Biotransformation of vine tea (*Ampelopsis grossedentata*) by solid-state fermentation using medicinal fungus *Poria cocos*

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Revised: 12 May 2016 / Accepted: 22 July 2016 / Published online: 25 August 2016
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Abstract Vine tea was bio-transformed using *Poria cocos* by solid-state fermentation in order to improve its taste and quality. Volatile components in vine tea were also identified by GC–MS. The changes of flavonoid, tea polyphenols and polysaccharides in fermented vine tea were evaluated. Flavonoid and polyphenols in vine tea were remained unchanged even after biotransformation, but content of polysaccharides increased to 3.9-fold than that of unfermented vine tea. Antioxidant activity such as DPPH free radical scavenging capacity (SR) was determined that there was a positive correlation between SR and content of polysaccharides in vine tea. Methyl 2-methylvalerate—a new volatile compound was identified and gave the vine tea rich delicate fragrance of fruits. The content of linolenic acid increased from 0.88 to 19.59 %. Biotransformation improved the taste and quality of vine tea.

Keywords Vine tea · Biotransformation · *Poria cocos* · Solid-state fermentation · Polysaccharides · Methyl 2-methylvalerate

Introduction

Vine tea are the dried leaves and stems of *Ampelopsis grossedentata* (family: *Vitaceae*; genus: *Ampelopsis Michx.*) which is a wild plant growing in mountainous areas of southern China, also called Mao Yan Mei or moyeam tea. Vine tea has been consumed as a health tea and herbal medicine for hundreds of years. However, there are only two or three decades for systematic study of vine tea till now. The study has been focused mainly on the nutritional profile of vine tea, including flavonoids and phenolics (Du et al. 2004; Wang et al. 2011), amino acid (Zheng 2008), dietary fiber (Zhang et al. 2013). Flavonoids are the main active substances in vine tea, mainly including dihydromyricetin (Liao et al. 2014; Ye et al. 2015) and myricetin (Zhong et al. 2014).

Vine tea has good pharmacological activities, such as antioxidant activity (Ye et al. 2015), anti-inflammatory (Hou et al. 2015), Hepatoprotective activity (Liu et al. 2014; Murakami et al. 2004), antitumor (Zhao et al. 2014), antityrosinase (Ye et al. 2010), anti-diabetics and anti-atherosclerosis (Chen et al. 2015). However, vine tea has adverse effects on human health. Consequently, vine tea is widely welcomed by consumers as a drink. The vine tea drink does not taste good because of the conventional production technology of vine tea, which limits the marketing promotion of vine tea for some consumer groups.

Poria cocos has a long history of medicinal use in Asian countries such as China, Japan and Korea. *P. cocos* is a kind of edible and pharmaceutical mushroom because of its

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chemical compositions including triterpenes, polysaccharides, steroids, amino acids, choline, histidine, etc. Pachyman is the mainly active compound (Sun 2014). *P. cocos* has many beneficial biological activities including anti-cancer, anti-inflammatory, antioxidant and antiviral activities (Sun 2014; Dong et al. 2015; Jeong et al. 2014; Tang et al. 2014). The activity of *P. cocos* is similar to that of vine tea. So, vine tea fermented by *P. cocos* should improve the activity, which has been implied by some studies (Roh 2014; Wu et al. 2012). There are no reports about biotransformation of vine tea using medicinal fungus *P. cocos* so far.

In this study, vine tea was bio-transformed using medicinal fungus *P. cocos* by solid-state fermentation to obtain higher bioactivity, to improve the taste, and to investigate the changes of volatile components by GC–MS.

Materials and methods

P. cocos and vine tea

The strain *P. cocos* was isolated from Shengnongjia Global Geopark P.R.China, and identified by the China General Microbiological Culture Collection Center in Beijing, China. The strain was now preserved as Accession Number PF-60 in Huazhong University of Science and Technology. Vine tea was purchased from Laifeng Elegant Vine Tea Biological Co., Ltd. in Hubei, China.

Biotransformation by solid-state fermentation

Vine tea (20 g, dry mass) was placed into 250-ml Erlenmeyer flasks, moistened with 24 mL distilled water, and inoculated with three discs cut (1 cm × 1 cm) from active *P. cocos* culture on potato extract agar plate after sterilization. The culture was fermented still at 28 °C for 15 days.

Analysis of flavonoid by HPLC method

The crushed and sieved vine tea, un-fermented or fermented sample (5 g, dry mass) immersed with 100 mL of distilled water for 30 min, and extracted by hot reflux method for 2 h. The filtrate from extract with filter paper was obtained to be used for analysis of flavonoid by HPLC method. The HPLC system (Shimadzu Corporation, Kyoto, Japan) was used, equipped with a 20 µL injector loop, an UV detector (SPD-10A VP Plus) set at a wavelength of 292 nm, and an ODS C18 reverse phase column (100 mm × 4.6 mm, i.d. 5 µm), two Liquid Chromatographs (LC-10AT), and a Communications Bus

Module (CBM-10A). A linear HPLC binary gradient was used as follows: solvent A was pure methanol (HPLC grade), and solvent B was 0.1 % formic acid in distilled water. Following the injection of 10 µL of a sample, solvent B was increased from 5 to 60 % over 55 min, held at 60 % for 7 min. The flow rate was held at 1.0 mL/min throughout the run time, which was a total of 62 min. Standard dihydromyricetin (CAS: 27200-12-0, 98 % of purity) and myricetin (CAS: 529-44-2, 98 % of purity) were purchased from Nanjing Jingzhu Biotechnology Co., Ltd. in Nanjing, China.

Analysis of tea polyphenols by UV method

The filtrate obtained from above assay for flavonoid method was also used for determination of tea polyphenols content using UV/vis spectrophotometric (754-type, Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China) at wavelength of 765 nm (Li and Jiang 2010).

Analysis of polysaccharide by phenol sulfuric acid method

The filtrate added five-times of pure ethanol was placed into 4 °C refrigerator for 12 h for full precipitation of polysaccharide. The precipitate was obtained by centrifugation at 5000 rpm for 10 min and dissolved again using the same volume of distilled water. The solution was centrifugated at 5000 rpm for 10 min to obtain the filtrate. Polysaccharide of the filtrate was determined by phenol sulfuric acid method (Masuko et al. 2005).

Analysis of DPPH radical scavenging capacity by UV method

Test tubes of 10 mL were added respectively with 2 mL of filtrate in test groups, in control group (CK) with 2 mL of filtrate and 2 mL of anhydrous ethanol, and in blank control group (BC) with 2 mL of anhydrous ethanol. Then the test tubes in test groups and BC group were added with 2 mL of 0.2 mmol/L DPPH solution, mixed well and reacted for 30 min in 25 °C water bath away from light. The absorbance in BC group (A_0), test group (A_1) and CK group (A_2) were measured by UV spectrophotometer (754-type, Shanghai Precision & Scientific Instrument Co., Ltd., Shanghai, China) at wavelength of 518 nm. The DPPH radical scavenging capacity was calculated according to the Eq. (1) as followed (Kaskoniene et al. 2015).

$$SR = \left[1 - \frac{A_1 - A_2}{A_0} \right] \times 100 \% \quad (1)$$

Analysis of volatile compounds by GC–MS method

The crushed and sieved vine tea, un-fermented or fermented, sample (8 g) was placed into 5 L sample bottle with 1 L distilled water, and 40 mL dichloromethane was added into extraction bottle. The extract of dichloromethane was obtained after 5 h by simultaneous distillation extraction method, and 2 mL volatile oils were obtained from the dichloromethane extract by concentration with a rotary evaporator (Model: Laborota 4003, Heidolph, Germany) and dewatering by anhydrous sodium sulfate. The compounds of volatile oils were analyzed by GC–MS. The GC–MS system (Agilent 7890A-5975C, Palo Alto, USA) was used, equipped with a flame ionization detector, NIST05 standard retrieval spectral library, and a HP-5 capillary column (30 m × 0.25 mm × 0.25 μm). Conditions of GC were as followed: 220 °C of inject temperature, 200 °C of detector temperature, 20:1 of split ratio, and 1 μL of injection volume. The oven program was executed by 50 °C of initial temp for 2 min, 5 °C/min to 190 °C, 10 °C/min to 250 °C, and remaining 250 °C for 4 min, the total run time was 40 min. Mass spectrometer conditions were EI of ionization mode, 70 eV of electron energy, 190 °C of interface temperature, 230 °C of ion source temperature, 20–500 AMU of mass scan range.

Statistical analysis

Each experiment was repeated for five times. The data were collected via Microsoft® excel® 2010 software, and the data were statistically treated by one-way ANOVA and Duncan's post hoc test ($p < 0.05$) with SPSS 19.0 software. Data were reported in the text as mean ± standard deviation, and the error lines in all figures corresponded to standard errors.

Results

Flavonoid

The biotransformation result are shown in Fig. 1. There was no obvious difference between liquid chromatogram of 0 day-fermentated and that of 9 days-fermentated sample with four main peaks (retention times were respectively 21.5, 31.5, 38.1 and 59.4 min). The peak at 31.5 min (dihydromyricetin) and 38.2 min (myricetin) changed very little. The peaks at 21.5 and 59.4 min decreased slightly. Therefore, flavonoid in vine tea was influenced slightly by biotransformation caused by *P. cocos*.

Fig. 1 Liquid chromatogram of 0 days-fermentated vine tea (a) and that of 9 days-fermentated vine tea (b)

Fig. 2 Kinetic curve of tea polyphenols in vine tea during biotransformation process

Tea polyphenols

Tea polyphenols is a kind of phenolic compound in tea, which is one of the main active compounds in tea. Tea polyphenols in vine tea was perhaps degraded by *P. cocos* during the fermentation process, which decreased the quality of vine tea. As shown in Fig. 2, with increase of biotransformation time, the polyphenols content in vine tea increased and reached the highest (292 μg/g) after 12 days of biotransformation, and then the content of tea polyphenols decreased. However, the change range of tea

polyphenols content was still within 5 % during the whole biotransformation process. Hence, there were small effects on tea polyphenols in vine tea by biotransformation using *P. cocos*.

Polysaccharide

As shown in Fig. 3, the content of polysaccharides in unfermented vine tea was only 178 $\mu\text{g/g}$. With increase of biotransformation time, the content of polysaccharides increased continuously, the highest content of 702 $\mu\text{g/g}$ was obtained at the end of biotransformation, which was 3.9-fold than that of unfermented vine tea. So, biotransformation with *P. cocos* was aimed to increase the content of polysaccharides in vine tea, which should improve the quality of vine tea.

DPPH free radical scavenging capacity

Flavonoid, tea polyphenols and polysaccharides all have antioxidant activity, and antioxidant activity of vine tea was evaluated using DPPH test in this study as shown in Fig. 4. With the increase of biotransformation time, DPPH free radical scavenging capacity (SR) was increased from 18 to 55 %. Comparing the SR and content of tea polyphenols and polysaccharides as shown in Fig. 4b, c, there was a good positive correlation between SR and content of polysaccharides, and the correlation coefficient was 0.96. However, the correlation between SR and content of tea polyphenols was poor, and the correlation coefficient was only about 0.6. Consequently, polysaccharide was the main reason of the increase of antioxidant activity of fermented vine tea during the biotransformation process.

Fig. 3 Kinetic curve of polysaccharides in vine tea during biotransformation process

Volatile components

The unfermented vine tea did not taste good, but the fermented vine tea had a pleasant taste, such as fragrance of fruits. By analysis of volatile components of vine tea in

Fig. 4 DPPH radical scavenging capacity of vine tea during biotransformation process (a) and the relationship between DPPH free radical scavenging capacity and content of tea polyphenols (b) and polysaccharides (c)

different biotransformation time by GC–MS, total ion chromatogram of volatile components of vine tea (0 and 6 days fermentation) as shown in Fig. 5, and twenty-six components identified are shown in Table 1. Ten hydrocarbons, four fatty acids, four alcohols, three esters, three ketones, one aldehyde and phenol were identified. Apart from six compounds including azulene, pentamethylbenzene, isophytol, (2,6-dimethyl-3-buten-2-one-methylnaphthalene), (2,7-dimethylnaphthalene) and (2,3-dihydro-4,7-dimethyl-1H-indene), all other compounds showed

significant differences by one-way ANOVA ($p < 0.001$) relative to biotransformation time.

The total content of hydrocarbons, fatty acids and esters were relatively higher than others. The total content of fatty acids and esters increased and then decreased with the increase of biotransformation time. The total content of hydrocarbons, ketones and phenol decreased from 42.88, 2.06 and 0.5 % to 25.95, 0.28 % and ND, respectively by biotransformation for 15 days. Three esters including dihydroactinidiolide, methyl 2-methylvalerate and dibutyl

Fig. 5 Total ion chromatogram of volatile components of vine tea by GC–MS. **a** 0 day-fermentation vine tea, **b** 6 days-fermentation vine tea

Table 1 Volatile components of vine tea during biotransformation process

Peaks time (min)	Volatile components	Relative contents (%) in different biotransformation time					p	
		0 day	3 days	6 days	9 days	12 days		15 days
03.71	Furfural	ND ^a	1.23 ± 0.17 ^a	2.56 ± 0.31 ^b	1.31 ± 0.16 ^a	1.24 ± 0.06 ^a	1.62 ± 0.15 ^c	<0.001
08.25	Benzyl alcohol	ND ^a	0.21 ± 0.11 ^a	0.42 ± 0.04 ^b	0.45 ± 0.06 ^b	0.29 ± 0.07 ^a	0.52 ± 0.09 ^b	<0.001
11.31	2,3-Dihydro-4-methyl-4-methyl-1H-indene	ND ^a	0.14 ± 0.09 ^c	0.25 ± 0.04 ^d	0.11 ± 0.04 ^{bc}	0.06 ± 0.02 ^{ab}	ND ^a	<0.001
11.38	1,2,3,4-Tetramethylbenzene	1.34 ± 0.07 ^a	1.52 ± 0.10 ^a	1.75 ± 0.11 ^b	1.79 ± 0.13 ^b	1.48 ± 0.19 ^a	1.36 ± 0.15 ^a	<0.001
12.25	Azulene	4.78 ± 0.62 ^a	4.92 ± 0.55 ^a	5.26 ± 0.62 ^a	5.61 ± 0.54 ^a	5.28 ± 0.42 ^a	4.87 ± 0.34 ^a	0.412
12.65	Pentamethylbenzene	0.94 ± 0.21 ^a	0.91 ± 0.19 ^a	1.13 ± 0.16 ^a	1.14 ± 0.20 ^a	1.09 ± 0.18 ^a	1.01 ± 0.14 ^a	0.524
14.04	2,3-Dihydro-4,7-dimethyl-1H-indene	0.94 ± 0.09 ^a	0.98 ± 0.12 ^a	1.14 ± 0.17 ^{ab}	1.21 ± 0.14 ^b	1.23 ± 0.02 ^b	1.11 ± 0.12 ^{ab}	0.052
15.30	1-Methylnaphthalene	19.45 ± 2.76 ^a	17.12 ± 2.71 ^a	12.90 ± 1.81 ^b	7.85 ± 2.30 ^c	5.29 ± 0.38 ^c	4.17 ± 0.34 ^c	<0.001
15.71	2-Methylnaphthalene	8.59 ± 1.02 ^a	8.27 ± 0.68 ^a	7.22 ± 0.49 ^b	5.49 ± 0.26 ^c	4.02 ± 0.26 ^d	3.17 ± 0.45 ^d	<0.001
18.10	2,7-Dimethylnaphthalene	3.35 ± 0.36 ^a	3.42 ± 0.36 ^a	3.51 ± 0.36 ^a	3.54 ± 0.29 ^a	3.57 ± 0.24 ^a	3.38 ± 0.21 ^a	0.879
18.46	2,6-Dimethyl-3-buten-2-one- methylnaphthalene	3.49 ± 0.30 ^a	3.44 ± 0.33 ^a	3.51 ± 0.16 ^a	3.53 ± 0.11 ^a	3.51 ± 0.27 ^a	3.42 ± 0.18 ^a	0.990
20.16	2-Pentadecanone	1.02 ± 0.20 ^a	0.86 ± 0.10 ^a	0.35 ± 0.11 ^b	0.25 ± 0.07 ^{bc}	0.14 ± 0.04 ^{cd}	ND ^d	<0.001
21.23	Dihydroactinidiolide	0.55 ± 0.09 ^a	0.78 ± 0.09 ^a	1.41 ± 0.12 ^b	1.89 ± 0.15 ^c	1.48 ± 0.17 ^b	1.09 ± 0.20 ^d	<0.001
22.87	Methyl 2-methylvalerate	ND ^a	2.56 ± 0.22 ^b	7.12 ± 0.72 ^c	22.74 ± 3.04 ^d	16.62 ± 1.15 ^e	7.44 ± 0.69 ^c	<0.001
26.47	Tetradecanoic acid	ND ^a	0.12 ± 0.07 ^{ab}	0.34 ± 0.11 ^{cd}	0.43 ± 0.14 ^d	0.36 ± 0.09 ^{cd}	0.21 ± 0.05 ^{bc}	<0.001
28.03	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-	0.52 ± 0.12 ^a	0.48 ± 0.12 ^a	0.33 ± 0.09 ^b	0.25 ± 0.04 ^b	0.19 ± 0.06 ^{bc}	0.07 ± 0.04 ^c	<0.001
29.51	6,10,14-Trimethyl-(E,E)-farnesyl acetone	0.52 ± 0.10 ^a	0.49 ± 0.04 ^a	0.43 ± 0.10 ^{ab}	0.35 ± 0.04 ^{bc}	0.26 ± 0.06 ^{cd}	0.21 ± 0.04 ^d	<0.001
30.06	Isophytol	0.50 ± 0.08 ^a	0.48 ± 0.08 ^a	0.46 ± 0.06 ^a	0.47 ± 0.08 ^a	0.45 ± 0.04 ^a	0.49 ± 0.08 ^a	0.954
30.39	Dibutyl phthalate	4.83 ± 0.66 ^a	4.19 ± 0.42 ^a	2.36 ± 0.47 ^b	1.32 ± 0.38 ^c	1.08 ± 0.13 ^c	0.77 ± 0.25 ^c	<0.001
30.89	n-Hexadecanoic acid	3.41 ± 0.21 ^a	7.26 ± 0.66 ^{ab}	10.50 ± 1.35 ^b	15.97 ± 3.31 ^c	20.86 ± 3.86 ^d	15.43 ± 1.60 ^c	<0.001
31.60	1-Trimethyl-nerolidol	ND ^a	0.24 ± 0.07 ^b	0.61 ± 0.15 ^{cd}	0.89 ± 0.20 ^e	0.73 ± 0.09 ^{de}	0.47 ± 0.04 ^c	<0.001
32.89	Phytol	1.57 ± 0.18 ^a	1.38 ± 0.14 ^a	0.92 ± 0.06 ^b	0.66 ± 0.10 ^c	0.37 ± 0.10 ^d	0.35 ± 0.09 ^d	<0.001
33.50	Linolenic acid	0.88 ± 0.08 ^a	3.67 ± 0.29 ^b	12.97 ± 1.38 ^c	18.37 ± 1.33 ^d	19.59 ± 2.26 ^d	15.27 ± 1.05 ^e	<0.001
33.74	Octadecanoic acid	ND ^a	0.36 ± 0.05 ^b	1.23 ± 0.07 ^c	1.48 ± 0.18 ^d	1.54 ± 0.11 ^d	1.48 ± 0.09 ^d	<0.001
35.28	n-Nonacosane	ND ^a	0.18 ± 0.04 ^b	0.43 ± 0.05 ^c	0.52 ± 0.12 ^{cd}	0.63 ± 0.15 ^d	0.46 ± 0.07 ^c	<0.001
36.40	2,2'-Methylenebis[6-(1,1-d imethylethyl)-4-methylphenol]	0.50 ± 0.06 ^a	0.42 ± 0.05 ^b	0.20 ± 0.02 ^c	ND ^d	ND ^d	ND ^d	<0.001

P value for one-way ANOVA. Lower case letters in ascending order denote significant differences for each component and biotransformation time (Duncan Ranking at $p < 0.05$)

phthalate were identified. The content of dibutyl phthalate decreased from 4.83 to 0.77 % by biotransformation for 15 days, and the content of dihydroactinidiolide increased slightly during biotransformation. A new ester methyl 2-methylvalerate with pleasant fruit aroma was found in fermented vine tea, and the content of methyl 2-methylvalerate increased from non-detectable to 22.74 % after 9 days of biotransformation, then decreased to 7.44 % 15 days later. The total fatty acids content increased from 4.29 to 42.35 % after 12 days of biotransformation, and then decreased to 32.39 % 15 later. The increase of total content of fatty acids was mainly caused by the increases of two fatty acids including *n*-hexadecanoic acid and linolenic acid. The content of linolenic acid increased from 0.88 to 19.59 % for 12 days of biotransformation.

Discussion

Biotransformation is a double-edged sword, which can degrade the active compounds to non-active small substances, or can modify the active compounds to new compounds by methylation, glycosidation and hydroxylation (Wu et al. 2012; Chen et al. 2013; Tronina et al. 2013). Tea polyphenols are polyhydroxy compounds including catechins, anthocyanins, phenolic acids, and polymeric phenol, and catechins is the main composition of tea polyphenols (Afzal et al. 2015). Tea polyphenols can be metabolized by extracellular polyphenol oxidase and peroxidase of some microorganism (Wang et al. 2014). *P. cocos* is a kind of brown-rot fungi, which can produce highly-active cellulase and hemi-cellulase without ligninolytic enzymes. Ligninolytic enzymes such as laccase, ligninase and Mn-peroxidase can degrade lignin which is a complex aromatic heteropolymer formed by radical polymerization of guaiacyl, syringyl, and *p*-hydroxyphenyl units linked by β -aryl ether linkages, biphenyl bonds and heterocyclic linkages (Pollegioni et al. 2015). Tea polyphenols and flavonoids such as dihydromyricetin and myricetin have a similar structure with lignin. Therefore, *P. cocos* could not degrade tea polyphenols and flavonoids just as shown in Figs. 1 and 2, which could keep the functional factors in vine tea intact, such as DPPH free radical scavenging capacity in Fig. 4.

Tea polysaccharide is one of the main bioactive components with anti-oxidant, anti-blood coagulation, anti-radiation, anti-cancer and immunological activities (Jin et al. 2015). Pachyman (a linear β -(1 $\rightarrow$ 3)-d-glucan PCM3) is a major polysaccharide in *P. cocos* mycelia, and can be produced by biotransformation of cellulose and hemicellulose in vine tea, which brought about the increase of content of polysaccharides as shown in Fig. 3, which led to increase of antioxidant activity of fermented vine tea as

shown in Fig. 4. Methyl 2-methylvalerate is a natural perfume, which was so far only found in baked potatoes. Methyl 2-methylvalerate was first identified in vine tea fermented by *P. cocos*, and methyl 2-methylvalerate also gives vine tea the rich delicate fragrance of fruits. Methyl 2-methylvalerate is the esterification product of 2-methylvaleric acid and methanol. 2-Methylvaleric acid was surmised as one of the metabolites from dihydroactinidiolide and isophytol, the contents of which were showed in Table 1. The biotransformation pathway of methyl 2-methylvalerate should be confirmed by radioactive isotope labeling experiment. Linolenic acid as the important member of fatty acids had been considered as the essential fatty acids (Zeng et al. 2015), which could be transformed perhaps from *n*-hexadecanoic acid as showed in Table 1. The increases of linolenic acid should also improve the quality of vine tea.

Biotransformation is a powerful method to obtain some new active compounds. Some active compounds in vine tea such as flavonoids and tea polyphenols were remained by biotransformation using *P. cocos*. Pachyman, methyl 2-methylvalerate and linolenic acid were produced, which gave the fermented vine tea high antioxidant activity and pleasant taste. Therefore, biotransformation improved the taste and quality of vine tea, which was helpful for development of vine tea high quality.

Conclusion

Biotransformation by *P. cocos*, changed flavonoid and polyphenols in vine tea to a little extent, and content of polysaccharides in vine tea increased to 3.9-fold than that of unfermented vine tea. DPPH free radical scavenging capacity was also increased with the increase of polysaccharides in vine tea. Fermented vine tea had pleasant taste such as fragrance of fruits because of methyl 2-methylvalerate which was formed during biotransformation process. Linolenic acid content increased from 0.88 to 19.59 % after biotransformation for 12 days. The taste and quality of vine tea improved by biotransformation.

Acknowledgments This work was supported by Natural Science Foundation of Jiangsu Province (Grant No. BK20141252) and Top-notch Academic Programs Project of Jiangsu Higher Education Institutions (PPZY2015A018).

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