Fermentation Production of Plant Alkaloids via Synthetic Biology and Development of Drug Discovery

Hiromichi MINAMI

Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University

Research objective

Plant secondary metabolites such as alkaloids, with a wide variety of pharmacological functions, are widely used in our lives and mainly produced via extraction from plants. However, details on them are not clear owing to their low content, and many of them are difficult to utilize on their own. If specific alkaloids can be produced efficiently, it would be possible to find new bioactivities that have not been revealed due to low content or foreign substances, and this could be an effective tool for drug discovery research.

The purpose of this study was to develop a practical alkaloid production system via a microbial fermentation that does not require the addition of expensive substrates and establish an efficient method for utilizing rare active ingredients of herbal medicines. In particular, we conducted a practical production of morphinan alkaloids (thebaine), an important pharmaceutical raw material, using a microbial fermentation method. In addition, we produced large amounts of rare alkaloids, such as magnoflorine and sanguinarine, which are active ingredients of crude drugs, and attempted to develop new drugs by discovering new bioactivities.

Furthermore, by combining biosynthetic genes (combinatorial biosynthesis) and constructing a unit-type production system, a practical production system can be established to produce novel biologically active substances in a short time and at a low cost.

Methods

To date, we have been able to construct an alkaloid production system using a microbial fermentation method and succeeded in producing 165.9 mg/L of (*S*)-reticuline, which is an important intermediate of isoquinoline alkaloids and contains only trace amounts in plants, from inexpensive glucose[1]. However, the production system that we constructed used three plasmids of biosynthetic enzymes, which cause cellular instability. To construct a more stable production system, an alkaloid biosynthetic pathway was constructed on the *Escherichia coli* chromosome. Each of the biosynthetic genes up to (*S*)-reticuline (12 genes) was sequentially inserted into the chromosome together with the T7 promoter to construct a (*S*)-reticuline production strain. By optimizing the culture conditions using a jar fermentor, we established a practical production system with a production volume in g/L.

Afterward, we investigated the production of morphinan alkaloids and rare alkaloids such as magnoflorine, which are important pharmaceutical raw materials. The biosynthetic enzymes for each alkaloid from (*S*)-reticuline were successfully expressed in *E. coli* using a plasmid. However, where it was difficult to construct a biosynthetic pathway on the chromosome, we considered constructing it on a plasmid.

Results

Each of the biosynthetic genes (12 types) up to (*S*)-reticuline was sequentially inserted into the *E. coli* chromosome along with the T7 promoter to establish a reticuline biosynthetic pathway from glucose (Fig. 1). As a result of optimizing the culture condition using a jar fermentor, 3 g/L of (*S*)-reticuline production was successfully achieved.

The production of the morphinan alkaloid thebaine was examined (Fig. 1). Eight biosynthetic genes from (*S*)-reticuline to thebaine were inserted into the constructed (*S*)-reticuline-high-producing *E. coli* chromosome to establish the thebaine biosynthetic pathway from glucose. However, the production of thebaine could not be confirmed. Therefore, we constructed a thebaine-producing strain by incorporating eight biosynthetic genes into three plasmids and introducing them into (*S*)-reticuline-high-producing *E. coli* cells. As a result,

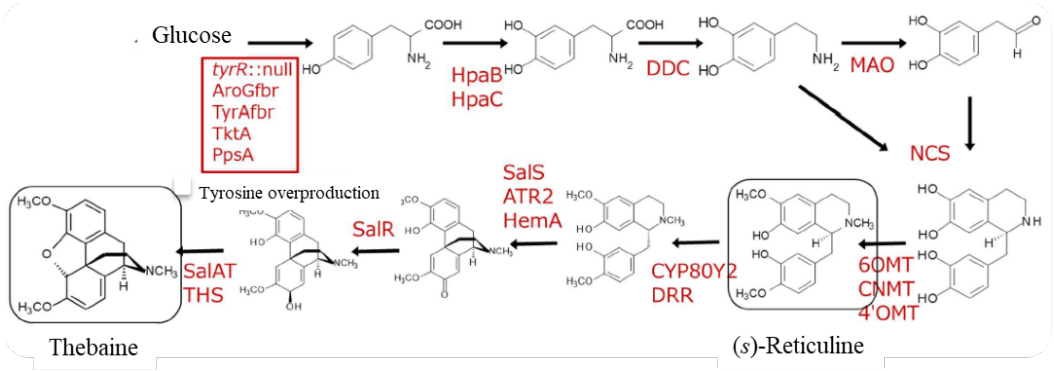

Figure 1 Construction of the thebain biosynthetic pathway in *E. coli*.

we succeeded in producing 40.6 mg/L of thebaine.

Furthermore, the (*S*)-reticuline-producing strain was used as a platform for the production of various isoquinoline alkaloids (Fig. 2). Therefore, by introducing two magnoflorine biosynthesis genes into the (*S*)-reticuline-producing strain, we investigated the production of magnoflorine from glucose and succeeded in producing 10 mg/L.

Conclusion

Microbial fermentation production of isoquinoline alkaloids ((*S*)-reticuline, thebaine, and magnoflorine) was successfully achieved using *E. coli* cells. This production system is a model case for introducing 20 levels of exogenous genes and enables us to obtain unique and superior

data and improve the accuracy of development. The production of 3 g/L of (*S*)-reticuline was successfully achieved, and the improvement in the production efficiency of other compounds is expected to lead to a wide range of practical production of various secondary metabolites via the microbial fermentation method, which has been difficult to achieve so far.

References

1) Matsumura E. *et al.* (2018) Microbial production of novel sulphated alkaloids for drug discovery. *Scientific Reports* 8: Article number 7980.

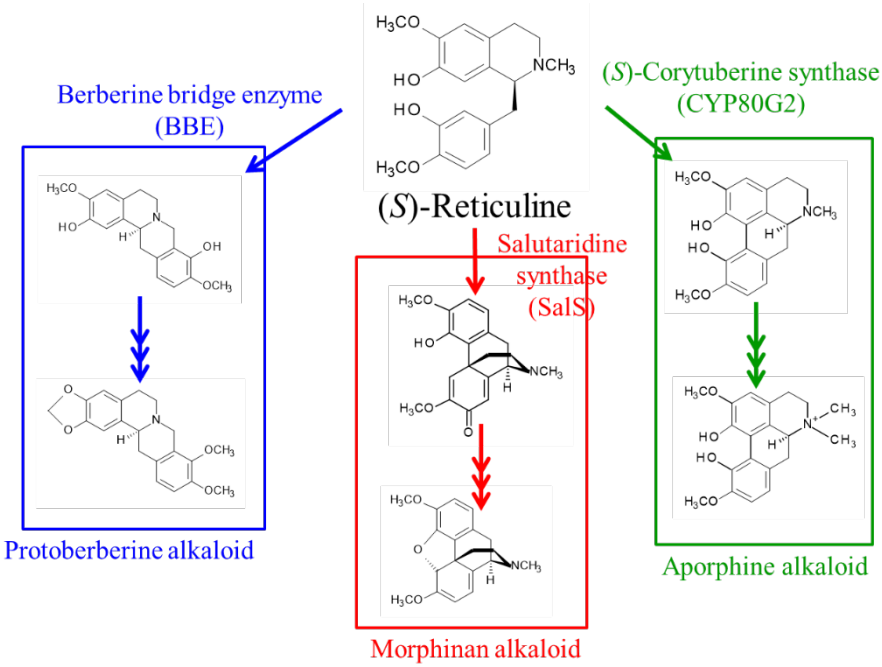

Figure 2 Three major groups of isoquinoline alkaloids