

Research advances in probiotic fermentation of Chinese herbal medicine

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Zhang, Xiaoling, Qin Miao, Chengxue Pan, Jia Yin, Leli Wang, Lingbo Qu, Yulong Yin*, Yongjun Wei*. Research advances in probiotics fermentation of Chinese herbal medicine. *iMeta* (2023): e93. <https://doi.org/10.1002/imt2.93>

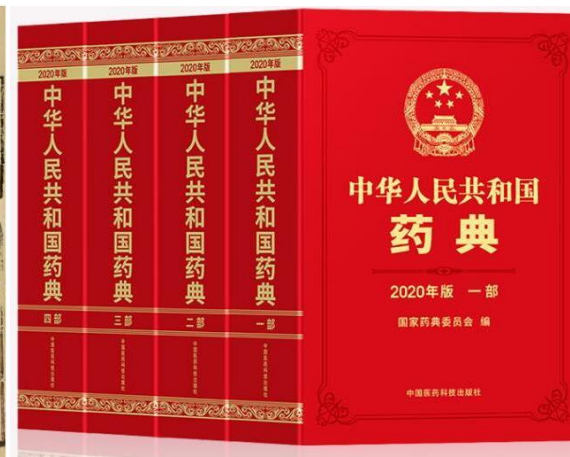
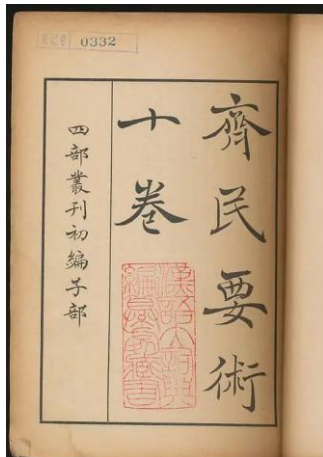
Introduction



- Chinese herbal medicines (CHM) refer to natural medicines and their processed products, and are mainly composed with plant medicines (including root, stem, leaf, and fruit) and mineral medicines.
- CHM have been used to treat human diseases in China and other Asian countries for thousands of years.

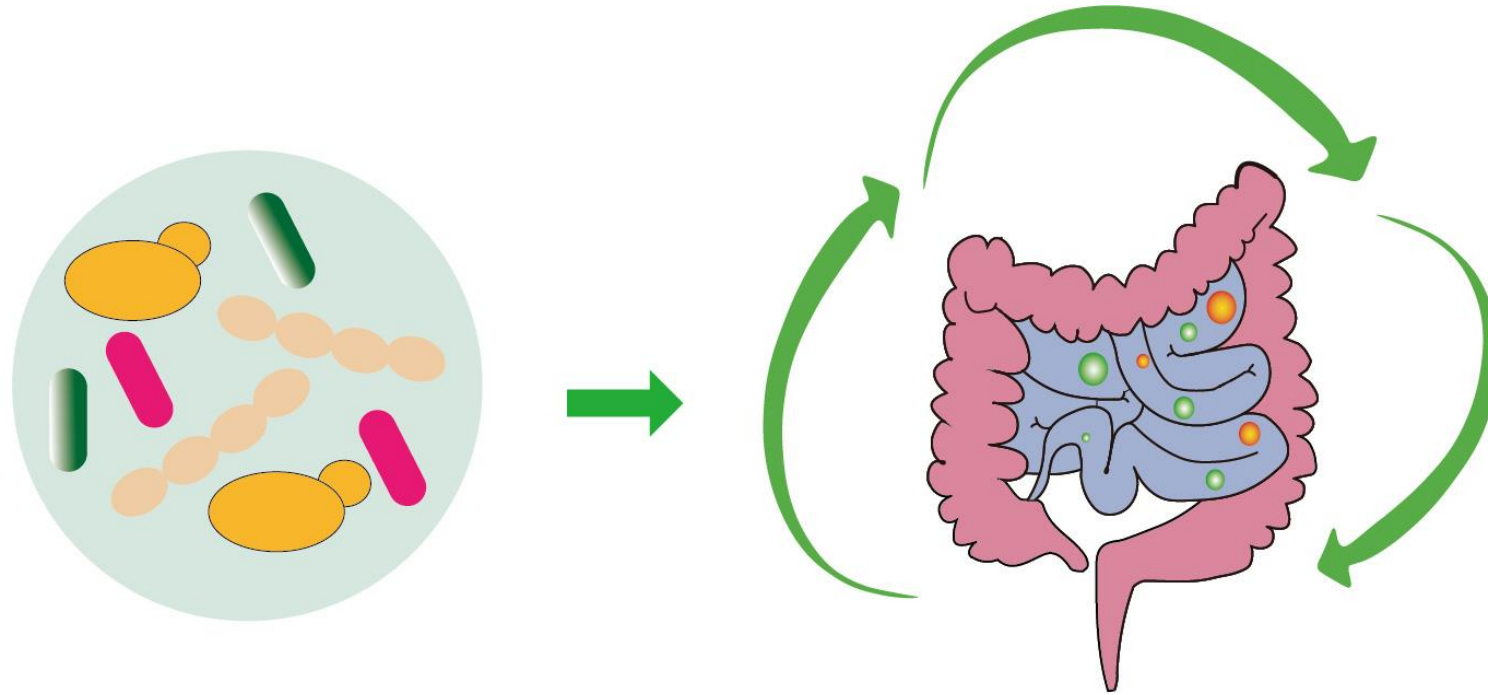
Introduction

- The contents of most bioactive ingredients in CHM are low, and some CHM components are toxic to humans and animals.
- Microbial fermentation is one of the traditional CHM processing techniques, which could increase pharmaceutical efficacy and reduce toxicity.



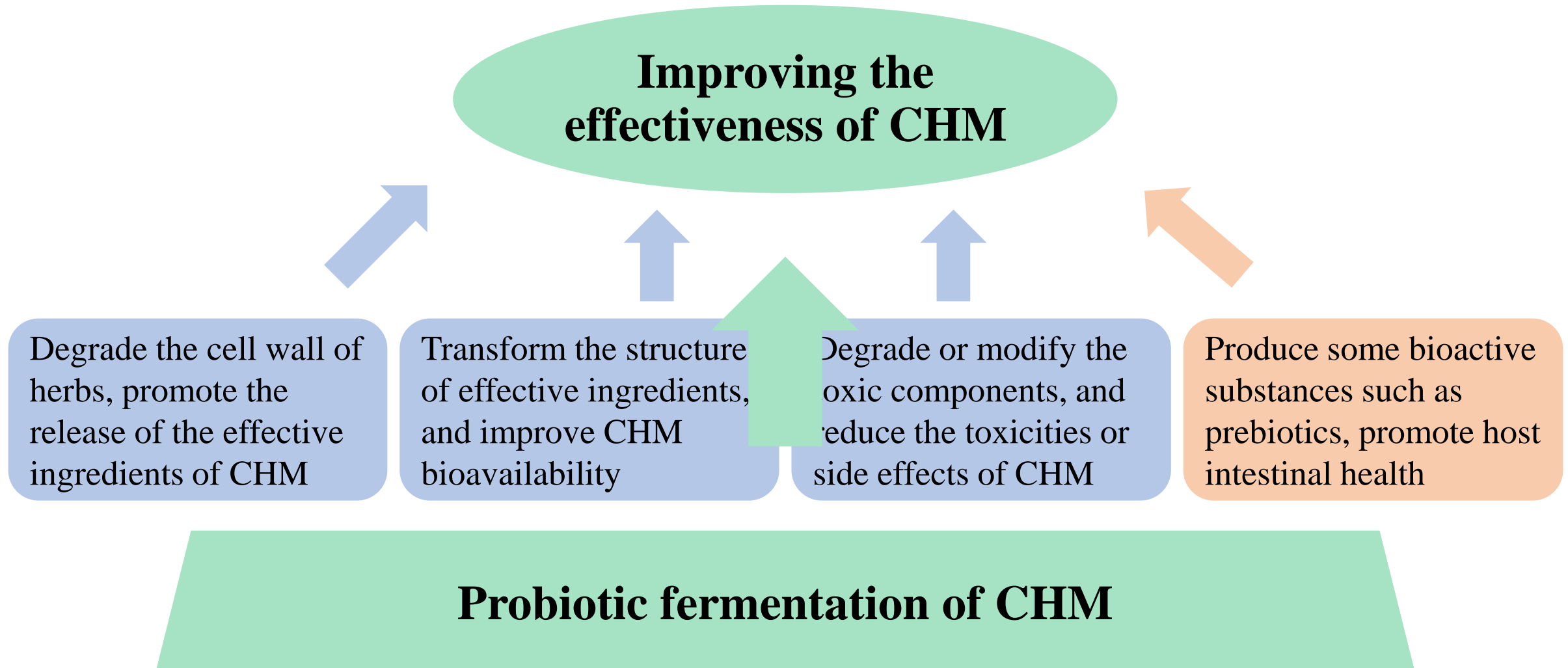
Some classical fermented CHM products are still available, including Pinelliae Rhizoma Qu, Shen Qu, Jian Shen Qu, Cai Yun Qu, Chen Xiang Qu, Semen Sojae Praeparatum, Bai Yao Jian, and Pien Tze Huang.

Introduction



- Probiotics are live microorganisms that have demonstrated beneficial effects on human health.
- Probiotics are beneficial for human gut microbes, intestinal epithelial barrier, and immune system, thus, fermentation of CHM with probiotics is of great interest.

Mechanisms of CHM fermentation by probiotics



The synergistic effect of CHM fermented with probiotics might enhance the effectiveness of CHM.

Lignocellulases and their functions in sealwort, ginseng, and other CHM fermentation

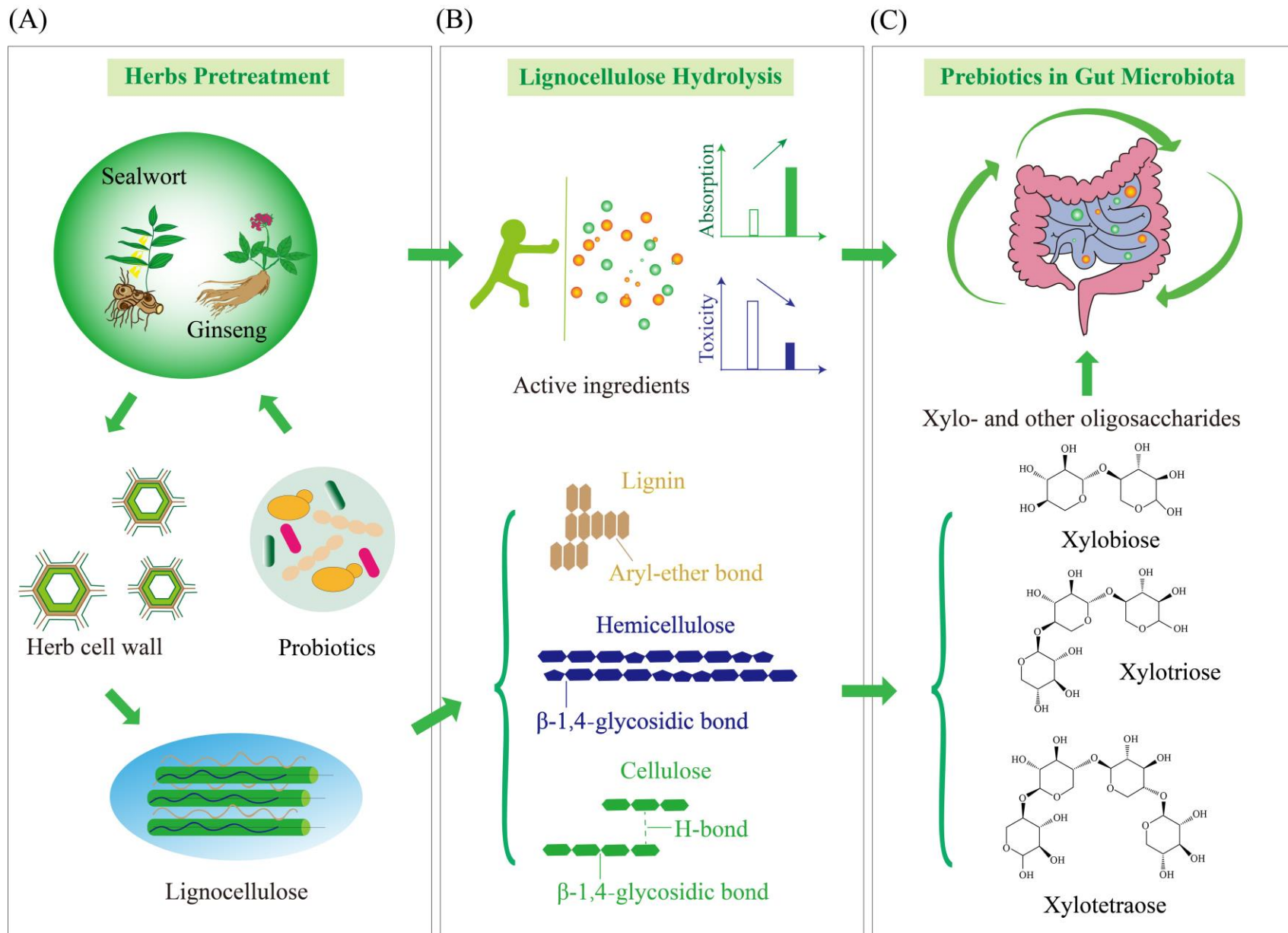


FIGURE 1 Lignocellulases and their functions in sealwort, ginseng, and other Chinese herbal medicine (CHM) fermentation. (A) The lignocellulose might prevent the release of bioactive ingredients of CHM, and lignocellulases derived from probiotics or other microbes can be used to degrade herb lignocellulose. (B) Lignocellulose hydrolysis releases bioactive ingredients in herbs, and leads to the generation of oligosaccharides prebiotics. (C) Bioactive ingredients and oligosaccharides are beneficial for the gut microbiota of humans and animals.

TABLE 1 Contents of effective Chinese herbal medicine ingredients increased after microbial fermentation.

| Herbs/herb formula | Microorganism | Increased bioactive natural products | Pharmacological effects | References |
|-------------------------------------|---|--|--|------------|
| Hwangryun-haedok-tang | <i>Lactobacillus curvatus</i> | Baicalin | Ovariectomy-induced bone loss ↓ | [48] |
| <i>Condonopsis lanceolata</i> | <i>Bifidobacterium longum</i> , <i>Lactobacillus acidophilus</i> , <i>Leuconostoc mesenteroides</i> | Gallic acid and vanillic acid | Neuroprotective effect ↑ Cognitive enhancing activity ↑ | [49] |
| <i>Artemisia princeps</i> Pampanini | <i>Lactobacillus plantarum</i> | Catechol and seco-tanaparthalide C | Anti-inflammatory activity ↑ | [50] |
| <i>Panax notoginseng</i> | <i>Lactobacillus helveticus</i> , <i>Lactobacillus rhamnosus</i> , <i>L. acidophilus</i> | Ginsenoside Rg3 and Rh1 | Anti-hepatocarcinoma activity ↑ | [51] |
| <i>Astragalus membranaceus</i> | <i>Enterococcus faecium</i> , <i>L. plantarum</i> | Astragalus polysaccharide, total saponins, and flavonoids | Not determined | [52] |
| <i>Polygonum cuspidatum</i> | <i>Aspergillus niger</i> , yeast | Resveratrol | Not determined | [53] |
| <i>Radix astragali</i> | <i>Aspergillus oryzae</i> | 3,4-Di(4'-hydroxyphenyl) isobutyric acid | Antioxidant activity ↑ | [54] |
| Red ginseng (the steamed ginseng) | <i>Phellinus linteus</i> | Ginsenosides Rg3, Rg5, Rk1, compound K, Rh1, F2, and Rg2 | Skin permeability ↑ | [55] |
| | <i>Lactobacillus brevis</i> | Ginsenosides Rg3, Rg5, Rk1, compound K, Rh1, F2, Rg2, and flavonoids | Antiwrinkle efficacy ↑ Skin sensitization ↓ | [56] |
| | <i>L. plantarum</i> | Ginsenoside Rd and total phenolic | Antioxidant activities ↑ | [57] |
| | <i>Lactobacillus paracasei</i> , <i>B. longum</i> | Ginsenosides Rg3, F2, Rh1, Rh2, and Rg2 | Ovalbumin-induced inflammation ↓ | [58] |
| <i>Panax ginseng</i> | <i>Ganoderma lucidum</i> mycelium | Polysaccharides | Immunological activity ↑ | [59] |
| | <i>Lactobacillus fermentum</i> | Rare ginsenosides (Rg2, Rg3, Rh1, Rh2, F2, and Ro) | Hyperlipidemia ↓ liver injury ↓ | [60] |
| <i>Dendrobium officinale</i> | <i>Bacillus</i> sp. DU-106 | Polysaccharides with high proportion of mannose | Immunoregulatory activities ↑ | [61] |

Biotransformation of main ginsenosides to active rare ginsenosides using efficient enzymes or probiotics

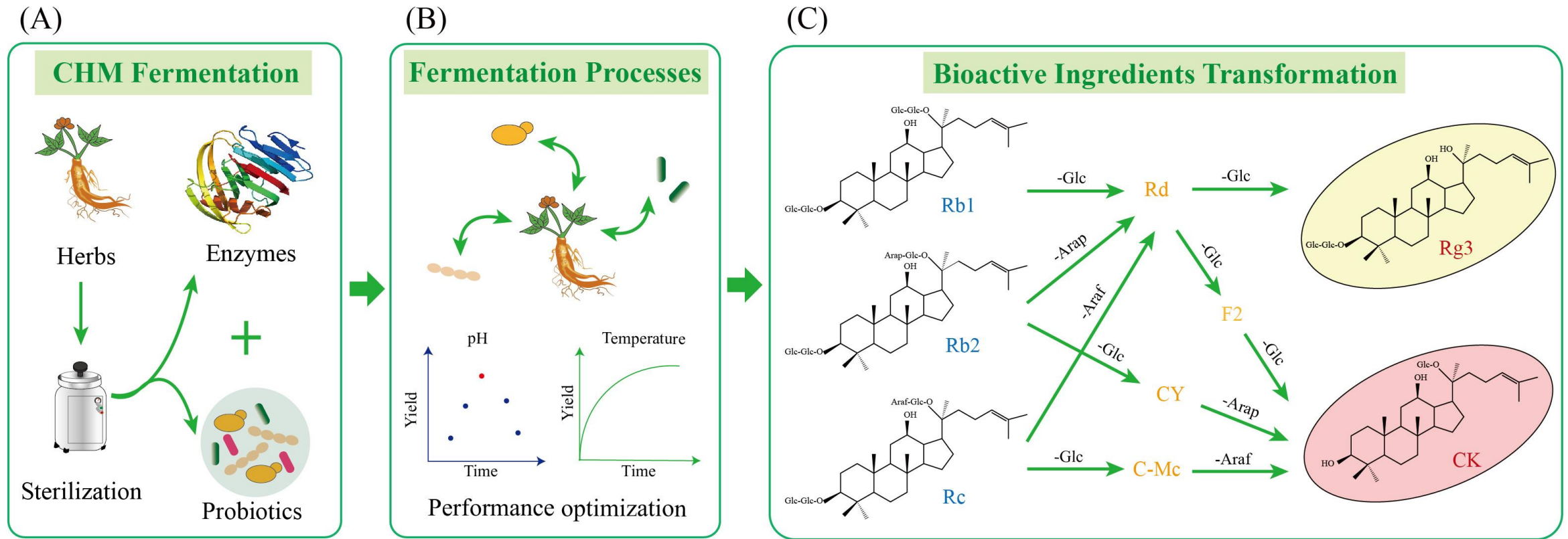


FIGURE2 Biotransformation of main ginsenosides to active rare ginsenosides using efficient enzymes or probiotics. (A) The herbs of *P. ginseng* are sterilized for probiotic fermentation, and the enzymes and probiotics are the main driving forces for CHM fermentation. (B) Probiotic performances during ginseng fermentation can be optimized to improve bioactive ingredients yield. (C) Main ginsenosides can be transformed to bioactive rare ginsenosides during CHM fermentation.

TABLE 2 List of probiotics, medicinal fungi, and a few industrial fungi used for Chinese herbal medicine fermentation.

| Category | Genus | Species | Herbs/Herb formulas used for fermentation | References |
|---------------|-------------------------|---|--|-------------------|
| Bacteria | Lactobacillus | <i>L. plantarum</i> | Red ginseng; Jianweixiaoshi tablets; Soshiho-tang; Rhizoma <i>Artactylodis macrocephalae</i> | [57, 81, 83, 86] |
| | | <i>L. acidophilus</i> | <i>Anoectochilus formosanus</i> Hayata; Jaeumganghwa-tang | [84, 87] |
| | | <i>L. casei</i> | <i>A. formosanus</i> Hayata; Hwangryun-haedok-tang | [84, 88] |
| | | <i>L. paracasei</i> | Red ginseng | [98] |
| | | <i>L. pentosus</i> | <i>Lespedeza cuneata</i> G. Don | [63] |
| | | <i>L. rhamnosus</i> | <i>Panax ginseng</i> ; <i>Salvia miltiorrhiza</i> Bunge | [75, 85] |
| | | <i>L. gasseri</i> | Ginseng seed | [89] |
| | | <i>L. fermentum</i> | <i>P. ginseng</i> | [21] |
| | Bifidobacterium | <i>B. breve</i> | Radix Puerariae | [64] |
| | | <i>B. longum</i> | <i>A. formosanus</i> Hayata; Red ginseng | [84, 98] |
| | | <i>B. lactis</i> | <i>P. ginseng</i> | [75] |
| | | <i>B. animalis</i> subsp. <i>lactis</i> | Red ginseng | [73] |
| | Bacillus | <i>B. subtilis</i> | <i>S. miltiorrhiza</i> Bunge; Ginseng seed; Deer antler; White ginseng roots | [85, 89, 99, 100] |
| | | <i>B. licheniformis</i> | Rhizoma <i>A. macrocephalae</i> | [90] |
| | Alcaligenes | <i>A. spiechaudii</i> | <i>Rhodiola rosea</i> ; <i>Lonicera japonica</i> | [101] |
| Lactococcus | <i>L. lactis</i> | <i>P. ginseng</i> | [98] | |
| Streptococcus | <i>S. thermophiles</i> | <i>Cyclopia intermedia</i> | [102] | |
| Leuconostoc | <i>L. mesenteroides</i> | <i>R. coptidis</i> | [103] | |
| Pediococcus | <i>P. pentosaceus</i> | Ginseng seed | [89] | |
| Fungi | Saccharomyces | <i>S. cerevisiae</i> | <i>Glycyrrhiza uralensis</i> Fisch; <i>Gegen Qinlian decoction</i> | [91, 92] |
| | | <i>S. boulardii</i> | <i>Dioscorea opposita</i> Thunb | [76] |
| | Kluyveromyces | <i>K. marxianus</i> | Rhubarb | [69] |
| | Trichoderma | <i>T. reesei</i> | White ginseng roots | [100] |
| | Ganoderma | <i>G. lucidum</i> | <i>Croton tiglium</i> ; <i>Tripterygium wilfordii</i> ; <i>Artemisia capillaris</i> leaves | [70, 71, 93] |
| | Trametes | <i>T. robiniophila</i> Murr | <i>Radix isatidis</i> | [104] |
| | Grifola | <i>G. frondosa</i> | <i>Rhizoma gastrodiae</i> | [105] |
| | Coprinus | <i>C. comatus</i> | <i>Sophora flavescens</i> | [106] |

Different probiotic CHM fermentation strategies and their characterization

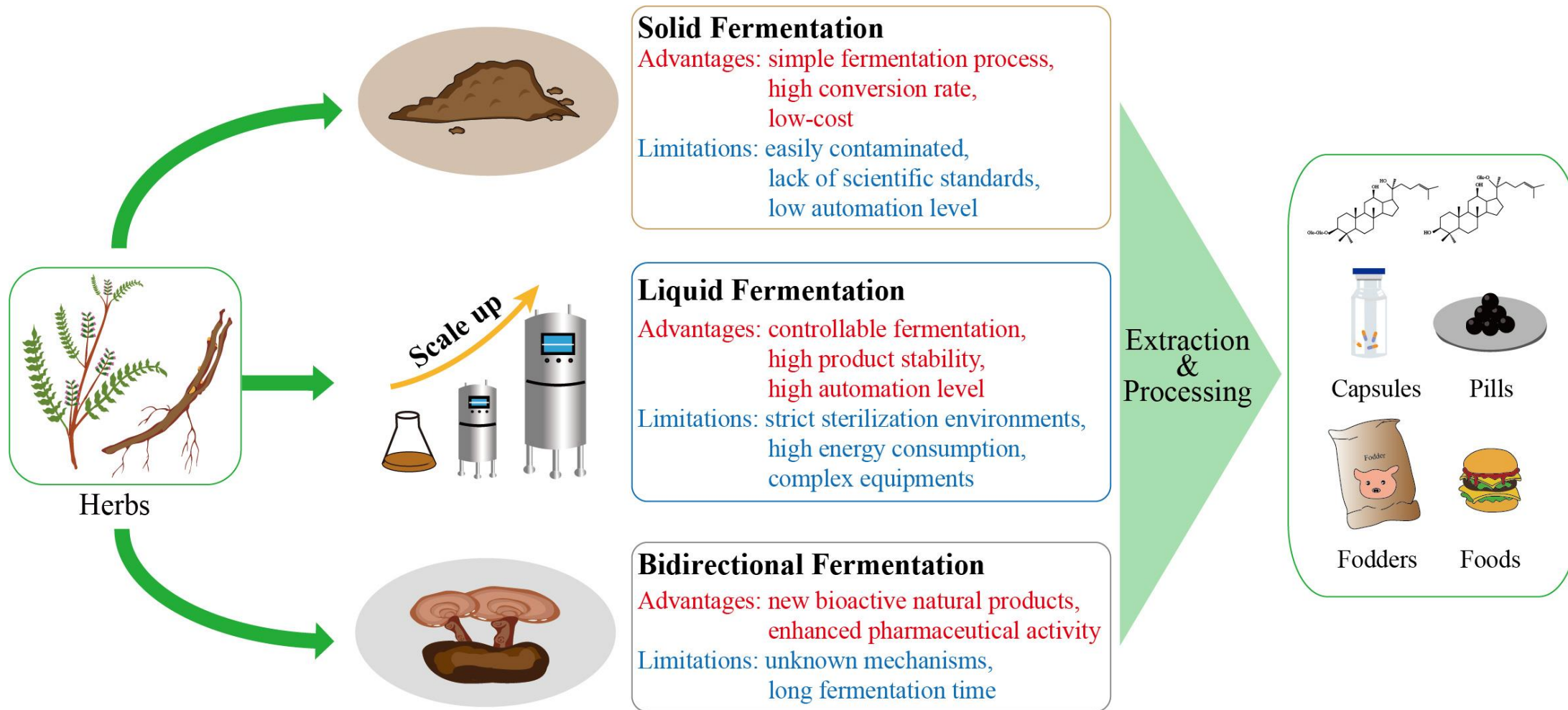


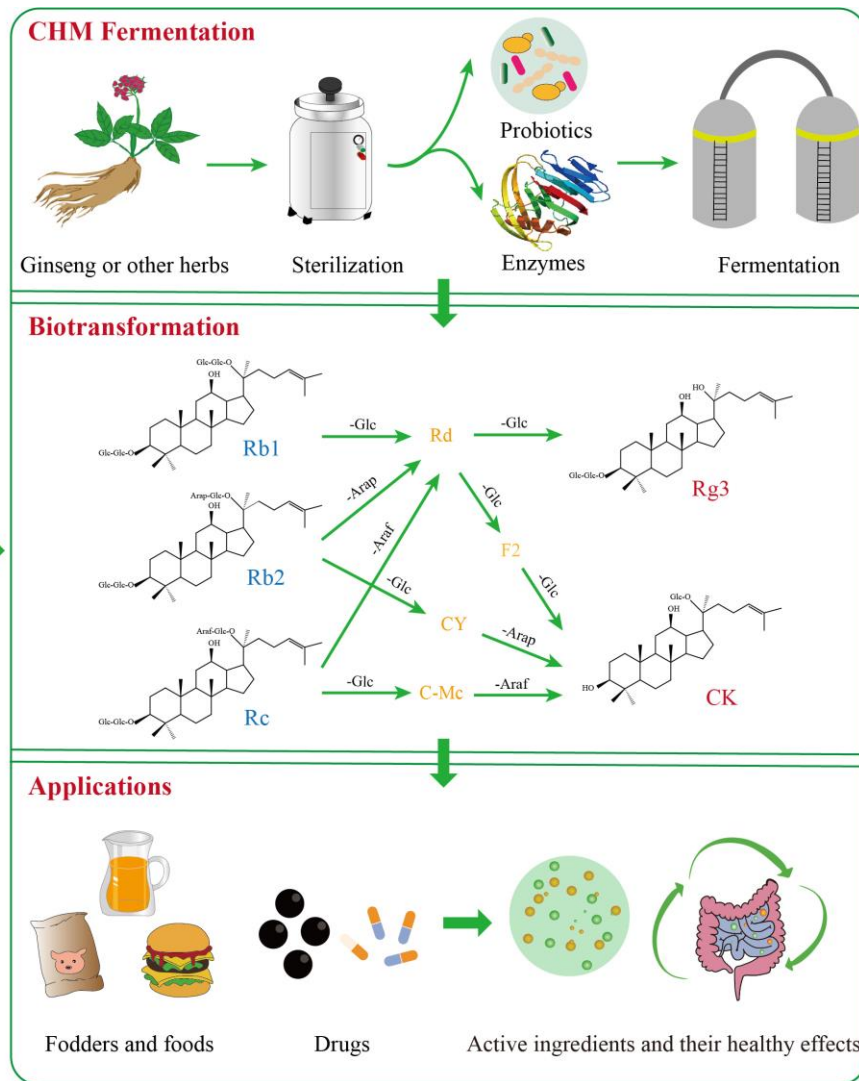
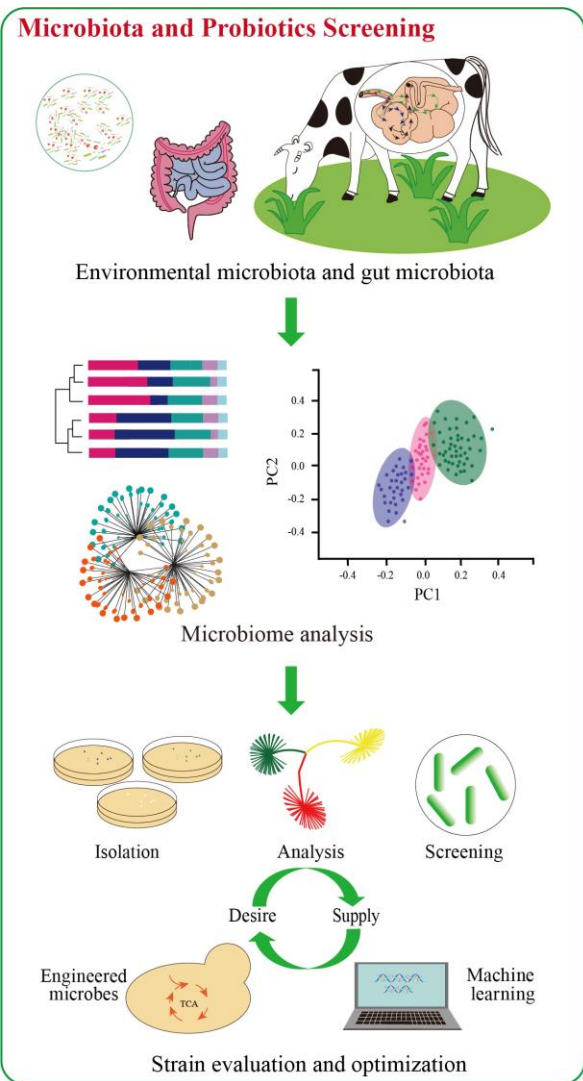
FIGURE 3 Different probiotic CHM fermentation strategies and their characterization. The liquid, solid, and bidirectional fermentation were used for CHM fermentation. After extraction and processing, the final products of CHM fermentation could be used as drugs, fodders, and foods.

The application of synthetic microbiology strategies for efficient probiotic CHM fermentation



FIGURE 4 Application of microbiome and synthetic biology strategies for efficient probiotic CHM fermentation. (A) Environmental microbiota, and human and animal gut microbiota are potential microbial sources for CHM fermentation. (B) Environmental microbiota and gut microbiota can be analyzed by microbiome strategies, and screened for probiotics. (C) Efficient probiotics can be isolated, analyzed, and screened for CHM fermentation. Moreover, the machine learning and metabolic engineering technologies can provide further efficient enzymes or microbes for CHM fermentation. (D) Fermentation process can be optimized, which can lead to the production of bioactive ingredients with high yield. (E) The obtained CHM fermentation products can be applied in foods, animal feeds, drugs, or other industries. The active ingredients would produce beneficial effects for humans and animals.

Summary



Highlights:

- Fermentation of Chinese herbal medicines (CHM) using probiotics can generate easily absorbed bioactive substances and reduce toxicities.
- Probiotic fermentation techniques for CHM are described and discussed.
- Microbiome, synthetic biology, and other cutting-edge biotechnologies improve probiotic fermentation of CHM.

Graphic abstract

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