ISSN: 2637-6067 DOI: 10.6918/IJOSSER.202206_5(6).0007

Application of Biotransformation Technology in Pharmaceutical

Lingmin Jiang[1], Yuefang Luo[2]

[1]Changde Vocational Technical College, Hunan Changde, 415000, China

[2]Xintian County Market Supervision Administration, Yongzhou, Hunan, 425700, China

Abstract

The use of exogenous natural or synthetic organic compounds as a substrate, added to the growing state in the biological system or enzyme system, under appropriate conditions for culture, the substrate and the enzyme in the biological system interaction,resulting in Structure change,this process known as biotransformation, also known as biocatalysis, its essence is the enzyme catalytic reaction.Compared with chemical catalysts, biotransformation with high specificity, conditions are mild, selective, less by-product and do not pollute the environment and so on. In recent years, biotransformation to structural modification of the chemical constituents of traditional Chinese medicine, novel structure, better lead compounds can be obtained, or are valuable intermediates.Biological transformation to the pharmaceutical industry in drug research and a series of ground-breaking application creates enormous health and economic value, has become an effective way to develop new drugs.

Keywords

Biotransformation; Suspension culture; Secondary metabolism; Microbial transformation; Enzymatic conversion.

1. Introduction

Plant cell suspension culture is one of the commonly used plant biotechnology, cell proliferation speed, good dispersion. Through the induction of explants and callus subculture, the establishment of a good suspension cell line, which can be biotransformation for drug production and new drug research and development.Microbial transformation is the use of microbial cells to produce one or more enzymes to a compound into a structure of the more economic value of the product, to complete the conventional chemical method is difficult to achieve the biochemical reaction, the essence of the use of micro-organisms produced by itself Enzyme catalyzed reaction of exogenous substrates. Enzyme, as a highly efficient, specific biocatalyst, with its unique advantages are widely used. The formation process

Of biotransformation products closer to or equal to thenatural process [1,2], American FDA has to include these products in natural products, which provides a good soft environment for the marketization of biotransformation. China, as the world's largest producer and exporter of chemical raw materials, workingin drug biotransformation are not carried out more. However, chemical production of high energy consumption, complex composition of waste, serious environmental pollution [3]. Along with the socio-economic development and the improvement of human civilization, is the development trend of green medicine, pharmaceutical companies will commit itself to take on green development mission.Although our country in the biotransformation of drugs work is not much, but has made some achievements, there is a certain accumulated, continue to develop and strengthen research in this area for pharmaceutical, functional food and natural skin care products research and development have a profound impact, also derived a series of innovative and efficient products.

ISSN: 2637-6067 DOI: 10.6918/IJOSSER.202206_5(6).0007

2. Results

Biotransformation technology can not only change the content of main components in traditional Chinese medicine, the structure of active substances, expand the scope of drug source, can also be toxic to traditional Chinese medicine for sustained attenuation. Traditional chemical synthesis uses a lot of organic solvents, such as acetone, pentane, chloroform etc, which possess corrosive, toxic and carcinogenicity, and they also can cause environmental pollution and menace human health. Therefore, some scientists have developed a prerequisite for the development of new reactions and technologies so as to reduce waste generation and solvent usage, minimize energy input, improve safety, and attain materical and cost efficiency [4].

Biotransformation is one such system that is catalyzed by whole cells (microbes, plant cells, animal cells), or by enzymatic or due to the high purity and high stereo selectivity in conjunction with the product and high enantiomeric excess [5]. Compared with chemical modifications, biotransformation is a useful tool to modify the structures of bioactive natural lead compounds (Table 1), with the advantages of high stereo and regioselectivity, mild conditions, and a simple operation procedure [6].

Table 1. Example for use of biotransformation system in pharmaceutical

Transformation system	Used for
Silymarin hairy roots culturing system[7]	culturing silymarin hairy roots and producing silibin
Transgenic hairy roots of Solanum lycopersicum[8]	provide consolidated base for low-cost preparation of improved oral vaccine against rabies
Agrobacterium rhizogenes was used to induce hairy roots of A. Annua[9]	Products artemisinin
S. baicalensis hairy roots[10]	Promoting flavone biosynthesis
Callus and cell suspension culture of Scrophularia striata Boiss[11]	obtain an in vitro acteoside producing cell line
cell suspension cultures of S. Involucrata[12]	production of bioactive compounds syringin, chlorogenic acid and 1,5-dicaffeoylquinic acid

Bioconversion system can be divided into the plant transformation systems, microbial transformation system, enzymatic transformation system.

3. Plant Transformation Systems

Plant transformation system has huge potential to produce specific secondary metabolites, and plant unique enzyme contains many microbes do not exist in, they can catalyze certain reactions to generate many complex compounds, even new chemical compounds[13,14]. Plants synthesize a large array of natural products or secondary metabolites which are important for them to survive and flourish in the natural environment. At the same time, different types of secondary metabolites, such as saponins and essential oils, were produced by plants throughout longterm co-evolution as a defence response mechanism against pathogens, probably including endophytic fungi [15,16]. Therefore, the use of plants for the production of drugs and new drug development has great potential.

Compared with the microbial biotransformation system, the plant biotransformation system has a long doubling time of biomass and less enzyme species.However, plant biotransformation also has its unique features: there are many unique enzymes in plants, and microorganisms do not have, they can catalyze a certain reaction to generate many complex compounds.Plant

ISSN: 2637-6067 DOI: 10.6918/IJOSSER.202206_5(6).0007

transformation systems including cell suspension culture system(Table 2), immobilized cell culture, the hairy root culture system and so on.

Free cells in suspension culture were the first to be developed using the system of bioconversion of plants, which can large-scale synthesis of secondary metabolites and the massive transformation of exogenous compounds, has advantages of direct use of precursors, less movement restrictions and does not affect cell viability and physiological state of the media [17], etc.

Table 2. Example of cell suspension culture system

Cell suspension cultures	Substrate	Product	Conversion rate (%)
Arnebia euchroma[18]	Rred naphthoquinone pigment	Alkannin/Shikonin	50.00
Centella asiatica[19]	/	Asiaticoside/Madecassoside	60.08
Polygonum multiflorum[20]	6-benzylaminopurine	Methyl jasmonate/Salicylic acid	81.67
Lactobacillus plantarum[21]	Total saponins	Ginsenoside Rd	35.00

Hairy root is a type of transformed root induced by the infection of wounded plant tissue with the soil bacterium Agrobacterium rhizogenes bearing the root-inducing (Ri) plasmid[22].

Plant hairy roots culture(Figure 1) is a kind of plant tissue cultures, growth does not depend on exogenous phytohormones, synthesis of secondary metabolites of strong and stable(Table3), and has the advantage of biological transformation function and the ability to propagate[23]. Agrobacterium rhizogenes induced hairy root cultures are entering into a new juncture of functional research in generating pharmaceutical lead compounds by bringing about chemical transformations aided through its inherent enzyme resources. Rational utilization of hairy root cultures as highly effective biotransformation systems has come into existence in the last twenty years involving a wide range of plant systems as well as exogenous substrates and diverse chemical reactions. To date, hairy root cultures are preferred over plant cell/callus and suspension cultures as biocatalyst due to their genetic/biochemical stability, hormone-autotrophy, multi-enzyme biosynthetic potential mimicking that of the parent plants and relatively low-cost cultural requirements[24,25]. The resultant biotransformed molecules, that are difficult to make by synthetic organic chemistry, can unearth notable practical efficacies by acquiring improved physico-chemical properties, bioavailability, lower toxicity and broader therapeutic properties.

Table 3. Example of plant hairy roots culture system

Plant hairy roots culture	Substrate	Days(d)	Product	Biomass accumulation (mg/L)
Prunella vulgari[26]	MS/BA/NAA	49	Ttotal flavonoids	6.615
Withania somnifera[27]	Salicylic acid	30	Withanolide A	0.647
Astragalus membranaceus[28]	MS	34	Isoflavonoids	0.235
tartary buckwheat [29]	MS	20	Rutin	0.590

ISSN: 2637-6067 DOI: 10.6918/IJOSSER.202206_5(6).0007

Biotransformation by plant cell has high selectivity and high catalytic efficiency, mild reaction conditions, fewer by-products as well as less pollution and so on. Synthesis of plant tissue as a bioreactor for converting natural active ingredients, the key lies in plant tissue culture medium of choice and determination of conversion condition, different kinds of plant tissue culture effects on substrate conversion rate considerably.

4. Microbial Transformation System

Microorganisms are widely distributed, proliferating quickly and easily, and they are very adaptable to the changes of the natural environment, and are rich in enzymes.The biotransformation of microorganisms and their enzymes can produce many useful compounds.The method has the advantages of short biomass doubling time, wide application of microbial gene manipulation methods, etc.

Microbial transformation by means of microbial cells to complex structural modification of the substrate, or by microbial metabolic processes generated in one or a series of enzyme-substrate reaction catalyzed by specific parts of it.It's a useful tool for organic chemists looking for new compounds, as a consequence of the variety of reactions for natural products. Microbial transformation includes hydroxylation, hydrogenation [30], dehydrogenation, epoxidationof redox reaction, hydrolysis, esterification, hydration, and acyl transfer, amination, dehydration, isomerization [31], aromatization and other kinds of chemical reactionStructural modification of steroids through whole-cell biocatalysis is an invaluable procedure for the production of active pharmaceutical ingredients (APIs) and key intermediates. Modifications could be carried out with region and stereospecificity at positions hardly available for chemical agents.

Response of different microbial transformation relates to diversity, which can produce a new compound, complex through a series of chemical reactions [31], therefore, people can find new medicinal value of derivatives by microbial transformation method, you can also find that these reactions in the microbialenzymes, and then used for industrial production. In recent years, pharmacy workers carried out extensive research system of microbial transformation, in order to study the active constituents of natural drugs as a starting point, and studies the structure modification on steroids[32], Terpenoids are a large family of natural products exhibiting a wide range of biological activities such as antibiotics, anti-inflammatory, anti-HIV and anti-tumor effects; hypotensive agents; sweeteners; insecticides; anti-feedants; phytotoxic agents; perfumery intermediates; and plant growth hormones[33].

Microorganism as a living organism, its volume is small, but has a complete enzyme system of its own. High recovery rate in the production process, low cost and bioconversion system can be used for industrial production[34].As a result of microbial culture is simple, wide variety, abundant enzyme, many advantages compared with chemical catalyst does not, therefore the use ofmicrobial transformation techniques in the study of active ingredients in medicinal plants is a hot research field of bio medicine technology content.Through the key enzyme purification and characterization of microorganisms, microbial conversion based on streamlined conversion process, to improve the efficiency.

5. Enzymatic Transformation System

The enzyme in biological system has high stereoselectivity, so it is a very effective and feasible method to select three-dimensional structure-specific compound with higher activity by screening and using microorganism or enzyme.In the method of enzyme synthesis(Table 4), less extracted from natural microbial enzymes, recombinant DNA technology can be used, a key enzyme gene expression through carriers to transfer to the new hosting, resulting in a large number of enzymes of goods [34].

ISSN: 2637-6067 DOI: 10.6918/IJOSSER.202206_5(6).0007

Table 4. Example of enzyme transformation system

Enzyme transformation system	Substrate	Temperature (°C)	pH	Product	Conversion rate (%)
Esteya vermicola CNU 120806[35]	Ginsenosides Rb1	50	5.0	Gypenoside LXXV	95.4
L-arabinose isomerase[36]	D-galactos	60	8.0	D-tagatose	43.9
Sucrose phosphorylase (EC 2.4.1.7, Sucrose phosphorylase, SPase) [37]	Hydroquinone	37	6-6.7	Alpha-arbutin	78.3

6. Conclusion and Discussion

At present, a large number of novel compounds have been obtained by biotransformation technology, including many derivatives of natural products with good activity, which provide valuable lead compounds for the development of new drugs. Biotransformation offers chemo enzymatic system to modify the compounds into their novel analogues which are difficult to synthesize by chemical methods, resulting biotransformed products represent interesting structural transformations.

To sum up, with the advance development of technology, multidisciplinary association for drugs research has become a leading trend. It is worth mentioning that biotransformation is a green technology and process in developing drugs which is far better than traditional chemical synthesis. It is also an effective method in obtaining novel or rare anti-cancer compounds which are difficult to synthesis in laboratories.

With the further development of Chinese medicine research, biotransformation technology has been different periods of medical experts continue to enrich and develop.However, there is still a long way for biotransformation technology as an emerging important tool. Firstly, although there are many kinds of enzymes and microorganisms, the biotransformation reaction and related mechanisms of these are still unclear, which means there are future development prospects. Secondly, some existing biotransformation parameters need further investigation and optimization so as to enhance the production . At the same time, we still need from the perspective of modern science to explore the biotransformation process and mechanism, greatly enriched traditional Chinese medicine fermentation practice and theory.

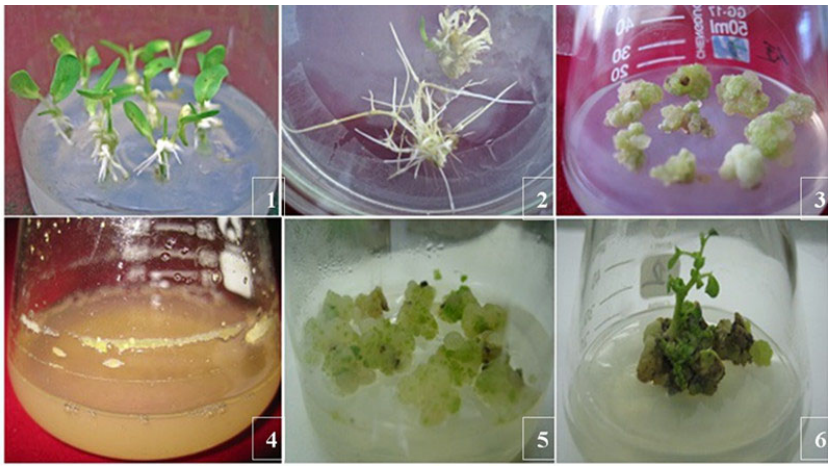

Figure 1. Licorice hairy root cluture

1.Licorice hairy root induction 2.Hairy root culture 3.Hairy root callus induction 4.Hairy root cell suspension culture 5.Induction training 6.Regeneration plant cultivation

ISSN: 2637-6067 DOI: 10.6918/IJOSSER.202206_5(6).0007

References

[1] Funes-Collado V, Morell-Garcia A, Rubio R, et al. Study of selenocompounds from selenium-enriched culture of edible sprouts. Food Chem, 2013,141(4):3738-43.

[2] Mao Z, Sun W, Fu L, et al. Natural dibenzo-alpha-pyrones and their bioactivities. Molecules, 2014,19(4):5088-108.

[3] Wang Y, Chen S, Yu O. Metabolic engineering of flavonoids in plants and microorganisms. Appl Microbiol Biotechnol, 2011,91(4):949-56.

[4] Cheng XD, Wei MG. Profiling the Metabolism of Astragaloside IV by Ultra Performance Liquid Chromatography Coupled with Quadrupole/Time-of-Flight Mass Spectrometry. Molecules, 2014,19(11):18881-96.

[5] Gao F, Zhang JM, Wang ZG, et al. Biotransformation, a promising technology for anti-cancer drug development. Asian Pac J Cancer Prev, 2013,14(10):5599-608.

[6] Tang YJ, Zhao W, Li HM. Novel tandem biotransformation process for the biosynthesis of a novel compound, 4-(2,3,5,6-tetramethylpyrazine-1)-4'-demethylepipodophyllotoxin. Appl Environ Microbiol, 2011,77(9):3023-34.

[7] Zhang SL, Zhang TZ, Yang SH. [Establishment of culture system of Silybum marianum hairy roots and determination of silybin]. Zhongguo Zhong Yao Za Zhi, 2014,39(11):2005-10.

[8] Singh A, Srivastava S, Chouksey A, et al. Expression of Rabies Glycoprotein and Ricin Toxin B Chain (RGP-RTB) Fusion Protein in Tomato Hairy Roots: A Step Towards Oral Vaccination for Rabies. Mol Biotechnol, 2014.

[9] Patra N, Srivastava AK. Enhanced production of artemisinin by hairy root cultivation of Artemisia annua in a modified stirred tank reactor. Appl Biochem Biotechnol, 2014,174(6):2209-22.

[10] Sharifi S, Sattari T N, Zebarjadi A, et al. The influence of Agrobacterium rhizogenes on induction of hairy roots and ß-carboline alkaloids production in Tribulus terrestris L[J]. Physiology and Molecular Biology of Plants, 2014, 20(1): 69-80.

[11] Khanpour-Ardestani N, Sharifi M, Behmanesh M. Establishment of callus and cell suspension culture of Scrophularia striata Boiss.: an in vitro approach for acteoside production. Cytotechnology, 2014.

[12] Chen RD, Liu X, Zou JH, et al. [Regulation of syringin, chlorogenic acid and 1,5-dicaffeoylquinic acid biosynthesis in cell suspension cultures of Saussurea involucrata]. Zhongguo Zhong Yao Za Zhi, 2014,39(12):2275-80.

[13] De Vijlder T, Valkenborg D, Dewaele D, et al. A generic approach for "shotgun" analysis of the soluble proteome of plant cell suspension cultures. J Chromatogr B Analyt Technol Biomed Life Sci, 2015,974:48-56.

[14] Cetin ES, Babalik Z, Hallac-Turk F, et al. The effects of cadmium chloride on secondary metabolite production in Vitis vinifera cv. cell suspension cultures. Biol Res, 2014,47(1):47.

[15] Mizzotti C, Ezquer I, Paolo D, et al. SEEDSTICK is a Master Regulator of Development and Metabolism in the Arabidopsis Seed Coat. PLoS Genet, 2014,10(12):e1004856.

[16] Gandhi SG, Mahajan V, Bedi YS. Changing trends in biotechnology of secondary metabolism in medicinal and aromatic plants. Planta, 2014.

[17] Cires S, Alvarez-Roa C, Heimann K. First use of the WAVE disposable rocking bioreactor for enhanced bioproduct synthesis by N -fixing cyanobacteria. Biotechnol Bioeng, 2014.

ISSN: 2637-6067 DOI: 10.6918/IJOSSER.202206_5(6).0007

[18] Syklowska-Baranek K, Pilarek M, Cichosz M, et al. Liquid perfluorodecalin application for in situ extraction and enhanced naphthoquinones production in Arnebia euchroma cell suspension cultures. Appl Biochem Biotechnol, 2014,172(5):2618-27.

[19] Loc NH, Nhat NT. Production of asiaticoside from centella (Centella asiatica L. Urban) cells in bioreactor. Asian Pac J Trop Biomed, 2013,3(10):806-10.

[20] Shao L, Zhao SJ, Cui TB, et al. 2,3,5,4'- tetrahydroxystilbene-2-O-beta-D-glycoside biosynthesis by suspension cells cultures of Polygonum multiflorum Thunb and production enhancement by methyl jasmonate and salicylic acid. Molecules, 2012,17(2):2240-7.

[21] Chen Y, Wang Y, Sun L, et al. [Fermentation transformed ginsenoside by Lactobacillus plantarum]. Zhongguo Zhong Yao Za Zhi, 2014,39(8):1435-40.

[22] Ming Q, Su C, Zheng C, et al. Elicitors from the endophytic fungus Trichoderma atroviride promote Salvia miltiorrhiza hairy root growth and tanshinone biosynthesis. J Exp Bot, 2013,64(18):5687-94.

[23] Zhang M, Gao W, Wang XJ. [Medicinal plant hairy roots generating and their applications]. Zhongguo Zhong Yao Za Zhi, 2014,39(11):1956-60.

[24] Lee M E, Aswani A, Han A S, et al. Expression-level optimization of a multi-enzyme pathway in the absence of a high-throughput assay[J]. Nucleic acids research, 2013: gkt809.

[25] Tusevski O, Petreska SJ, Stefova M, et al. Phenolic profile of dark-grown and photoperiod-exposed Hypericum perforatum L. Hairy root cultures. ScientificWorldJournal, 2013,2013:602752.

[26] Fazal H, Abbasi BH, Ahmad N. Optimization of Adventitious Root Culture for Production of Biomass and Secondary Metabolites in Prunella vulgaris L. Appl Biochem Biotechnol, 2014,174(6):2086-95.

[27] Sivanandhan G, Arun M, Mayavan S, et al. Optimization of elicitation conditions with methyl jasmonate and salicylic acid to improve the productivity of withanolides in the adventitious root culture of Withania somnifera (L.) Dunal. Appl Biochem Biotechnol, 2012,168(3):681-96.

[28] Li Z, Zhang L, He W, et al. Astragalus membranaceus inhibits peritoneal fibrosis via monocyte chemoattractant protein (MCP)-1 and the transforming growth factor-尾1 (TGF-尾1) pathway in rats submitted to peritoneal dialysis[J]. International journal of molecular sciences, 2014, 15(7): 12959-12971.

[29] Thwe AA, Kim Y, Li X, et al. Accumulation of Phenylpropanoids and Correlated Gene Expression in Hairy Roots of Tartary Buckwheat under Light and Dark Conditions. Appl Biochem Biotechnol, 2014,174(7):2537-47.

[30] Park JW, Park SR, Han AR, et al. Microbial transformation of trichostatin A to 2,3-dihydrotrichostatin A. J Nat Prod, 2011,74(5):1272-4.

[31] Khan NT, Bibi M, Yousuf S, et al. Synthesis of some potent immunomodulatory and anti-inflammatory metabolites by fungal transformation of anabolic steroid oxymetholone. Chem Cent J, 2012,6(1):153.

[32] Hosseinabadi T, Vahidi H, Nickavar B, et al. Fungal Transformation of Androsta-1,4-diene-3,17-dione by Aspergillus brasiliensis. Daru, 2014,22(1):71.

[33] Lin XH, Cao MN, He WN, et al. Biotransformation of 20(R)-panaxadiol by the fungus Rhizopus chinensis. Phytochemistry, 2014,105:129-34.

[34] Quan LH, Kim YJ, Li GH, et al. Microbial transformation of ginsenoside Rb1 to compound K by Lactobacillus paralimentarius. World J Microbiol Biotechnol, 2013,29(6):1001-7.

[35] Hou JG, Xue JJ, Sun MQ, et al. Highly selective microbial transformation of major ginsenoside Rb1 to gypenoside LXXV by Esteya vermicola CNU120806. J Appl Microbiol, 2012,113(4):807-14.

ISSN: 2637-6067 DOI: 10.6918/IJOSSER.202206_5(6).0007

[36] Liang M, Chen M, Liu X, et al. Bioconversion of D-galactose to D-tagatose: continuous packed bed reaction with an immobilized thermostable L-arabinose isomerase and efficient purification by selective microbial degradation. Appl Microbiol Biotechnol, 2012,93(4):1469-74.

[37] De Winter K, Soetaert W, Desmet T. An imprinted cross-linked enzyme aggregate (iCLEA) of sucrose phosphorylase: combining improved stability with altered specificity[J]. International journal of molecular sciences, 2012, 13(9): 11333-11342.