydrolytic pathway by β-glucosidase and the phosphorolytic pathway by phosphorylase are both needed for intracellular cellobiose metabolism in *Myceliophthora thermophila* with glucose as an end product (Li et al., 2019). With enzymatic degradation of cellulose, importance has grown in the development of green fuel, researchers have discovered a new class of enzymes and cellulosome (Datta, 2024).

With the recalcitrance of lignin to microbial action, white rot fungi are the best-known degraders with the aid of extracellular laccase and peroxidase. This led to the bioprospecting of more lignin degraders, leading to the discovery of bacterial liginin valorizaters (Bugg et al., 2011, Xu et al., 2019, Liu et al., 2019).

Microbial growth and metabolites

Metabolite production by microorganisms is related to the microbial growth phases. Modulation of growth phase effects metabolite production. Microbially produced metabolites have manifold uses (Tamano, 2014). Different techniques has been employed to increase metabolite production, like controlled ultrasound technology in microbial growth (Behzadnia, et al., 2020). Metabolite engineering through different molecules can aid in formation of different

molecules, like organic acids that helps in breakdown of organic wastes. The process is also helpful in establishment of plant growth promoting microbes and their beneficial effects on crop plants (Lopes and Dias-Filho, 2021). Microbial metabolites plays important role not only in agriculture, but also in nutrition and healthcare systems (Singh et al., 2017).

Physico-Chemical Factors in metabolite production

Physical and chemical conditions in fermentation affects the production of metabolites from *Athlia rolfsii* (Suharsanti et al., 2022). Reduced light increases secondary metabolite production of *Trichoderma atroviride* (Missbach et al., 2023). Cyanobacterial metabolites have been put forth as an alternate source of sunscreens and moisturizers to the current synthetic derivatives (Derivkand et al., 2016). The physical and chemical properties of metabolites, like aqeous solubility, gaseous volatility, and others depends also on the physico-chemical properties of habitat, as well as on the nature and success of ecological interactions (Angelo and Adriana, 2020). In cropping systems, application of fertilizers like, nitrogen and phosphorus modulate the physical, chemical and metabolic characteristics of sugar maple leaves (Young et al., 2023).

Metabolites and metabolic reprogramming in waste matter decomposition

Decomposition of waste matter proceeds with microbial and enzymatic activity. The dynamics change with every stage wherein end products are produced. The mode starts with aerobic mode and can change to anaerobic mode. Composting is the one of the best methods to decompose waste matter. The end products of aerobic and anaerobic vary in composition. However, in both the modes, there is formation of total solids (TS), Fixed solids (FS or ash), and volatile solids (VS) (Hamilton, 2016). Methane formation is one of the features in anaerobic decomposition, and the process involves four stages, namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (Li et al., 2019). Reports have observed that methanogenesis is the rate-limiting step for easily biodegradable substances, and hydrolysis acts as the rate-limiting step for complex organic wastes because of formation of toxic by-products like complex heterocyclic compounds and volatile fatty acids (VFAs). Researchers have reported that methanogenesis is the rate-limiting step for easily biodegradable substances, whereas hydrolysis is the rate-limiting step for complex organic wastes due to formation of toxic by-products like complex heterocyclic compounds and undesirable volatile fatty acids (Ghasemzadeh et al., 2017). In rose waste composting, concentration of polysaccharides and terpenes decreased with more than 60 % in first two months, indicating the adoption of co-metabolism of lignin (Nijs et al.,

2023). Breakdown of plastic has been reported to simpler forms, like monomers or dimers, or smaller molecules by depolymerization and fragmentation. This breakdown was initiated upon by the secretion of metabolites, poly hydroxyl alkanoate depolymerases along with the abiotic factors. Furthermore, *Arcobacter* and *Colwellia* are not only one of the best hydrocarbon degraders, but also best restorers of aquatic pelagic and benthic levels. Exemplarary microbes to degrade petroleum based plastic wastes are *Aspergillus, Penicillium, Moraxella, Nocardia,* etc. (Kumari et al., 2024).

Application of municipal solid waste in soil not only restores soil health and boosts plant productivity but also effects soil microbial and plant metabolic pathways (Abbey et al., 2022). Though microbial decomposition brings up differences across ecosystems, the same process also increases similarity of molecular soil organic matter composition (Davenport et al., 2023). Reports have suggested use of organic matter decomposition and ecosystem metabolism as a tool to assess the functional integrity of aquatic ecosystems (Ferreira et al., 2020). Frequent application of municipal solid waste compost reprograms microbial metabolic pathways (Abbey et al., 2022).

Conclusion

Metabolic reprogramming refers to the adoption of altered metabolic pathway. Organisms at cellular level adapts this to survive and proliferate in a changed environment. Microorganisms are no exception the process of metabolic reprogramming. Substrate breakdown by altered metabolism leads to the development of valorization. Often, product concentration can act as feedback inhibitor for the substrate utilization, like in cellulose breakdown. It has been suggested to practice organism development for consolidated bioprocessing, wherein all the steps of cellulolytic enzyme production, biomass hydrolysis and sugar fermentation can occur in one step (Lynd et al., 2002). Metabolically engineered microorganisms (MEMs) are the other alternatives that can be used to breakdown substrates and yield high value products. (Pena-Castro et al., 2023).

Bacterial metabolic reprogramming occurs both in aerobic and anaerobic conditions. There are essentially two critical factors that determines the bacterial metabolic pathway in oxygen deprived microaerobic and anaerobic conditions, namely redox homeostasis and maximal energy conservation. The process is regulated by the regulatory factors that can be used to engineer metabolic pathways, and under anaerobic conditions this engineered organisms will help to obtain products with optimum yield under desired fermentation conditions (Shan et al., 2012). This activity will also help in the study of microbial evolution (Bhattacharya, 2023a, Biswas, 2023). Though, microbial production

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of secondary metabolite is not an important characteristic feature to regulate microbial primary metabolism, but it is necessary to change physical properties of the environment surrounding bacterial as well as microbial community and population (Santamaria et al., 2022). Therapeutic application of micro-organisms offers a number of benefits when used along with advanced technologies like omics and genetic engineering, and together these modulates microbial metabolic reprogramming. Combinatorically, these offers synergy towards a sustainable greener agriculture and restoration practices (Iqbal et al., 2023). Microbial population and communities vary with regard to carbon decomposed and mass loss. Consecutively, bacterial communities change very fast than fungal communities in response to climate changes. This information is essential to predict global terrestrial carbon models and prediction of ecosystem responses to climate changes (Glassman et al., 2018).

In soil, composting of waste is decomposition of the waste matters, and with changed stages, there is evolution of changed flora due to varied secretory and intermediate products at each stage. Though application of compost in soil can also bring up reprogrammed microbial metabolism (Abbey et al., 2022), this leads to the boosting of soil health, microflora, rhizosphere activity and improvement of plant health. Thus, compost application in soil acts as an effector material of microbial metabolic reprogramming. Biochar, is an alternative, as well as addendum to this resultant beneficial activity. Though these activities might hold good for a tropical country like India, but it needs to be looked at if the same hypothesis will good for different other climatic situations. Metabolic reprogramming will also help in bioprospecting of microorganisms in terms of diversified or specific functions.

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