Biotechnol Lett
https://doi.org/10.1007/s10529-018-2531-6

REVIEW

Yield enhancement strategies of rare pharmaceutical metabolites from endophytes

Fangxue Xu · Shiyuan Wang · Yujuan Li · Mengmeng Zheng · Xiaozhi Xi · Hui Cao · Xiaowei Cui · Hong Guo · Chunchao Han

Received: 30 November 2017 / Accepted: 28 February 2018

Abstract Endophytes are barely untapped as vital sources in the medicine. They are microorganisms which mostly exist in plants. As they are exploited, it is accepted that endophytes can produce active metabolites that possess same function as their hosts such as taxol, podophyllotoxin, hypericin, and azadirachtin. These metabolites have been promising potential usefulness in safety and human health concerns. We are supposed to adopt measures to raise production for the low yield of metabolites. This paper summarizes the latest advances in various bioprocess optimization strategies. These techniques can overcome the limitations associated with rare pharmaceutical metabolite-producing endophytic fungi. These strategies include strain improvement, genome shuffling, medium optimization, fermentation conditions optimization, addition of specific factor, addition of solid sorbent, and co-culturing. It will enable endophytes to produce high and sustainable production of rare pharmaceutical metabolites.

F. Xu · Y. Li · M. Zheng · X. Xi · H. Cao · X. Cui · H. Guo · C. Han (✉)
School of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan 250355, People's Republic of China
e-mail: chunchaoh@126.com

S. Wang
School of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Jinan 250355, People's Republic of China

Keywords Yield improvement · Develop strategies · Pharmaceutical metabolites · Endophytes

Introduction

The requirement for new agents that are of high efficiency and low toxicity is ever-increasing. However, extraction from natural sources presents biotic and abiotic stresses. Chemical synthesis of these compounds presents ecological issues. Therefore, alternative assays for the production of these rare metabolites are demanded.

Endophytes are comparatively untapped as fresh resources in the filed of medicine. Many pharmaceutical metabolites can be resynthesised by endophytes which possess the function of treating numerous diseases. (Strobel and Daisy 2003; Strobel et al. 2004; Zhang et al. 2006; Kusari et al. 2009, 2012). There have been plenty of studies that elaborate the significance of endophytes as the means for promotion of secondary metabolites. For example, using the search filters "endophytes" and "anti-cancer" in PubMed, the papers accessed were 92 on June 1, 2017 (Fig. 1A). Using the search filters "endophytes" and "antibiotics" in PubMed, the papers accessed were 150 on June 1, 2017 (Fig. 1B). Endophytes are able to produce same pharmaceutical metabolites as their hosts such as azadirachtin, podophyllotoxin, hypericin, and taxol (A. A. Leslie Gunatilaka 2006;

Published online: 31 March 2018

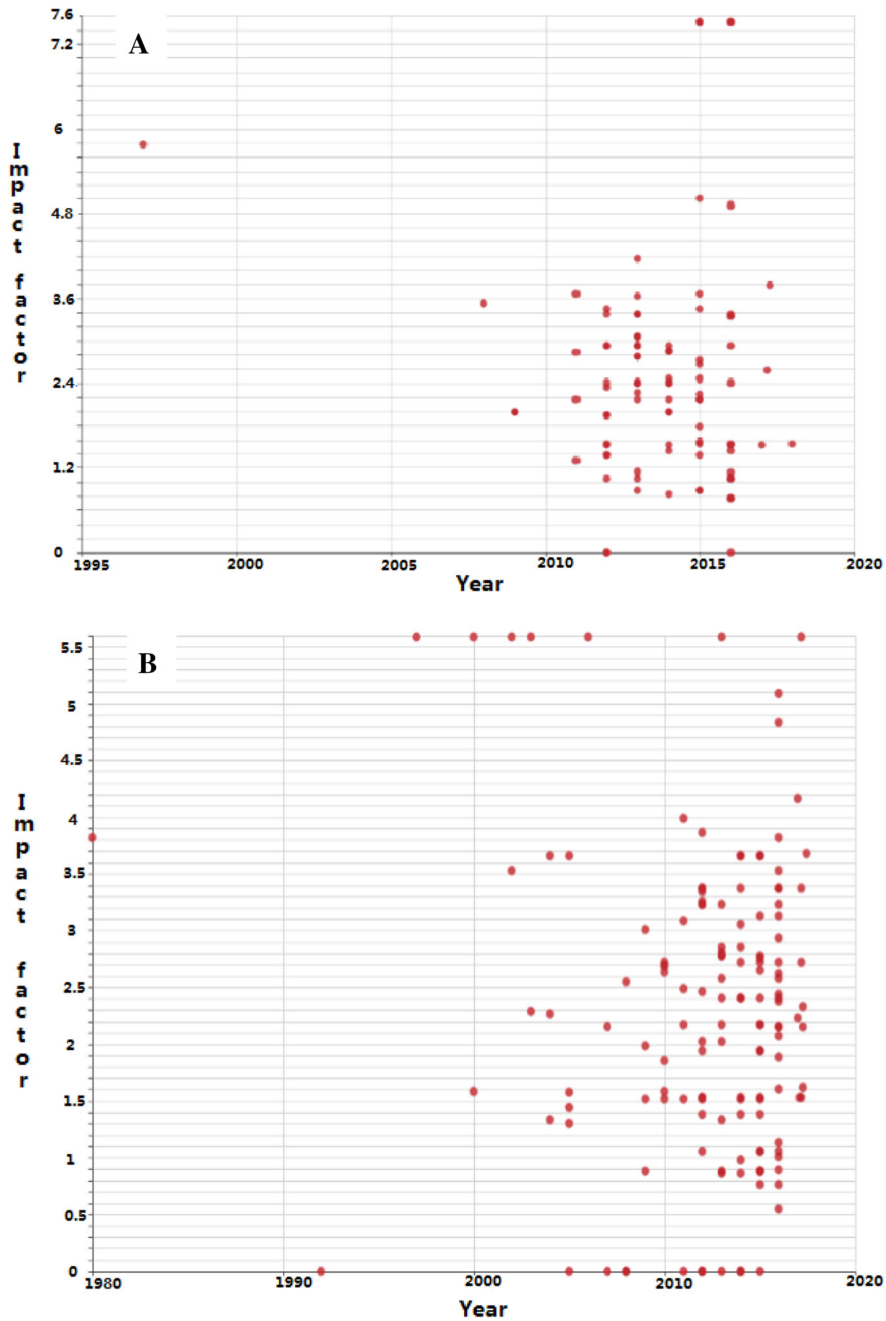

Fig. 1 Examples of endophytes as the tools for production of secondary metabolites. (**A** anticancer; **B** antibiotics)

Kharwar et al. 2011; Kusari et al. 2008; Findlay et al. 1997; Puri et al. 2006; Stierle et al. 1995). This review presents recent advances in strategies enhanced production of pharmaceutical metabolites in endophytes.

Strain improvement

Strain improvement has many advantages such as increasing fermentation yield and decreasing costs greatly. The means to generate new genetic characters include mutation and genetic recombination techniques (Parekh 2000). We summarize some samples that strain improvement was successfully applied to various metabolites produced from endophytes in Table 1.

Mutation has been mainly applied to boost the productivity of industrial cultures (Parekh et al. 2000). The commonly used mutagens include nitrosoguanidine (NTG), methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS), hydroxylamine (HA), and ultraviolet light (UV) (Fig. 2). Taxol (shown in Fig. 3A) is an anticancer drug which has a fairly high demand all over the world. Mutagenesis has been successfully utilized to raise the yield of taxol. Zhou et al. (2001a, b) successfully separated endophytic fungi that can produce taxol higher than wild strains from Taxus cuspidata.

Genome shuffling

Cell fusion is a mighty technique for the direct evolution of whole organisms and complex phenotypes (Bajwa et al. 2010). Genome shuffling is applied to promoting strains for high production of secondary metabolites (Table 2). Deacetylmycoepoxydiene (DAM) (Fig. 3B) is an antitumor agent separated from *Phomopsis* sp. A123 (Zhu et al. 2015; Sommart et al. 2009). Wang et al. (2016) utilized eight parental protoplasts to cell fusion for yield improvement of DAM. After 1980, there was a keen concern that genetic recombination was utilized to enhance the production of metabolites (Thompson et al. 1982). At present, there are numerous papers on genetic manipulation of taxol-producing endophytes (Table 3). Bian et al. (2017) formed the taxadiene-producing platform into *A. alternate* TPF6. The yield of taxadiene remarkably increased to a maximum concentration of 61.9 ± 6.3 μg/L.

Medium optimization

It turned out that medium optimization is another useful method to enhance yield of pharmaceutical metabolites in endophytes. The yield of metabolites can be increased largely by optimizing medium. This can be carried out by using plenty of methods such as orthogonal test, response surface, artificial neural network (RSM), genetic algorithm, and so on (Franco-Lara et al. 2006). It is well known that fungal polysaccharides is a crucial kind of bioactive compounds (Cho et al. 2006). The yield of exopolysaccharide obtained in the present study increased two times by medium optimization (Mahapatra and Banerjee 2013). Zofimarin (Fig. 3C) has powerful activity against pathogenic yeasts. Wetwitaklung et al. (2009) successfully separated zofimarin-producing endophytic fungus named Xylaria sp. Acra L38. Chaichanan et al. (2014) obtained the maximum production of zofimarin by optimizing the composition of medium in the orthogonal experiment.

Fermentation conditions optimization

Camptothecin (CPT) (Fig. 3D) was the first class of antineoplastic agent and widespreadly applied in the treating various tumors (Puri et al. 2005). They explored the fermentation parameters on CPT production for increasing its yield (Venugopalan et al.

Table 1 Examples on usage of strain improvement for improvement in product yield from endophytes

Product	Wild-type strain	Mutant strain	Mutagen	Yield enhancement	Reference
Taxol	*Fusarium maire* strain Y1117	*F. maire* K178	UV + DES	8.6-fold	Xu et al. (2006a, b)
Berberine	S6	S-NU-302	UV, X-rays and $NaNO_2$	170%	Gao et al. (2008)
Tanshinone IIA	*Emericella foeniculicola* TR21	*E. foeniculicola* NU152	UV + $NaNO_2$	1.46-fold	Ma et al. (2011)
L-asparaginase activity	Trichoderma sp. Gen 9 and Cladosporium sp. Gen 20	AYA 20-1	ethanol	2.11-fold	El-Gendy et al. (2017)
L-glutaminase activities	Trichoderma sp. Gen 9 and Cladosporium sp. Gen 20	AYA 20-1	ethanol	2.58-fold	El-Gendy et al. (2017)

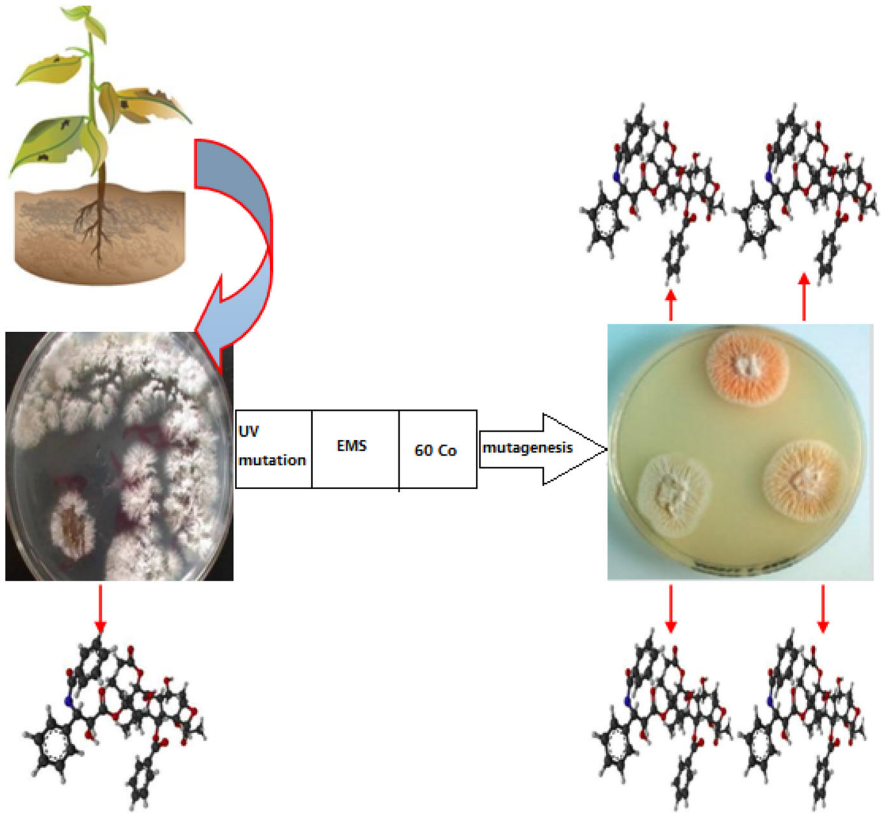

Fig. 2 A rational approach toward the exploitation of strain improvement of endophytes for secondary metabolite production

2016). The good CPT harvest received in the optimized fermentation condition was increased up to 152 times. Proteases are one of the main enzymes applied generally as detergents in the pharmaceutical industry (Rani et al. 2012; Rocha et al. 2012; Ueda et al. 2007). Zaferanloo et al. (2014) studied three fungal endophytes that were assessed for yield of proteases. They evaluated protease activity under the optimized fermentation condition. Amylase is capable of catalyzing the hydrolysis of internal α-1,4-O-glycosidic bonds in polysaccharides. It is regarded as starch-degrading enzyme on the basis of its function (Sivaramakrishnan et al. 2006). Zaferanloo et al. (2013) separated amylase-producing endophytic fungus named as *Preussia minima*. Acetylcholinesterase inhibitor (AChEI) is applied to treating Alzheimer's disease as enzyme inhibitor. Bhagat et al. (2016) found that the yield of AChEI was increased under the optimized fermentation condition. In addition, the AChE inhibition enhanced 1.3 times by optimizing fermentation parameters compared to previous condition.

Specific factor addition (Table 4)

The elicitor is the extracellular signal factor of plant cells that trigger plant defense responses in synthesis (Cheng et al. 1996). Benzoate was added to the medium of *Periconia* sp. leading to the enhancement of taxol production up to 8 times higher than that in control (Parra et al. 2005). Besides, Xu et al. (2006a, b) utilized sodium acetate to prominently enhance taxol production from a Fusarium strain. Addition of serine enhanced taxol yield by *Epicoccum nigrum* with a 29.6-time yield increase over the control (Somjaipeng et al. 2016). In the case of HupA (Fig. 3E), it is a pharmaceutical metabolite of high value which is generally applied in curing Alzheimer's disease (AD) (Zhao et al. 2013). They indicated that ethanol could significantly enhance the HupA yield compared to control (51.89% increasement) (Fig. 4) (Kern et al. 2009; Ebel and Cosio 1994). As for CPT (Fig. 3D), supplement of BSA resulted in maximum CPT concentration (41.9 μg/L) compared to control (12.3 μg/L) (Zhang et al. 2011).

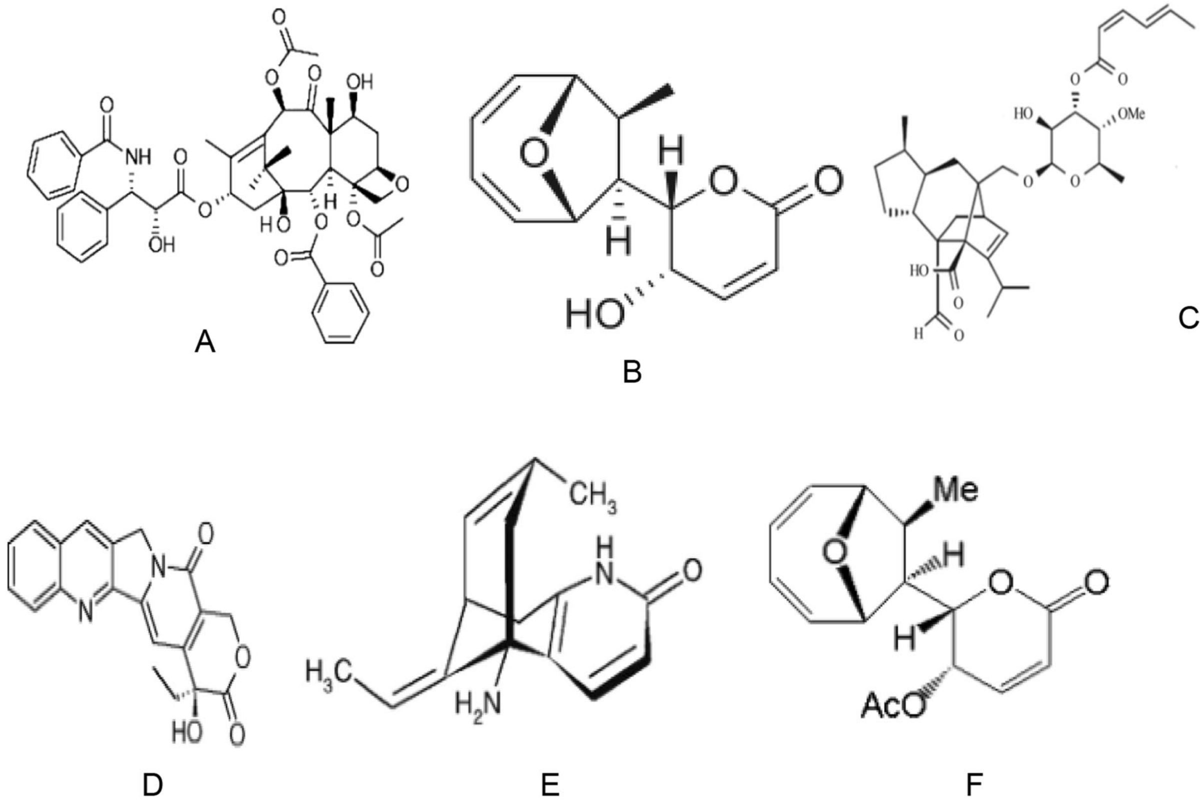

Fig. 3 The chemical structure of taxol, deacetylmycoepoxydiene, zofimarin, camptothecin, hupA, mycoepoxydiene

Precursor feeding is another strategy to enhance the desired metabolite yield (Fig. 5). In the case of CPT (Fig. 3D), supplement of 0.5 mM tryptamine resulted in the maximum CPT concentration (153.6 μg/L). The amount of the maximum CPT concentration was 4.5 times compared to control (Zhang et al. 2011). In addition to elicitors and precursor, metabolic inhibitor has also been used for improving the production of metabolite in endophytic fungi. Li et al. (1998) obtained the yield of taxol 50-fold higher than that in control by utilizing paclobutrazol in taxol biosynthesis process.

Solid sorbent addition

It is known that we can enhance the yield of high-value metabolite by adding solid sorbent to the medium (Bigelis et al. 2006). Luo et al. (2014) investigated the influence on botrallin and TMC-264 yield by adding polystyrene resin DM-301 to the suspension. It turned out that the yield of botrallin and TMC-264 enhanced up to 2.29 times and 11.76 times, respectively, compared to control. In addition, the production of beauvericin and palmarumycin C13 increased up to 1.8 times and 1.4 times by adding polystyrene resin X-5 and polystyrene resin AB-8 (Xu et al. 2009; Zhao et al. 2011). As far as CPT concerned (Fig. 3D), they found that the CPT production increased up to 11 times by adding sorbent resin X-5 compared to control (Pu et al. 2013). Besides, mycoepoxydiene (Fig. 3F) exhibited potent cytotoxic activities toward some cancer cell lines. A cellulose paper disc was added to the culture medium leading to the increase of the yield of mycoepoxydiene up to level of 354 mg/L (Thammajaruk et al. 2011).

Co-culturing

Co-culture is a way to influence growth by utilizing ‘auxiliary’ strains through the presence of bioactive molecules. It exploits the interrelated characteristics of some microorganisms (Table 5). Soliman and Raizada (2013) studied the influence on taxol production of co-culturing SSM001 with other fungi. To our

Table 2 Examples on usage of genome shuffling for improvement in product yield from endophytes

Product	Wild-type strain	Mutant strain	Rounds	Yield enhancement	Reference
Tylosin	*Streptomyces fradiae* strain 1	GS1 and GS2	2 rounds	9-fold	Zhang et al. (2002)
Riboflavin	*Bacillus. subtilis* DB104 and *Bacillus. subtilis* 24/pM X45	*B. subtilis* RH33/pMX45	2 rounds	2-fold	Chen et al. (2004)
lipase	*Aspergillus tamarii*	*Penicillium expansum*	2 rounds	317% more	Lin et al. (2007)
epothilones	*Sorangium cellulosum* So0157-2	*S. cellulosum* mutant GSUV3-205	Several rounds	130 times	Gong et al. (2007)
Teicoplanin	*Actinoplanes teichomyceticus*	*Actinoplanes teichomyceticus* mutant strains	Several rounds	65.3% more	Xu et al. (2006)
Taxol	*Nodulisporium sylviform* NCEU-1	*Nodulisporium sylviform* rearrangement strain	Four rounds	31.52–44.72% more	Zhao et al. (2008)
Hydroxycitric acid	*Streptomyces* sp. U121	Mutant strains of *Streptomyces* sp. U121	Three rounds	5-fold	Hida et al. (2007)
Spinosad	*Saccharopolyspora spinosa* y-2	*S. spinosa* 4-7	Four rounds	436.27% more	Jin et al. (2009)
DAM	Phomopsis sp. strain A123	G2-119	–	243-fold	Wang et al. (2016)
	Phomopsis sp. strain A123	G2-448	–	241-fold	
	Phomopsis sp. strain A123	G2-866	–	225-fold	
	Phomopsis sp. strain A123	G2-919	–	275-fold	

Table 3 Genetic manipulation of endophytes on taxol-producing endophytes

Wild-type strain	Transformant	Strategies	Reference
Ozonium sp. EFY-21	*Ozonium* sp. EFY-21 transformants	PEG-mediated transformation	Wei et al. (2010)
Ozonium sp. EFY-21	*Ozonium* sp. EFY-21 transformants	Key enzyme gene transformation	Yamin et al. (2012)
Ozonium sp. EFY21	Hygromycin B-resistant transformants	*Agrobacterium tumefaciens*-mediated genetic transformation method	Liu et al. (2013)
Cladosporium cladosporioides strain MD2	*Cladosporium cladosporioides* strain MD2 transformants	*Agrobacterium tumefaciens*-mediated transformation	Zhang et al. (2011)
Ozonium sp. strain BT2	BT2 transformants	Restriction enzyme-mediated integration	Wang et al. (2007)
Cladosporium cladosporioides MD2	*C. cladosporioides* MD2 transformants	*Agrobacterium tumefaciens*-mediated genetic transformation method	Zhang et al. (2011)

surprise, the yield of taxol increased 3 times by co-culturing of SSM001 with a bark fungus. In addition, Li and Tao (2009) indicated that the production of taxol could enhance up to 38-fold by co-culturing *Fusarium* with *Taxus* suspension cells. Unexpectedly, Vinale et al. (2017) separated and purified new tetramic acid by co-culturing *T. harzianum* M10 with *T. pinophilus* F36CF. This method solved the problem that it could not be separated from natural sources or chemical synthesis. The yield of enniatins A1 and B1 enhanced up to 78-fold compared to the fungal

Table 4 Examples on usage of specific factor addition for enhanced production of pharmaceutical metabolites in endophytes

Product	Organism	Factor	Yield enhancement	Reference
Taxol	Periconia sp.	Benzoate	8-fold	Parra et al. (2005)
Taxol	*Fusarium maire* strain Y1117	Sodium acetate	11.26-fold	Xu et al. (2006)
Taxol	*Epicoccum nigrum*	Serine	29.6-fold	Somjaipeng et al. (2016)
HupA	*Colletotrichum gloeosporioides* ES026	Methanol and ethanol	51.89% increment	Kern et al. (2009)
CPT	F. solani	BSA	3.4-fold	Zhang et al. (2011)
CPT	F. solani	Tryptamine	4.5-fold	Zhang et al. (2011)
Taxol	*Pestalotiopsis microspora* Ne32	Sterol biosynthesis inhibitors	50-fold	Li et al. (1998)

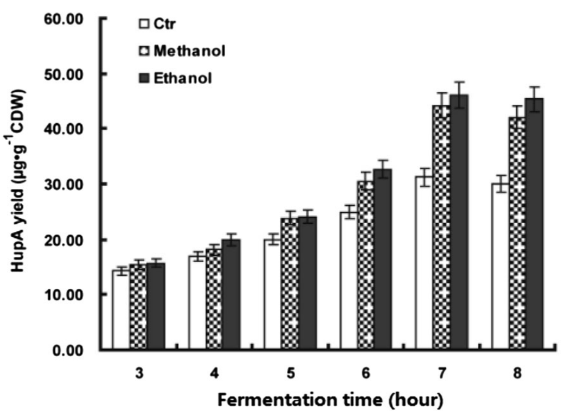

Fig. 4 The effect of 1% methanol and 1% ethanol on *Colletotrichum gloeosporioides* ES026 fermentation for HupA

endophyte alone by co-culturing Fusarium tricinctum with Bacillus subtilis 168 trpC2 (Ola et al. 2013).

Secondary metabolites produced by endophytes used commercially for the production of therapy

Using strain improvement, genome shuffling, medium optimization, fermentation conditions optimization, addition of specific factor, addition of solid sorbent, and co-culturing, pharmaceutical metabolites produced by endophytes may be used commercially for the production of therapy. Wang et al. (2015) purified taxol from endophytic fungi that could lead to apoptosis in several cancer cells. It can be used as taxol injection, paclitaxel liposome for injection, and so on. They offered a new resource for the production of a vital anticancer drug by endophytic fermentation. In addition, the drug camptothecin produced by endophytes has a wide range of antitumor effects in various cancers (Wen et al. 2017). It can be used as camptothecin injection, camptothecin ointment, camptothecin patch, and so on.

Fig. 5 Effect of addition of precursors on biomass and CPT production

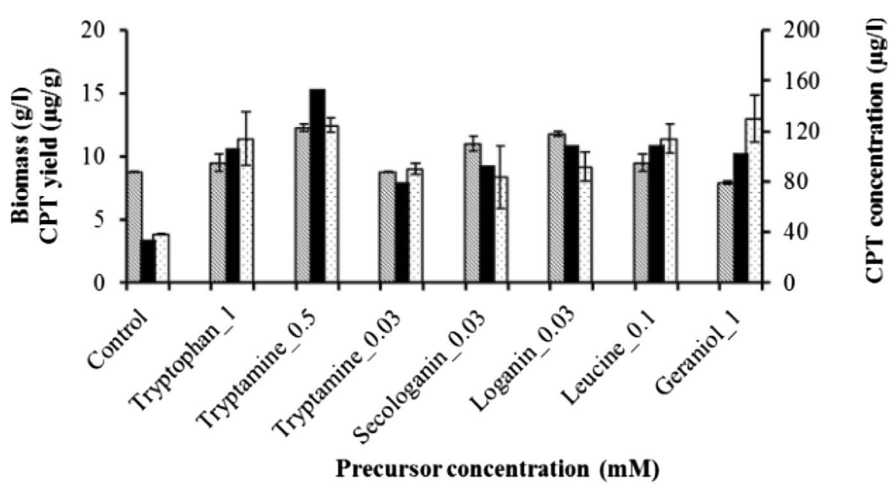

Table 5 Examples on usage of co-culturing for enhanced production of pharmaceutical metabolites in endophytes

Product	Co-culturing strain	Original species	Yield enhancement	Reference
Taxol	*Paraconiothyrium* SSM001	*Alternaria alternata*	3-fold	Soliman and Raizada (2013)
Taxol	*Fusarium*	*Taxus* suspension cells	38-fold	Li et al. (2009)
Tetramic acid	*T. harzianum* M10	*T. pinophilus* F36CF	–	Vinale et al. (2017)
Enniatins A1 and B1	*Fusarium tricinctum*	*Bacillus subtilis* 168 trpC2	78-fold	Ola et al. (2013)

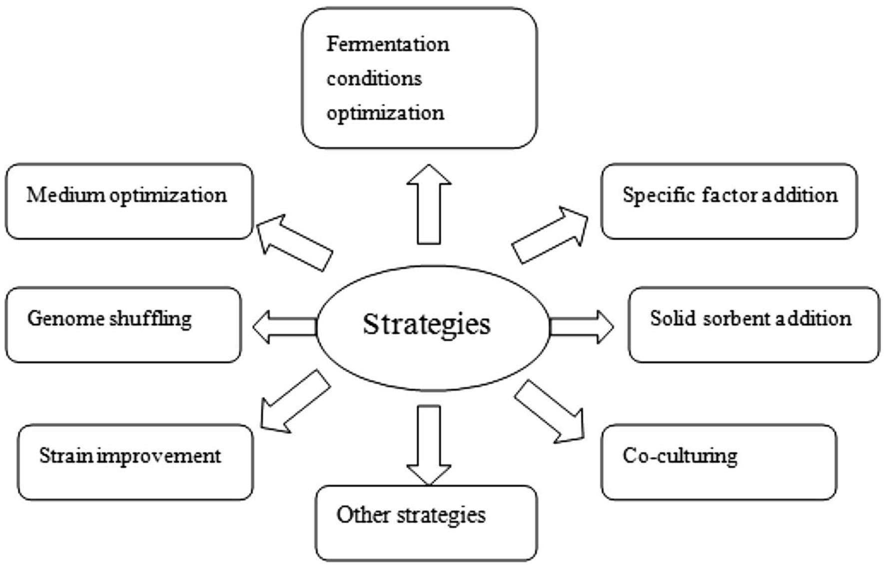

Fig. 6 Yield enhancement strategies of rare pharmaceutical metabolites from endophytes

Conclusions

Although pharmaceutical metabolites produced by endophytes have been a hopeful way to the treatment of several diseases, the amount of secondary metabolites produced by endophytes is usually very low. Therefore, strategies and technologies are required to increase the yield of these pharmaceutical metabolites from endophytes. This paper summarized recent advances in various bioprocess optimization strategies and techniques to overcome the limitations associated with pharmaceutical metabolite-producing endophytes. These strategies will enable endophytes to produce high yield of pharmaceutical metabolites (Fig. 6). These high-value metabolites have promising potential use in safety and human health concerns.

Acknowledgments This work was supported by Project of Shandong Province Key Research and Development Program (2017YYSP030). The authors would also like to thank the anonymous reviewers for their helpful comments and suggestions that greatly improved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human and animal studies This article does not contain any studies with human participants or animals performed by any of the authors.

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