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Review

Analysis of traditional Chinese medicine based on microbial fermentation and transformation

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Microbial fermentation and alteration of traditional Chinese medicine (TCM) is an important way to achieve a amalgamation of modern biotechnology and TCM's research, and gaining an increasing worldwide popularity as a new type of traditional Chinese medicinal processing approach to develop pharmaceuticals in therapeutic applications. This new technology not only broadens the scope of TCM's research and application, but also provides us a new idea and way to understand and use TCM scientifically and effectively. This paper reviews the current status of microbial fermentation and transformation of TCM at home and abroad, mainly from TCM's ideas, principles, existed problems and significances of research. The focus is on medicinal fungi fermentation and transformation of TCM. In the discussion of specific microorganisms, the emphasis is placed on *Grifola frondosa* (*G. frondosa* or Maitake) and *Ganoderma lucidum* (*G. lucidum*), for which experimental work has been on-going actively in the authors' laboratories. Other selected microorganisms will be discussed only briefly, aiming primarily to illustrate the current status of research in this area.

Key words: Microbial fermentation, transformation, *Grifola frondosa*, microorganism, biotectnology

INTRODUCTION

The microbial fermentation is one of the bio-transformations, long applied to TCM. The Chinese "Compendium of Materia Medica" recorded that the ancients used yeast to make wine more before yeast was used for drug. Subsequently, this "yeast" was called "Shenqu (*Massa Medicata Fermentata*)" in ancient China, and its efficacy was far better. That is to say that the microbiological has been applied to concoct and transformed into TCM in long ancient China (Chen et al., 2010). Therefore, as early as more than a thousand years ago, Chinese have begun to use microbial fermentation of TCM so as to enhance its efficacy, change the potency and reduce toxicity by microorganism. For example, *Massa Medicata Fermentata*, *Semen Sojae Preparatum* (SSP), *Rhizoma Pinelliae Fermentata* and *Pien Tze Huang* are all made by the native single or combined herbs through microbiological solid-state fermentation (Li et al., 2004). An appropriate microbial fermentation of TCM not only can contribute to enhancing immune activity, but plays an important role on bio-transformation for TCM significantly. So we can use microorganism, especially medicinal fungi, to ferment and transform TCM, and accordingly produce a new medicinal material or formulation with varieties of active ingredients.

The medicinal fungi has a powerful enzyme system, which can decompose and utilize the Chinese medicines, such as protein, fiber and carbohydrates so on, and also may bio-transform some secondary metabolites. Meanwhile, some compositions in TCM not only can promote or inhibit the

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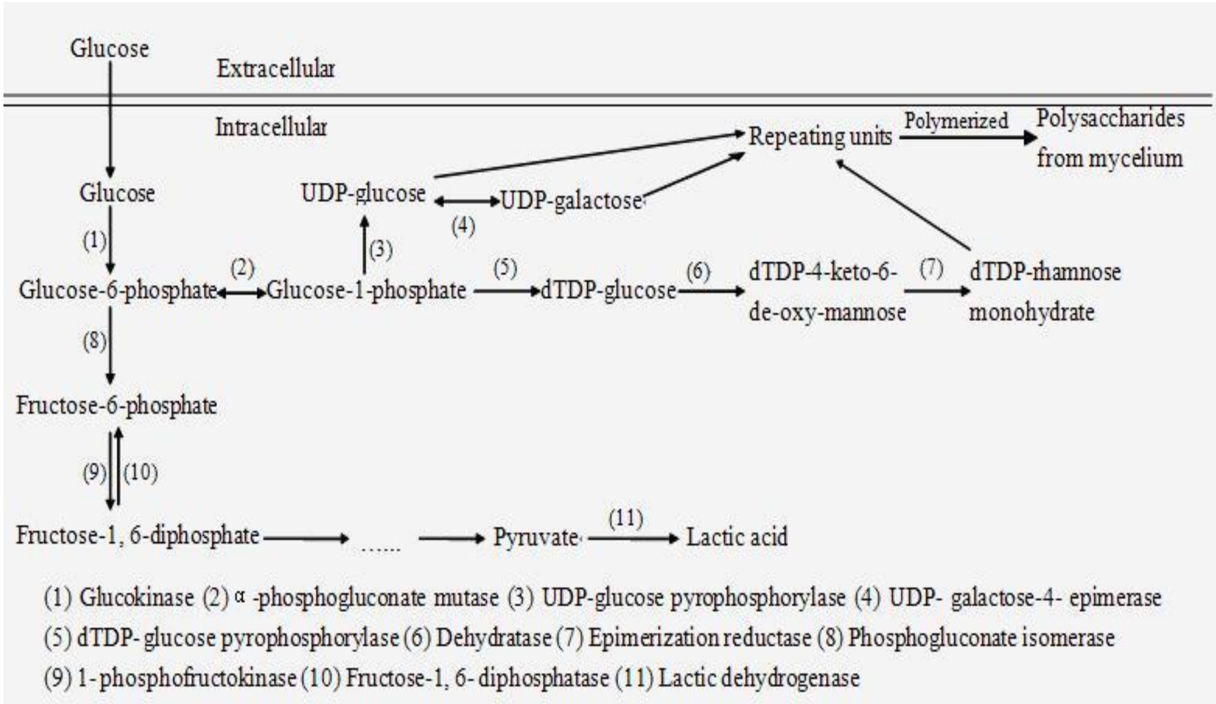

Figure 1. A synthesis pathway of exopolysaccharides from medicinal fungi (Vandamme et al., 2004).

growth of medicinal fungi and generate metabolites, producing some new active compounds by an inter-action between them, but also may reduce some side effects of toxin after biotransformation with medicinal fungi (Yang et al., 2005). Now, it has become a hot topic for medicinal fungi fermentation and transformation of TCM, which also provides a new and modern way to develop and protect TCM in China. In summary, with the pace of progress in the microbial fermentation technology and in-depth research on the modernization of TCM, it will be certain that microbial fermentation and transformation of TCM is being taken seriously, and becomes one method to produce new Chinese drugs and obtain new active compounds.

IDEAS OF RESEARCH ON MICROBIAL FERMENTATION AND TRANSFORMATION OF TCM

Studies have shown that microbial polysaccharides, especially polysaccharides from medicinal fungi, have a very strong biological activity, such as polysaccharides of *G. frondosa* (Nanba, 1995; Hsieh et al., 2008; Huang and Chen, 2009), polysaccharides of *G. lucidum* (Yang et al., 2004; Liu and Zhang, 2007), and polysaccharides of *Phellinus igniarius* (PL) (Zou et al., 2006). Therefore, it has been a concern widely to most researchers on how to get the maximum amount of polysaccharides from medicinal fungi (Yang and Liau, 1998; Tang and Zhong, 2002, 2003).

Li et al. (2006) had reported that when medicinal fungi fermented and transformed TCM, the ingredients of TCM not only could stimulate the growth of microbial cells, but participate in the metabolic processes of microorganisms, and increase the biological activity of microbial secondary metabolites (such as extracellular polysaccharides, EPS).

Moreover, Vandamme et al. (2004) in their books pointed out that α-phosphoglucose mutase (α-PGM) ((2) in Figure 1) and phosphoglucose isomerase (PGI) ((8) in Figure 1) are the two key enzymes in medicinal fungi polysaccharide synthesis and glycolysis. Degeest and De (2000) found that it was the enhanced activity of *α*-PGM for *streptococcus thermophilus* that made carbon flux to increase a higher level in a synthetic pathway from EPS to EMP, so as to enhance the yield of EPS. Here, carbon fluxes referred to glucose-6-phosphate (G-6-P), which was the precursor through synthesis of EPS. Meanwhile, Tang and Zhong's experiment also showed the same result that showed an association between the vitality of *α*-PGM and the production of EPS in the metabolism of *G. lucidum* (2002).

Recently, Xu et al. (2012) reported that the pathway from the glucose-1-phosphate (G-1-P) to UDP-glucose, a branch of polysaccharides of *G. frondosa*, mainly synthesized glucose and/or galactose as a basic unit, and UDP-glucose pyrophosphorylase ((3) in Figure 1) and dTDP-glucose pyrophosphorylase ((5) in Figure 1) are also two key enzymes which could affect the types of polysaccharides from medicinal fungi and monosaccharides. It also confirmed that the vitality of *α*-

PGM in the experimental group increased two times than the control group's, but the vitality of PGI in the experimental group decreased than the control group's.

So this result suggested that there exist a relationship between *α*-PGM and the synthesis of EPS.

Thus, if we could measure the activities of *α*-PGM, PGI, UDP-glucose pyrophosphorylase and dTDP-glucose pyrophosphorylase to plot these kinetic curves of enzymatic activity, and then analyze and compare among these curves, combined with some changes in the compositions of single ingredient in TCM and the product of polysaccharides, we would clarify traditional Chinese herbal ingredients' effects on medicinal fungi and interpret the ideas of research on microbial fermentation and transformation of TCM.

PRINCIPLES OF RESEARCH ON MICROBIAL FERMENTATION AND TRANSFORMATION OF TCM

TCM is rich in chemical compositions, and microbial fermentation and transformation of TCM can obtain new compounds by an interaction between micro-organisms and Chinese herbal ingredients so as to enhance activity and reduce toxicity for TCM (Hsu et al., 2007; Dong et al., 2003).

Principles of microbial fermentation and transformation of TCM can be summarized into the following aspects: (1) Microorganism in the growth process can produce a variety of biological substances, such as protease, amylase, cellulase, esterase and amidase so on, which contain synthase and decomposing enzyme, and these enzymes are the material basis of a chemical reaction in a medicinal fermentation process, which can decompose Chinese medicines and transform them into new ingredients. For example, fermented soybean, as a kind of TCM, is called for *Semen Sojae Preparatum*, and its glycosides have also been transformed into the free aglycones; (2) Microorganism can use active TCM's ingredients as a precursor to form new compounds, at the same time, the secondary metabolites of microorganism and Chinese medicines will also interact to form new compounds; (3) Some ingredients in TCM may change the metabolic pathways of microorganism, accordingly form a new ingredient; (4) Chinese medicines may be concentrated because microorganism will consume proteins, sugars and other substances during growth such as the extraction from dioscins; it is by fermentation that starch is removed from *Dioscorea opposite*.

ADVANCES IN THE STUDY ON MIICROBIAL FERMENTATION AND TRANSFORMATION OF TCM

Effects of TCM on *G. frondosa* by submerged culture

G. frondosa is a kind of edible medical fungi, and its fruiting bodies are rich in polysaccharides, especially its EPS which has various physiological activities, such as anti-tumor, immunity activity, antioxidant and superoxide anion scavenging (Suzuki et al., 1989; Deng et al., 2009; Kodama et al., 2003; Nanba et al., 2000; Lin, 2011). So, it has become a hot topic how to accelerate the mycelia biomass (BIO) and EPS of *G. frondosa* in maximum amount, and increase the production of active products in recent years (Hsieh et al., 2006, 2008), in which the way of adding proper amount of TCM into the fungal culture has been running successfully (Table 1).

However, the above also listed in Table 1 showed us different effects of different TCMs on *G. frondosa*, meanwhile, there exists an interaction between TCM and microorganism. For some effects of *G. frondosa* on TCM, especially effects of *G. frondosa* on some main ingredients of *R. gastrodiae*, our groups had analyzed the main ingredients in the ethanol extract of fresh *R. gastrodiae* qualitatively and quantitatively by HPLC and LC-MS in detail, and some changes in the components of *R. gastrodiae* before and after Maitake's fermentation (Xu et al., 2012; Wang et al., 2012; Zhang et al., 2012). Our results suggested that *G. frondosa* itself in the fermentation process may decompose and utilize some *R. gastrodiae*'s ingredients. Furthermore, the pure gastrodin (GA), hydroxybenzyl alcohol (HA) and *p*-hydroxybenzaldehyde (HBA) with different concentrations were respectively added into *G. frondosa* fermentation system, however, the pure of GA could hardly promote the cell growth of *G. frondosa* and its synthesis of EPS, on the contrary, 10 mg/L HA and 20 mg/L HBA would significantly promote the growth of Maitake and its synthesis of EPS; especially, 20 mg/L HBA played a more significant role. So we concluded that HA and HBA were the two key ingredients for promoting Maitake's fermentation.

Meanwhile, we also found another interesting experimental phenomenon. After sterilization, 5 %(v/v) *R. gastrodiae* alcohol extract was added into the fermentation broth of *G. frondosa*; GA could re-synthesize parishin by submerged fermentation (Wang et al., 2012). This interesting and important discovery not only certifies that *G. frondosa* could utilize own cells or enzymes to re-synthesize gastrodin into parishin by submerged fermentation, but has an important theoretical and practical significance for inter-transformation between fungal microorganisms and TCM.

Effects of TCM on *G. lucidum* by submerged culture

Like *G. frondosa*, a kind of edible medical fungi, *G. lucidum* is also a hot topic (Table 2).

Effects of TCM on other microorganisms by fermentation

Apart from effects of TCMs on *G. frondosa* and *G. lucidum*,

Table 1. Research for TCM's effects on *G. frondosa* in recent years.

Samples of TCM	Effects on *G. frondosa*
Buckwheat (Zhao, 2008)	With addition of *Buckwheat* into the submerged culture of *G. frondosa*, and the EPS biosynthesis of *G. frondosa* increased by 0.5 g/L.
R. gastrodiae (Zhao, 2008); (He and Wu, 2011);	(1) With addition of *R. gastrodiae* into the submerged culture of *G. frondosa*, the EPS biosynthesis of *G. frondosa* increased by 2.1 g/L; (2) After screen and optimize the medium of Maitake's fermentation and transformation of *R. gastrodiae* by Plackett-Burman, a further result showed that 3.91 g/L EPS could be obtained in the submerged culture of *G. frondosa* with adding the ethanol extract of *R. gastrodiae*, which increased by 3.4% compared with no addition of *R. gastrodiae* in the control group.
Yam (Zhao, 2008)	With addition of *Yam* into the submerged culture of *G. frondosa*, and the EPS biosynthesis of *G. frondosa* increased by 1.2 g/L.
Fructus arctii (Kim et al., 2010)	(1) Some enzymes secreted from *G. frondosa*, such as β-glucosidase, would convert the glycosides (arctiin and caffeic acid derivatives) into aglycones (arctigenin and caffeic acid); (2) The fermented *Fructus arctii* extract with *G. frondosa* (G-FAE) had antioxidant and 5-lipoxygenase inhibitory activities.
R. gastrodiae (Zhang et al., 2012)	With addition of 5 %(v/v) ethanol extract of fresh *R. gastrodiae* into the submerged culture of *G. frondosa*: (1) The BIO and EPS biosynthesis of *G. frondosa* were both promoted from 0.564±0.09 to 1.324±0.25 g/L and from 71.69±0.53 to 107.08±0.85 mg/L, separately increased by 134.75% and 49.37%, respectively; (2) However, intracellular polysaccharides (IPS) content declined from 60.38±0.87 to 45.71±0.66 mg/g, which decreased by 24.30% compared with no addition of *R. gastrodiae* in the control group, respectively.
R. gastrodiae (Wang et al., 2012)	From a perspective of fermentation kinetics, with addition of 7 %(v/v) ethanol extract of *R. gastrodiae*: (1) The highest amount of mycelia and EPS were increased to 2.0630±0.0520 g/L and 89.3846±3.2422 mg/L, accordingly increased by 169.32% and 52.49% compared with the control without addition of *R. gastrodiae*; (2) The respective top of BIO and EPS appeared on the 10th and 8th day, whereas in the control, BIO and EPS simultaneously reached their tops on the 10th day; (3) So the synthesis of EPS seemed to be more synchronize with the cell growth on the whole in the control, perhaps the extract stimulated an earlier EPS production, that is to say *R. gastrodiae* can reduce the cycle of Maitake's EPS synthesis.
R. gastrodiae (Xu et al., 2012)	From a point of microbial metabolism, with addition of *Gastrodia* tuber components in culture medium of *G. frondosa*: (1) Two enzymes related to EPS synthesis, α-phosphoglucomutase (α-PGM) and phosphoglucose isomerase, the activity of α-PGM increased, while the activity of phosphoglucose isomerase decreased; (2) Mixture components of ethanol extract of *Gastrodia* tuber was more effective than pure gastrodine; (3) A maximum dry cell weight of 138.5 mg/L and the EPS at 0.606 g/L were obtained when the unprocessed *Gastrodia* tuber culture was added.

we also have briefly summarized effects of other TCMs on other microorganisms by fermentation and transformation (Table 3).

Additionally, some complexes or preparations of TCM fermented with some microorganisms also produce lots of wonderful and beneficial effects, for example: (1) Liuweidihuang (LWDH), consisting of six kinds of TCMs: *Rehmannia glutinosa*, *Cornus officinalis*, *Dioscorea opposite*, *Rhizoma alismatis*, *Cortex moutan radicis* and *Wolfiporia cocos*, was fermented with the microbial so that the data showed that the improving effects of fermented decoction of LWDH on the cellular immunity and humoral immunity were potent than those of decoction of LWDH (Guo et al., 2001); (2) Kangqiang capsule is a kind of traditional Chinese medicinal preparation, and a combination and application of Photosynthetic bacteria (PSB) and Kangqiang capsule had a stronger antitumor effect compared with the pure Chinese herbal compounds (Shi and Zhang, 2003).

PROBLEMS THAT EXISTED IN MICROBIAL FERMENTATION AND TRANSFORMATION OF TCM

The technical means of medicinal fungi fermentation and transformation of TCM have been widely recognized and applied at home and abroad. As mentioned in above "ADVANCES", there have also been a lot of literatures to support that medicinal fungi transformation of TCM will have a stronger biological activity and new pharmacological efficiencies.

Although many TCMs are effective in treating diseases, their remedial mechanism is not well understood. The analysis of active components in Chinese medicinal extracts is a key to unlocking the secret of their effectiveness (Cai et al., 2002). However, presently there is still a lack of more in-depth and systematic study for the interaction between these active ingredients and the metabolic process of medicinal fungi, as well as the synthesized mechanism of these active ingredients.

Table 2. Research for TCM's effects on *G. lucidum* in recent years.

Samples of TCM	**Effects on *G. lucidum***
Eupolyphaga sinensis (Liu and Zhang, 2007)	The ethyl acetate extract of *Eupolyphaga sinensis* at 55 mg/L would lead to significant increase in both BIO and IPS concentration of *G. lucidum*, from 8.53±0.41 to 14.16±0.43 g/L and 1.28±0.09 to 2.13±0.11 g/L, respectively.
Catharsius molossus (*C. molossus*) (Liu and Zhang, 2007); (Liu et al., 2011)	(1) The ethyl acetate extract of *C. molossus* at 55 mg/L would significantly enhance EPS yield, which increased from 350.9±14.1 to 475.1±15.3 mg/L; (2) *Cis*-9,10-methylenehexadecanoic acid and hexadecanoic acid, identified of the two key ingredients of *C. molossus* by GC-MS, would play a catalytic and promoted role in both the growth of *G. lucidum* and the synthesis of triterpenoids, especially *cis*-9,10-methylenehexadecanoic acid could significantly promote the synthesis of triterpenoids.
Angelicae sinensis (Yang et al., 2003)	Addition of the ethanol extracts from *Angelicae sinensis* promoted the yield of EPS, which reached to 572.80 mg/L compared with 231.10 mg/L in the control group, but inhibited the growth of *G. lucidum*.
Dioscorea opposite (or Chinese yam) (Yang et al., 2003)	The BIO and EPS of *G. lucidum* in group with addition of the ethanol extracts from *Dioscorea opposite* reached separately to 22.30 g/L and 350.20 mg/L, compared with 12.80 g/L and 231.10 mg/L in the control group.
Root of pilose asiabell (Yang et al., 2003)	The BIO and EPS of *G. lucidum* in group with addition of the ethanol extracts from *Root of pilose asiabell* reached separately to 26.70 g/L and 440.90 mg/L, compared with 12.80 g/L and 231.10 mg/L in the control group.
Radix achyranthis bidentatae (*R. achyranthis bidentatae*) (Yang et al., 2003)	The BIO and EPS of *G. lucidum* in group with addition of the ethanol extracts from *R. achyranthis bidentatae* reached separately to 28.60 g/L and 417.10 mg/L, compared with 12.80 g/L and 231.10 mg/L in the control group.
Dendrobium (Yang et al., 2003)	A little like *Angelicae sinensis*, addition of the ethanol extracts from *Dendrobium* promoted the yield of EPS, which reached to 378.90 mg/L compared with 231.10 mg/L in the control group, but inhibited the growth of *G. lucidum*.
Astragalus mongholicus (*A. mongholium*) (Wang et al., 2005)	With the optimal addition of 1.0 g/dL, the wet weight of bacteria reached to 86.4 g/dL, and crude IPS and EPS also increased to 281.5 mg/dL and 463.9 mg/dL, seperately. At the same time, a analysis by Sephadex G-75 showed that the components in produced polysaccharides had also been changed.
Semen coicis (Bi et al., 2011)	*Coix lacryma-jobi* oil and *Coixenolide,* two components of *Semen coicis*, both could improve *G. lucidum* cell growth, production of polysaccharides and ganoderic acids: (1) The BIO, EPS, IPS and ganoderic acids with addition of 2 %(v/v) *Semen coicis* oil were separately 3.58, 2.44, 2.24 and 4.04 times than the control group; (2) The BIO and IPS with addition of 0.50 %(v/v) coixenolide were 1.17 and 1.60 times than the control group, respectively; (3) The EPS with addition of 0.05 %(v/v) coixenolide was 2.4 times than the control group; (4) The ganoderic acids with additon of 0.20 %(v/v) coixenolide was 16.42 times than the control group.
Flos lonicerae (Wang et al., 2004)	The BIO and EPS of *G. lucidum* in group with addition of 100 g/L powders of *Flos lonicerae* reached separately to 7.10 g/L and 236 mg/L, compared with 13.70 g/L and 318 mg/L in the control group.
Fructus forsythiae (Wang et al., 2004)	The BIO and EPS of *G. lucidum* in group with addition of 100 g/L powders of *Fructus forsythiae* reached separately to 12.80 g/L and 229 mg/L, compared with 13.70 g/L and 318 mg/L in the control group.
Ephedrasinica stapf (Wang et al., 2004)	The BIO and EPS of *G. lucidum* in group with addition of 100 g/L powders of *Ephedrasinica stapf* reached separately to 31.30 g/L and 1451 mg/L, compared with 13.70 g/L and 318 mg/L in the control group.
Semen raphani (Wang et al., 2004)	The BIO and EPS of *G. lucidum* in group with addition of 100 g/L powders of *Semen raphani* reached separately to 26.30 g/L and 1177 mg/L, compared with 13.70 g/L and 318 mg/L in the control group.
Radix scutellariae (*R. scutellariae*) (Wang et al., 2004)	Inhibition on the growth of *G. lucidum* was observed when 100 g/L powders of *R. scutellariae* were used.
Radix sophorae flavescentis (*R. sophorae flavescentis*) (Li et al., 2006)	The cultured broth supplemented with aqueous extract of *R. sophorae flavescentis* had effects of anti-hepatitis B virus activity *in vitro* and protected mice from liver damage *in vivo*, meanwhile, the co-fermentation broth of *G. lucidum* in the presence of aqueous extract of *R. sophorae flavescentis* had better medicinal effects than simply mixing these two ingredients together, suggesting a potential novel way to prepare Chinese herbal mixtures.

Table 3. Research for TCM's effects on other microorganism in recent years.

Samples of TCM	Samples of microorganism	Effects
Panax ginseng (*P. ginseng*) (Kim et al., 2010)	*Hericium erinaceum*	*Hericium erinaceum*, a kind of bearded tooth mushroom, its mycelia cultivated in mushroom complete medium (MCM) supplemented with *ginseng* extract (GE) enhanced the immuno-stimulation and anti-metastasis.
Carthamus tinctorius (He et al., 2005)	*Bacillus sp.* C2-13	A processing method to enhance thrombolytic effect of *Carthamus tinctorius* using a fermentation technology with *bacillus sp.* C2-13 showed that *Carthamus tinctorius* by fermentation not only increased its fibrinolytic activity and anticoagulant effect, but enhanced thrombolytic efficacy.
Viscum coloratum (or *Mistletoe*) (Yang and Zhang, 2006)	Photosynthetic bacteria (PSB)	Antitumor activity of PSB transforming *Mistletoe* preparation showed that PSB's transformation could reduce the toxicity of *Mistletoe*, and have a synergistic effect in terms of anti-tumor.
Fructus arctii (Xu et al., 2007)	HB-2 (A strain screened from *Fructus arctii*)	Screening strains HB-2 could ferment and transform of *Fructus arctii* to obtain a wealth of arctigenin with the β-glucosidase activity.
Panax notoginseng (Han et al., 2007)	*Fusarium sacchari*	Han et al. (2007) also utilized screened *Fusarium sacchari* from planted ginseng soil to biotransform the active component notoginseng triterpenes in *Panax notoginseng* stalks and leaves, and three kinds of rare ginsenosides with anti-tumor activity (CK, C-Mx and G-Mc) were obtained in a high yield.

Accordingly, these immaturity mentioned above have led to this technical means not been widely recognized at home and more abroad (Liu et al., 2011; Zhang et al., 2012). Furthermore, how to work and interact between Chinese medicines and fungal metabolism, and how to enhance the biological activity of fermented products are puzzled now, and these further breakthroughs and progresses will lay a solid scientific basis for the development of modern TCM. Therefore now, the complex mechanism of microbial fermentation and transformation of Chinese medicines is the most urgent problem that needs to be resolved.

CONCLUSION

In recent years, with the advances in microbial fermentation technology and the depth in research on the modernization of TCM, the study on microbial fermentation and transformation of TCM is being gradually taken seriously and has become a new way to produce new drugs and get new active compounds in TCM. The significances of microbial fermentation and transformation of TCM are mainly as following:

(1) The method of microbial fermentation and transformation of TCM can get faster new drugs with own intellectual property rights, and may obtain the high efficiency of 1+1>2. Moreover, there is almost no side effect in the obtained drugs, and the fermented products can be utilized comprehensively without leftovers or waste residues (You et al., 2005);

(2) The method of microbial fermentation and transformation of TCM is a perfect combination of modern biotechnology and traditional Chinese medicinal research, and it has become a hot topic. The microbial fermentation into TCM not only expands a new space for TCM's research and development, but also provides a new idea for the protection and utilization of TCM resources (Li et al., 2004).

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