Endophytes Acting on Secondary Metabolites in Plants

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Endophytes, which are widely found in host plants and have no harmful effects, are a vital biological resource. Plant endophytes promote plant growth and enhance plants' resistance to diseases, pests, and environmental stresses. In addition, they enhance the synthesis of important secondary metabolites in plants and improve the potential applicability of plants in agriculture, medicine, food, and horticulture. Secondary metabolites (SMs) are the products formed by interactions with the environment during plant growth and development. SMs mainly include alkaloids, flavonoids, terpenoids, peptides, phenols, sterols, and additional minor molecular organic compounds. As essential substances used by plants for self-protection to cope with their environment, SMs have various physiological functions, such as regulating plant growth and biological defense, and they are also involved in the plant response abiotic stresses, such as drought, low temperature, salinity, and metals

endophytes biosynthesis mechanism secondary metabolites SynComs metaomics

1. Construction of the SynComs Model for Endophyte–Plant Interactions

Microorganisms rarely exist alone, and there is a positive correlation between species diversity and community productivity in nature. The ubiquity of these microbial communities in nature highlights the possible advantages of endophytic strains in coculture [1]. However, the study of plant secondary metabolites relying on only the naturally formed endophyte community is becoming increasingly limited. Researchers have gradually shifted their focus to identifying methods to better study the occurrence and accumulation of secondary metabolites under the interaction between plants and endophytes. SynComs is a process of artificially mixing different microorganisms with distinct species and functions in certain proportions under certain conditions to create a stable microbial community with distinct functions. With the development and implementation of SynComs, research on plants and endophytes advanced further (**Figure 1**). SynComs consider multiple types of interactions and the functions of different microorganisms under certain conditions to determine the proportion of mixed, stable, and functional microbial communities that can promote plant growth, nutrient uptake, and stress resistance. These interactions play an important role in such aspects of secondary metabolite production and can be applied to industrial production.

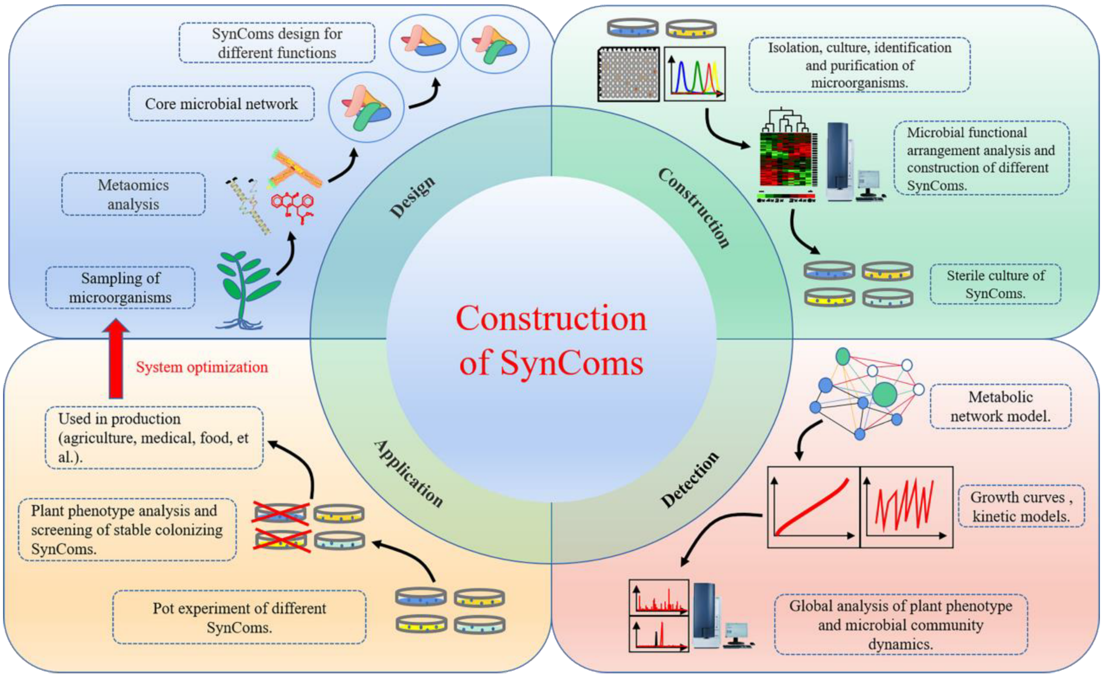

Figure 1. Construction of SynComs. SynComs with specific functions are formed by the recombination of microorganisms with different functions, depending on the needs of the plant. Functional analysis of the recombinant microbial community was performed using an omics approach. Core microbial communities that can play a stable and efficient role in plant growth and development, synthesis, and accumulation of secondary metabolites can be screened for commercial mass production. The function and composition of the SynComs were also optimized through practice.

SynComs were first reported in the early 21st century. *Saccharomyces cerevisiae* was genetically modified through genetic hybridization, and two nonmating strains, R and Y, with different metabolic abilities were obtained. R synthesized lysine but needed adenine for growth, and Y synthesized adenine but needed lysine for growth [2]. A stable and sustained cooperative relationship was formed after coculture of the two strains, which laid a foundation for the development of SynComs in the future. Further studies showed that internal and external factors played an important role in the stability of the SynComs [3][4][5][6]. In 2014, researchers developed the concept of SynComs and defined SynComs as a coculture system established by two or more microorganisms in a substrate with a clear composition [7]. Subsequently, the importance of host genotypes and core microorganisms for the construction of SynComs was further revealed [8][9][10]. The above research results greatly promoted the development of SynComs technology and laid a solid foundation for the use of SynComs technology to reveal the function of plant endophyte communities and the interaction mechanisms with plants. The SynCom construction process is regulated by multiple factors, such as the microorganisms themselves, plant hosts, and environment, among which the effects of microorganisms themselves include interspecies interactions, metabolism, and spatial structures [11]. Therefore, the construction of SynComs requires comprehensive consideration of the interactions among species, metabolism, and spatial structures to ensure the stability of SynComs.

2. Application of Omics to Elucidate the Mechanisms by Which Endophytes Promote the Occurrence and Accumulation of Plant Secondary Metabolites

Endophytes promote the synthesis of plant secondary metabolites in various complex ways, and traditional research methods have been insufficient for studying secondary metabolites through these relationships [12]. Studies on plant secondary metabolites have focused on their genetics, responses to stress, metabolism, and structures (**Figure 2**). Among existing studies and analyses, the main research methods used to explore the interactions between endophytes and plants include next-generation sequencing (NGS) and omics analysis. To date, NGS has been widely used to study the diversity of microbial communities in various environments [13][14][15]. In addition, this technology can also be used for large-scale genome sequencing [13][16], gene expression analysis [17][18], the identification of noncoding small RNAs [19][20], the screening of transcription factor target genes [21][22], and DNA methylation [23][24]. However, NGS has limitations, such as the possible detection of plant DNA when studying microbial communities interacting with plants, thereby reducing the amount of data acquired for microbes. Thus, to avoid interference with plant sequences, we can increase the specificity of the primers or improve the ability of high-throughput data acquisition to obtain additional information. With technological advancement, NGS will gradually improve. Currently, omics, as an effective complement to this technique, plays an essential role in the analysis of plant secondary metabolites, particularly the joint effect of multiomics as a directional indicator to study the interactions between endophytes and plants.

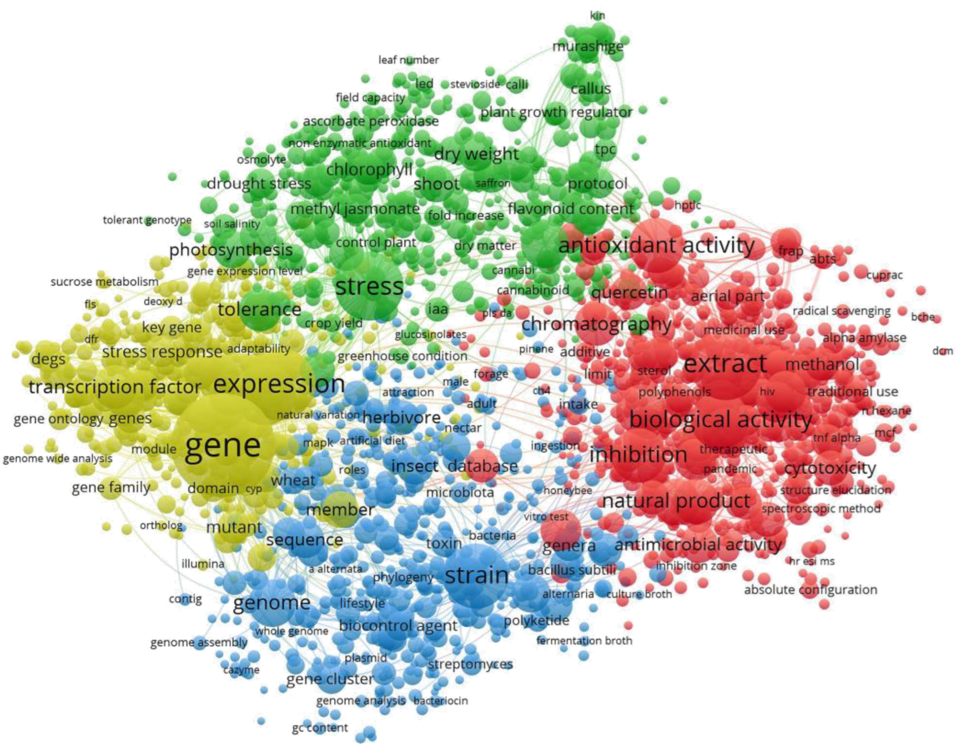

Figure 2. Visualization of the popular research topics related to plant secondary metabolites in the past five years. VOSviewer software was used to analyze the titles and abstracts of 12,745 articles in the Web of Science

database from the past five years (1 January 2018–11 October 2022). The results showed that the current popular research topics mainly focus on three aspects of plant genes: type, stress, and metabolism. The retrieval conditions used in the database are (ALL = (Secondary metabolites)) AND ALL = (plant), the export format is a TAB delimited file, and the record content is “Full record and referenced references”. The lowest frequency of keywords screened by VOSviewer software was set as 15, and 4523 keywords were screened in total. Among 2714 keywords with correlations greater than or equal to 60%, 351 uninformative keywords were manually removed, and 2363 keywords were finally used for visual analysis.

To date, the application of multiomic technology in studies on secondary metabolism to determine the interactions between plants and endophytes is becoming more widespread [22][25]. In research, multomics generally takes the form of metaomics, which mainly includes metagenomics, macrotranscriptomics, and macroproteomics. By combining metagenomic, macrotranscriptomic, and macroproteomic methods, metaomics can be used to not only predict the potential functions of endophytic communities, but also to determine the functional activity of endophytic communities [26][27]. At the same time, a more comprehensive understanding of endophytic communities can be promoted by determining the intraspecific relationships of communities, understanding nutrient competition between plants and endophytes, and examining community development [28][29]. In recent years, metaomics has been the focus and frontier of research in the field of plant–endophyte interactions, with remarkable results. With the development of whole-metagenome shotgun (WMS) sequencing technology, research using metaomics has changed from high-depth sequencing, which can only reflect the characteristics of one sample, to large-sample sequencing, which can reflect the differences between different samples. Studies have shifted from sample sequencing at one time point to dynamic process sequencing in response to different external environments [27][30].

However, the current metaomic research methods still have some shortcomings and inadequacies. Metaomic analysis of plant samples, while potentially capable of representing all the diversity in gene sequence and function, has not been able to elucidate all the genes and functions of a single microorganism, and this has presented great difficulties in exploring the functions of plant endophytes. Fortunately, modern methods such as single-cell genome sequencing (SCGS) have been slowly developed [31]. Therefore, we should look forward to the development of SCGS and alternative techniques to address these difficult issues in studying the interaction between plants and endophytes and to further elucidate the mechanisms by which endophytes facilitate the occurrence and accumulation of secondary metabolites in plants.

3. Endophytes Advance the Application of Plant SMs

Endophytes are ideal resources for facilitating the formation and accumulation of secondary metabolites in plants. Earth is a large biological resource bank, with approximately 270,000 plant species, and the number of endophytes that can be isolated exceeds one million based on the calculation that there are approximately four distinct endophytes in each plant [32]. The study of endophytes has opened up new areas for research into the application of microorganisms. The current work focuses on the use of screening to promote the formation and accumulation of plant secondary metabolites in host strains and the transformation of strains to improve the breeding process, industrialized production, and production capacity, as well as to improve the direct application of plant secondary metabolites in food, agriculture, and other areas [33]. In addition, various plant secondary metabolites have the potential to be indirectly exploited as biopesticides, pharmaceuticals, or pharmaceutical precursors, and these secondary metabolites possess a wide range of activities, including antimicrobial, antioxidant, and biocontrol activities [34][35]. At the same time, the interaction between endophytes and plants produces new bioactive secondary metabolites, providing feasible opportunities for the development of improved drugs and more effective ways for humans to cure diseases in the future [36]. This result clearly shows that using endophytes promotes the synthesis of host plant secondary metabolites and the generation of new active substances. This solves the issues with using traditional methods involving the long growth cycle and restrictions on the active ingredients needed to produce plant resources. This method is expected to provide a new way to quickly produce active substances in plants and will have great application value and development potential.

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