METABOLIC ENGINEERING IS A PROMISING WAY TO GENERATE HIGHLY EFFECTIVE PRODUCERS OF BIOACTIVE SUBSTANCES

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Medicines play an indisputable role in life extension and improvement of the quality of life. To obtain medicinal compounds, researchers traditionally rely on natural sources and chemical synthesis, however, currently developing biotechnological methods allow one to introduce the group of genes encoding new metabolic pathways into the genomes of heterologous hosts and regulate activity of the hosts' intrinsic metabolic pathways. Such an approach makes it possible to reproduce biosynthesis of bioactive substances in heterologous hosts, the approach combines the benefits of conventional methods and works around the shorcomings of those. In our view, the use of metabolic engineering to obtain medicinal compounds is becoming increasingly important for their production.

Keywords: heterologous gene expression, metabolic pathways, metabolic engineering, biosynthesis of medicinal compounds

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МЕТАБОЛИЧЕСКАЯ ИНЖЕНЕРИЯ — ПЕРСПЕКТИВНЫЙ ПУТЬ ПОЛУЧЕНИЯ ВЫСОКОЭФФЕКТИВНЫХ ПРОДУЦЕНТОВ БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ

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Лекарственные препараты играют неоспоримую роль в продлении жизни и повышении ее качества. Для получения лекарственных соединений исследователи традиционно обращаются к природным источникам и химическому синтезу, однако в настоящее время активно развиваются биотехнологические методы, позволяющие внедрять группы генов, кодирующие новые метаболические пути, в геномы гетерологических хозяев и регулировать активность их собственных метаболических путей. Такой подход дает возможность воспроизводить биосинтез биологически активных соединений в гетерологических хозяевах, сочетает достоинства традиционных методов их получения и обходит недостатки этих методов. С нашей точки зрения, применение метаболической инженерии для получения лекарственных соединений приобретает все большее значение в производстве.

Ключевые слова: гетерологическая экспрессия генов, метаболические пути, метаболическая инженерия, биосинтез лекарственных соединений

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Living organisms, especially fungi and plants, are conventional sources of bioactive compounds and medicines. However, extraction of these compounds from natural sources can be a complex and costly process due to low content of compounds. Development of the organic synthesis techniques has provided a breakthrough in drug manufacturing [1], however, it is not rational to obtain all natural compounds chemically due to multistage synthetic pathways, requirements for optical activity, and low yield of pure substance [2, 3]. Biotechnology offers an alternative approach that allows to produce medicinal compounds in heterologous hosts [4], such as bacteria, yeast, plants, algae, and mold fingi, many of which combine the features of rapid growth, simplicity and low cost of cultivation.

Heterologous gene expression provides opportunities to program new properties of the host at the cellular and organism levels, including the large-scale production of atypical substances [5]. Since bioactive compounds cannot usually be encoded by single genes, the projects of metabolic engineering of entire biochemical pathways are becoming increasingly popular. Integration of long multigene constructs into the host genome is supported by the development of methods for DNA assembly [6] and delivery [7]. Regulation of the host's intrinsic metabolic pathways has a significant impact on the success of such projects, since it allows the host cell to produce appropriate amounts of essential intermediate metabolites. Engineering of autotrophic yeast, Pichia pastoris, is one such example [8].

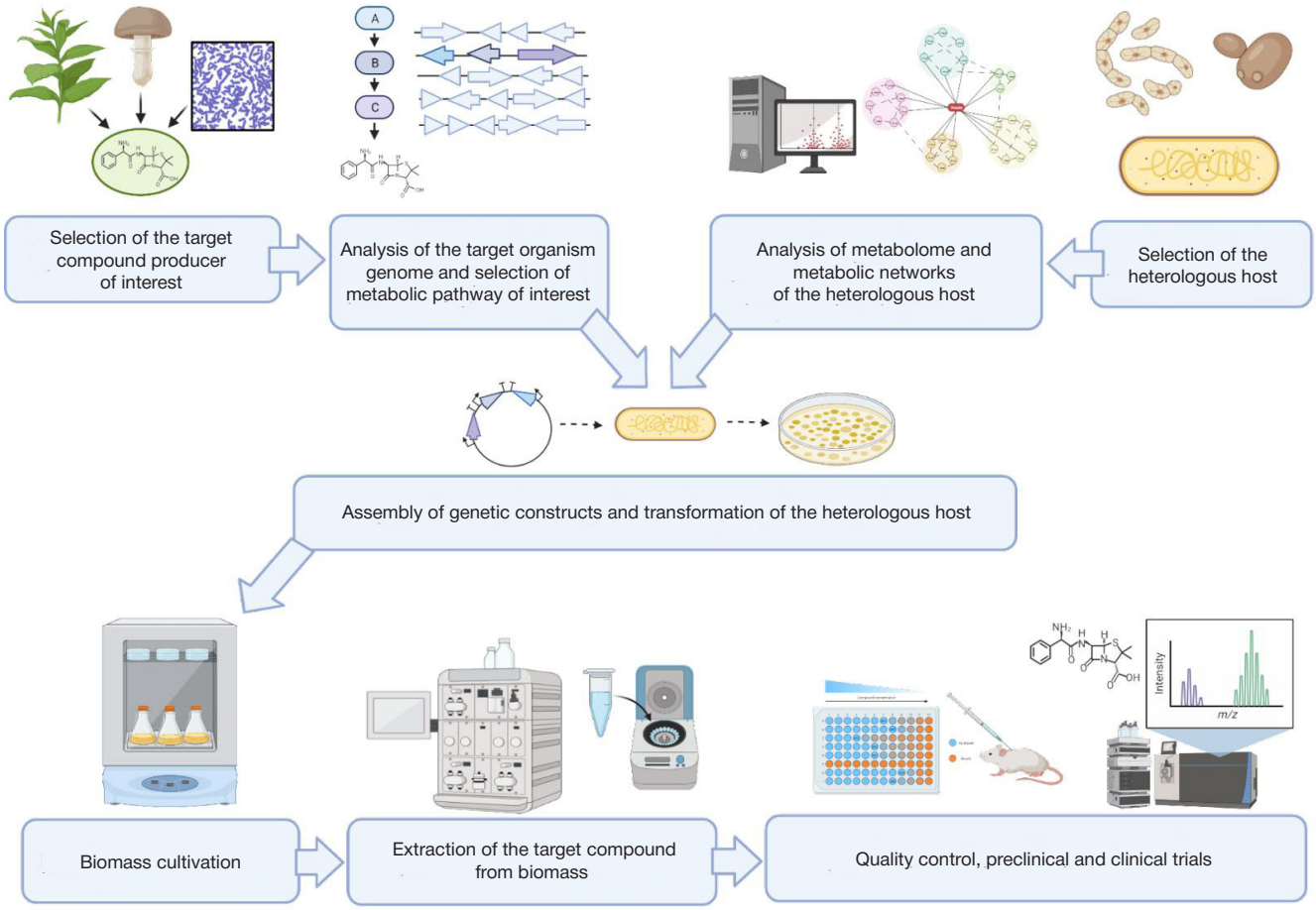

Fig. Scheme of contemporary approach of generating bioactive compounds in heterologous hosts

Metabolic pathways of any organism form complex metabolic networks, that is why it is extremely important to have a detailed picture of the enzyme components of certain biosynthetic pathways and their products to select the points of metabolic regulation in the heterologous host. For this purpose, various databases, such as KEGG (Kyoto Encyclopedia of Genes and Genomes) [9], BRENDA (BRaunschweig ENzyme DAtabase) [10], and PathBank [11] can be used, along with the gene co-expression databases, such as ATTED-II (Arabidopsis thaliana trans-factor and cis-element prediction database) that has been designed for Arabidopsis [12]. However, the built-in algorithms of these databases may not be informative enough when dealing with the understudied genes or organisms; the bioinformatics tools based on the machine learning algorithms, the predictive power of which improves with time, are used in such cases [13].

After defining the points of metabolic regulation, biochemical pathways can be tuned by site-specific genome editing and/or epigenetic regulation. Considering the general trends of switching from omnidirectional effects to more specific ones, fine tuning of the host gene expression levels is becoming increasingly popular and accessible. In particular, the guide RNAs and artificial Cas9-based transcription factors can be used for gene activation [14, 15].

The combined approach involving implementation of heterologous metabolic pathways and rerouting of the host's intrinsic metabolic pathways (see Figure) yields spectacular results of the target bioactive compound biosynthesis in the heterologous host [4].

CONCLUSION

The success of the medicinal compound biosynthesis is based on the combination of several orthogonal approaches. Successful implementation requires determining the shortest metabolic path to the desired substance or property using databases, defining the limiting stages using conventional biochemical models or machine learning algorithms, selecting the optimal method for host genome transformation, constructing genetic constructs that are compatible with the selected host, characterizing the host's metabolic landscape, and regulating the activity of the host's intrinsic metabolic pathways aimed at directing metabolic flows towards heterologous pathway. The development of modern technology makes it possible to increase the efficiency of each stage and eventually results in production of the new source of medicinal compound.

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