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采用大孔树脂富集内生真菌 *Hyalodendriella* sp. 中的 Botrallin 和 TMC-264

毛子翎,李　京,于瑞婷,徐　丹,周开谊,赖道万,周立刚*

中国农业大学农学与生物技术学院,北京 100193

摘　要:研究了6种大孔吸附树脂对内生真菌 *Hyalodendriella* sp. Ponipodef12 中活性成分 Botrallin 和 TMC-264 的富集作用,其中树脂 SP825L 表现出最好的吸附和解吸附作用。采用树脂 SP825L,优化的吸附条件为:洗脱系统为水,洗脱体积为 28 BV、流速为 3 BV/h、pH 值为 5.0,温度为 25 ℃;优化的解吸附条件为:洗脱系统为乙醇-水(50∶50,v/v)、洗脱体积为 7 BV、流速为 4 BV/h。从 1600 mg 乙酸乙酯粗提物中得到 150 mg 产物,Botrallin 和 TMC-264 的含量由树脂处理前的 2.18% 和 0.65% 分别提高到处理后的 20.66% 和 6.58%,Botrallin 和 TMC-264 的回收率分别为 88.85% 和 94.90%。结果表明,采用树脂 SP825L 从粗提物中富集 Botrallin 和 TMC-264 是一种有效的方法,有利于将来从内生真菌 *Hyalodendriella* sp. Ponipodef12 中大规模制备 Botrallin 和 TMC-264。

关键词:内生真菌;*Hyalodendriella* sp.;大孔树脂;树脂 SP825L;botrallin;TMC-264

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Enrichment of Botrallin and TMC-264 from Endophytic Fungus *Hyalodendriella* sp. by Macroporous Resins

MAO Zi-ling, LI Jing, YU Rui-ting, XU Dan, ZHOU Kai-yi, LAI Dao-wan, ZHOU Li-gang*

College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China

Abstract: The present study described a rapid and cost-effective method for the enrichment of botrallin and TMC-264 as major bioactive dibenzo-α-pyrones in the endophytic fungus *Hyalodendriella* sp. Ponipodef12 by macroporous resins. Resin SP825L displayed the best adsorption and desorption properties for botrallin and TMC-264 among 6 resins. The optimal conditions for resin SP825L adsorption of botrallin and TMC-264 were processing bed volumes (BV) as 28 BV, flow rate as 3 BV/h, pH value as 5.0, and temperature as 25 ℃. Those for desorption were ethanol-water elution system at the ratio of 50∶50 (v/v), eluent volume as 7 BV, and flow rate as 4 BV/h. After one round treatment with resin SP825L, the contents of botrallin and TMC-264 were increased from 2.18% and 0.65% in the crude extract to 20.66% and 6.58% in the final product, respectively. About 150 mg of the final product was obtained from 1600 mg of crude ethyl acetate extract. The recoveries for botrallin and TMC-264 were 88.85% and 94.90%, respectively. The results demonstrated that resin SP825L chromatography can be an appropriate strategy for obtaining botrallin and TMC-264 from the crude extract. The optimized process will be beneficial for large-scale preparation of botrallin and TMC-264 from *Hyalodendriella* sp. Ponipodef12.

Key words: endophytic fungus; *Hyalodendriella* sp.; macroporous resins; resin SP825L; botrallin; TMC-264

Introduction

Plant endophytic fungi are a special group of microorganisms which spend all or part of their lifecycles inter- and/or intracellularly colonizing the healthy tissues of their host plants, typically causing no apparent disease symptoms [1]. They are a class of new microbial resources not fully studied and have been proved to be a promising and largely untapped reservoir of natural products with the great chemical diversity and potential applications in medicine, agriculture and food industry [2-5].

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* Corresponding author Tel: 86-10-62731199; E-mail: lgzhou@cau.edu.cn

Botrallin and TMC-264 belong to the members of dibenzo-α-pyrones (also named benzo-α-pyranones, 6*H*-benzo[*c*]chromen-6-ones, and 6*H*-dibenzo[*b*,*d*]pyran-6-ones)[6]. Botrallin has been isolated from the fungi *Botrytis allii*[7], *Microsphaeropsis olivacea*[8] and *Hyalodendriella* sp.[9] with antimicrobial, antinematodal and acetylcholinesterase inhibitory activities[8,9]. TMC-264 has been isolated from the fungi *Phoma* sp.[10] and *Hyalodendriella* sp.[11]. TMC-264 also showed antimicrobial and antinematodal activities[11]. In addition, TMC-264 selectively inhibited interleukin-4 (IL-4) signaling by interfering phosphorylation of the signal transducer and activator of transcription six (STAT6), as well as binding phosphorylated STAT6 to the recognition sequence. So it might be an inhibitor of IL-4 signaling for the treatment of allergic disease[12]. Both botrallin and TMC-264 have been identified as the main bioactive dibenzo-α-pyrones from the ethyl acetate extract of the endophytic fungus *Hyalodendriella* sp. Ponipodef12 isolated from the hybrid 'Neva' of *Populus deltoides* Marsh × *P. nigra* L in our previous studies[9,11]. In order to speed up the development and application of botrallin and TMC-264, one of the most important approaches is to efficiently separate and purify these two compounds from the crude extract. However, lack of an efficient method for the separation of these two compounds from mycelia limits their final yield and industrialization.

The conventional methods for the separation of dibenzo-α-pyrones including botrallin and TMC-264 have been performed by means of solid-liquid extraction from the fungal materials, then liquid-liquid extraction was conducted by using different solvents with various polarities, and then followed by multistep column chromatography with the gradient solvent system[9,12]. However, due to their relative low handling capacity in one cycle, these separation methods are inefficient, and are also troubled with various other disadvantages such as bulk amount of solvent wastage, low capacity, time-consuming, low yields, special instruments needed, even safety and environmental problems. These deficits make them unsuitable for large-scale industrial production. In view of the important biological properties and the difficulties connected with the existing purification methods, the development of environment-friendly separation strategies with high recovery yield and low-cost technology to obtain botrallin and TMC-264 from the endophytic fungus *Hyalodendriella* sp. Ponipodef12 is urgently required.

Macroporous resins are high cross-linked polymers with large specific surface areas and numerous permanent pores. The adsorption-desorption processes on macroporous resins have been recently received great attention from the scientific community. They have been widely used for separation and purification of various natural compounds from microorganisms and plants due to their high efficiency, low operational cost, easy regeneration, and environment-friendly features[13-21]. To the best of our knowledge, there was no information available about simultaneous enrichment and recovery of botrallin and TMC-264 from the crude extracts by using macroporous resins. The purpose of this investigation was to develop an efficient method for the preparative enrichment and recovery of botrallin and TMC-264 from the crude extract of the endophytic fungus *Hyalodendriella* sp. Ponipodef12 to meet their future development and application. The adsorption and desorption behaviors of botrallin and TMC-264 on the macroporous resins with different physicochemical properties were studied in detail. The processing parameters for the dynamic adsorption and desorption of the screened resin SP825L were further optimized to enrich botrallin and TMC-264 from the crude extract.

Materials and Methods

Chemicals and reagents

Botrallin and TMC-264 standards were purified and identified from the fermentation cultures of the endophytic fungus *Hyalodendriella* sp. Ponipodef12 in our previous studies[9,11]. The organic solvents used for analytical HPLC were of chromatographic grade and purchased from Tianjin Tianhao Chemical Company, Tianjin, China. Water was purified by a Milli-Q system (TTL-30C, Tongtai, Beijing, China). Methanol, ethanol, ethyl acetate and other chemicals and reagents were of analytical grade.

Macroporous resins

The macroporous resins including SP70, SP700, SP850, SP825L, SP207 and HP2MGL were purchased from Mitsubishi Chemical Holdings (Tokyo, Japan). The chemical and physical properties of the macroporous resins were summarized in Table 1. The resins were pre-treated successively by 1 mol/L HCl and then 1 mol/L NaOH solutions to remove the monomers and porogenic agents trapped inside the pores during the synthesis process. Then the resins were soaked in ethanol, shaken for 24 h and then washed with distilled water thoroughly before use [16]. The moisture content of each resin was determined as follows. Three samples of each resin were weighted and placed in a drying oven at 60 ℃ to constant dry weight. The moisture contents were then determined.

Table 1 Chemical and physical properties of the resins employed

Resin	Polymer chemistry	Polarity	Particle size (mm)	Surface area (m^2/g)	Average pore diameter (nm)	Moisture content (%)
SP207	Polystyrene divinyl benzene	Non-polar	≥ 0.25	1260	22	50.33 ± 0.05
SP70	Polystyrene divinyl benzene	Weak-polar	≥ 0.25	880	14	61.30 ± 0.08
SP700	Polystyrene divinyl benzene	Weak-polar	≥ 0.25	1200	18	67.29 ± 0.12
SP850	Polystyrene divinyl benzene	Weak-polar	≥ 0.25	930	9	48.27 ± 0.07
SP825L	Polystyrene divinyl benzene	Weak-polar	≥ 0.25	1050	14	57.72 ± 0.09
HP_2MGL	Polymethacrylate	Middle-polar	≥ 0.30	470	48	64.51 ± 0.22

Note: The information was provided by the manufactures except moisture content.

Preparation of crude extracts

The endophytic fungus *Hyalodendriella* sp. Ponipodef12 (GenBank accession number HQ731647) was isolated from the healthy stems of the 'Neva' hybrid of *Populus deltoides* Marsh × *P. nigra* L. in our previous study [22]. The colony front and back views of *Hyalodendriella* sp. Ponipodef12 on potato dextrose agar (PDA) plate along with its mycelia were shown in Fig. 1.

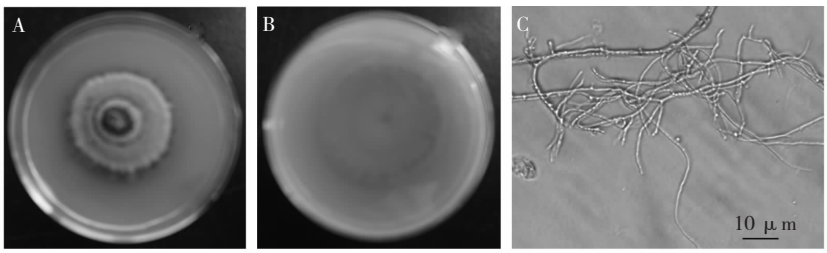

Fig. 1 The colony front review (A), back review (B) and mycelia (C) of *Hyalodendriella* sp. Ponipodef12

Note: The period of culture at 25 ℃ on PDA was in 20 days.

The fungus *Hyalodendriella* sp. Ponipodef12 was stored both on PDA slants at 4 ℃ and in 40% glycerol at -70 ℃ in the Herbarium of the College of Agronomy and Biotechnology, China Agricultural University (Beijing, China). The fungus at 4 ℃ was activated on PDA plates at 25 ℃ for 8-10 days before being used. For seed culture, 4-5 plugs of agar medium (0.5 × 0.5 cm) with fungal cultures were inoculated in each 250-mL Erlenmeyer flask containing 100 mL potato dextrose broth (PDB) medium, and incubated on a rotary shaker at 150 rpm and 25 ℃ for 7 days. For fermentation culture, the endophytic fungus strain Ponipodef12 was inoculated in each 1000-mL Erlenmeyer flask containing 300 mL PDB medium which was inoculated and mixed with the mycelia pellets, and incubated on a rotary shaker at 150 rpm and 25 ℃ for 30 days. Afterwards, a total of 30 L fermentation broth was harvested. The mycelia separated from the culture filtrate by filtration was lyophilized and ground to obtain 350 g of mycelia powder which was extracted with methanol (3 × 2 L) by sonication in an ultrasonic bath. After filtration, the filtrate was concentrated under vacuum at 50 ℃, the brown residue was suspended in water (0.7 L) and fractioned successively with ethyl acetate (3 × 1

L) to obtain the crude ethyl acetate fraction (24 g). The contents of botrallin and TMC-264 in the extracts were 2.18% and 0.65%, deionized water was added to get sample solutions at the concentration of 0.087 mg/mL for botrallin and 0.026 mg/mL for TMC-264, respectively.

HPLC analysis of botrallin and TMC-264

A prominence LC-20A high-performance liquid chromatographic (HPLC) system (Shimadzu, Japan) was used to determine the content of botrallin and TMC-264. Chromatographic separation was carried out by a reversed-phase Ultimate TM XB C_{18} column (250 × 4.6 mm, 5 μm, Welch Materials, Inc., Ellicott, MD, USA). The mobile phase composed of methanol-water (60∶40, v/v) was eluted at a flow rate of 1.0 mL/min; the column temperature was maintained at 40 ℃; the targeted compounds were detected at a wavelength of 234 nm with an SPD-M20A photodiode array detection (DAD) system and the retention times of botrallin and TMC-264 were 7.83 min and 11.43 min, respectively. The injection volume was 10 μL. The LC solution multi-PDA workstation was employed to acquire and process chromatographic data. The calibration curves of two targeted compounds showed good linearity. The linear equation for botrallin by HPLC analysis was $Y = 5.71711 \times 106X\text{-}85792.5$ ($R^2 = 0.9997$), and that for TMC-264 was $Y = 1.62393 \times 10^7 X - 137986$ ($R^2 = 0.9981$), where Y is the peak area, X is quality (μg) of the sample injected for each time, and R is the correlation coefficient.

Adsorption and desorption properties of resins

The static adsorption process of the crude ethyl acetate extract from *Hyalodendriella* sp. Ponipodef12 on macroporous resins was carried out as follows: 0.5 g of each test resin (dry weight basis) together with 40 mL of sample solutions at 2 mg/mL were added into a flask, shaken (100 rpm) for 8 h at 25 ℃. The initial concentrations of botrallin and TMC-264 in the sample solutions as well as their concentrations after adsorption were analyzed by HPLC.

The desorption process was conducted as follows. After adsorption equilibrium was reached, the residual solution was removed, the resins were first washed with 50 mL of deionized water, and shaken at 100 rpm for 2 h, and then desorbed with 40 mL of 95% ethanol solution. After the flask was shaken at 100 rpm and 25 ℃ for 4 h, the desorption solution was analyzed by HPLC. The process was repeated for three times.

The resins were firstly evaluated for their adsorption capacities, and the ratios of adsorption and desorption. The equations for quantification of these parameters were expressed as follows:

Adsorption capacity: $Q_e = (C_0 - C_e) \times (V_i/W)$ (1)

Absorption ratio: $A(\%) = (C_0 - C_e)/C_0 \times 100$ (2)

Desorption ratio: $D(\%) = C_d \times [V_d/(C_0 - C_e)V_i] \times 100$ (3)

where Q_e is the adsorption capacity at adsorption equilibrium (μg/g resin); A and D are the adsorption ratio (%) and desorption ratio (%), respectively; C_0 and C_e are the initial and equilibrium concentrations of botrallin or TMC-264 in the solutions, respectively (μg/mL); C_d is the concentration of botrallin or TMC-264 in the effluent solutions of desorption (μg/mL); V_i is the volume of the initial sample solution (mL); V_d is the volume of the desorption solution (mL); W is the weight of the dry resin (g).

Static adsorption kinetics of resin SP825L

The adsorption kinetic curves of the screened resin SP825L were studied as the static adsorption test described above, except for a change of adsorption time to 3.5 h until adsorption equilibrium. The respective concentrations of botrallin and TMC-264 in the sample solutions after adsorption in a certain time were monitored by HPLC at equal time intervals till equilibration.

Adsorption isotherms

The adsorption isotherms of botrallin and TMC-264 on resin SP825L at different temperatures were also studied by adding 40 mL sample solutions of the crude ethyl acetate extract at different concentrations with 0.5 g resin (dry weight basis) in shakers for 8 h at 25, 30 and 35 ℃, respectively, and their degrees of fitness to Freundlich and Langmuir equations were evaluated[23]. The initial and equilibrium concentrations at different temperatures were determined by HPLC. Both Freundlich and Langmuir equations for describing the interaction of botrallin and TMC-264 with resin SP825L are as

follows [18].

Freundlich isotherm equation: $Q_e = K_F \cdot C_e^{1/n}$ (4)

A linearized form of equation (4) can be written as:

$\log Q_e = 1/n \log C_e + \log K_F$ (5)

where K_F is a constant, an indicator of adsorption capacity; $1/n$ is an empirical constant related to the magnitude of the adsorption driving force; Q_e is the adsorption capacity at adsorption equilibrium (μg/g resin) and C_e is the equilibrium concentration of solutes in the solutions.

Langmuir isotherm equation: $C_e/Q_e = C_e/Q_{max} + 1/(k \cdot Q_{max})$ (6)

Equation (6) can be rearranged to the following linear form: $1/Q_e = 1/(K_L \cdot C_e) + 1/Q_{max}$ (7)

where Q_{max} is the theoretically calculated maximum adsorption capacity (μg/g resin); k and K_L are constants; Q_e and C_e are the same as those defined in Equations (4) and (5).

Effects of pH values of sample solution on the adsorption capacity

The effects of initial pH values of sample solution on the adsorption capacity of resin SP825L were also studied using the same procedure described in the above sub-section "Adsorption and desorption properties of resins". The initial pH values of sample solution were 3, 4, 5, 6, 7, 8, 9 and 10, and the adsorption ratio was calculated to reveal the adsorption capacity. The initial and equilibrium concentrations of the solutions were determined by HPLC.

Effects of ethanol concentrations on desorption ratios of resins and purities of botrallin and TMC-264

The effects of ethanol concentrations on desorption ratios and purities of botrallin and TMC-264 were also studied using the same procedure described in the above sub-section "Adsorption and desorption properties of resins". The ethanol concentrations were 10, 20, 30, 40, 50 and 70%, respectively. The concentrations and purities of botrallin and TMC-264 in the desorption solution were determined by HPLC.

Dynamic adsorption and desorption of botrallin and TMC-264 on resin SP825L

The dynamic adsorption and desorption tests were conducted in a glass column (16 × 400 mm) wet-packed with 10.0 g (dry weight) of resin SP825L. The bed volume (BV) of the resin was 14 mL. For breakthrough volume experiment, the sample solution through the column was at the flow rate of 2, 3 and 4 BV/h, respectively, and the concentrations of botrallin and TMC-264 in the eluents were monitored by HPLC analysis. Once adsorption reached saturation, the loading of the sample solution was stopped.

For desorption experiment, after adsorption reached equilibrium, the columns were firstly washed with deionized water and then eluted with ethanol-water solution at the flow rate of 3, 4 and 5 BV/h, respectively. The concentrations and purities of botrallin and TMC-264 in the desorption solution were determined by HPLC. The effluent solutions were concentrated under vacuum at 60 ℃. The recoveries and the purities of botrallin and TMC-264 in the concentrate treated with resin SP825L were calculated. The following equation was used to calculate the recovery:

$R(\%) = (m/M) \times 100$ (8)

where R is the recovery (%), M is the weight of the targeted component laden onto the selected adsorbent, m is the weight of the targeted component in the product collected

Statistical analysis

All tests were carried out in triplicate, and the results were represented by their mean values and the standard deviations (SD). The data were submitted to analysis of variance (one-way ANOVA) to detect significant differences by PROC ANOVA of SAS version 8.2. The term significant has been used to denote the differences for which $P \leqslant 0.05$.

Results and Discussion

Adsorption and desorption properties of the resins for botrallin and TMC-264

According to the structural characters, botrallin and TMC-264 have both non-polar and polar groups. Six macroporous resins, ranging from non- to middle-polarity, were chosen for testing the adsorption and desorption capacities for botrallin and TMC-264 with the results shown in Table 2. All the resins showed high desorption ratio for the two compounds. Among them, res-

in SP825L showed the best adsorption and desorption capacities. According to the "like dissolves like" principle, resin SP825L has a similar polarity to botrallin and TMC-264, also it has larger surface area (1050 m^2/g) and smaller pore size (14 nm), thus make it suitable for purifying small and low-polarity molecules [24,25]. Obviously, the middle-polar resin HP_2MGL with low specific surface area and big average pore diameter possessed unsatisfactory adsorption and desorption capabilities for botrallin and TMC-264. By considering both adsorption and desorption properties, resin SP825L was selected for the following adsorption kinetics experiments.

Table 2 Adsorption capacities, adsorption desorption ratios of the resins for botrallin and TMC-264

Resin	Botrallin			TMC-264		
	Adsorption capacity (μg/g)	Adsorption ratio (%)	Desorption ratio (%)	Adsorption capacity (μg/g)	Adsorption ratio (%)	Desorption ratio (%)
SP207	897.18 ± 11.51^{d}	63.25 ± 0.81^{c}	91.35 ± 2.54^{a}	52.40 ± 2.17^{c}	69.66 ± 2.89^{d}	92.03 ± 3.87^{a}
SP70	1007.97 ± 10.33^{c}	71.06 ± 0.73^{b}	86.94 ± 2.94^{b}	51.27 ± 2.40^{c}	68.15 ± 3.19^{d}	79.43 ± 2.28^{c}
SP700	1169.87 ± 9.52^{b}	75.12 ± 0.80^{b}	85.07 ± 2.03^{b}	63.72 ± 1.18^{b}	84.70 ± 1.56^{b}	86.06 ± 4.64^{b}
SP850	1101.80 ± 5.99^{b}	77.67 ± 0.42^{b}	93.86 ± 2.95^{a}	58.48 ± 1.26^{bc}	77.73 ± 1.68^{c}	88.56 ± 3.69^{b}
SP825L	1300.54 ± 11.94^{a}	91.68 ± 0.84^{a}	91.13 ± 2.84^{a}	71.21 ± 1.38^{a}	94.64 ± 1.83^{a}	93.11 ± 1.71^{a}
HP_2MGL	431.69 ± 9.77^{e}	30.43 ± 0.69^{d}	90.45 ± 2.19^{a}	37.84 ± 1.69^{d}	50.30 ± 2.25^{e}	94.79 ± 3.25^{a}

Note: The values are expressed as means ± standard deviations ($n = 3$). Different letters indicate significant differences among the treatments in each column at $P \leqslant 0.05$.

Static adsorption kinetics of resin SP825L for botrallin and TMC-264

The static adsorption kinetics curves of resin SP825L for botrallin and TMC-264 were shown in Fig. 2. Botrallin and TMC-264 reached equilibrium approximately at 2.0 and 1.5 h, respectively. Within the first 1 h, adsorption capacity of botrallin on resin SP825L increased quickly. However, a gradual increase was observed for TMC-264. This indicates that resin SP825L possesses good adsorption for botrallin and TMC-264, and 2 h was sufficient to reach adsorption equilibrium. So the duration of adsorption was set to more than 2 h in the following study.

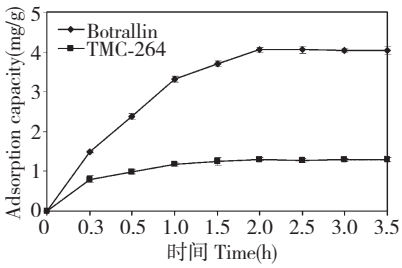

Fig. 2 Static adsorption kinetics curves of resin SP825L for botrallin and TMC-264

Adsorption isotherms of resin SP825L for botrallin and TMC-264

Equilibrium adsorption isotherms of botrallin and TMC-264 on resin SP825L were investigated at 25, 30 and 35 ℃. The initial concentrations of botrallin were 0.022, 0.033, 0.044, 0.065, 0.087 and 0.109 mg/mL, respectively. The initial concentrations of TMC-264 were 0.007, 0.010, 0.013, 0.020, 0.026 and 0.033 mg/mL, respectively. As shown in Fig. 3, the adsorption capacities for botrallin and TMC-264 on resin SP825L increased with the increasing of equilibrium concentration, and reached saturation status at the turning point. The concentration at this point, which corresponds to the equilibrium concentration, was chosen as the proper initial concentration [14]. Therefore, the initial concentrations of botrallin and TMC-264 in sample solution for adsorption were selected as 0.087 and 0.026 mg/mL, respectively. The adsorption capacity of SP825L was weakened with the increase of temperature. This phenomenon indicates that the increase in temperature is a disadvantage for a physical adsorption process between polystyrene resins and adsorbates, implying that the adsorption process is an exothermic process. Therefore, 25 ℃ was selected as the adsorption temperature.

Both Langmuir and Freundlich equation parameters of botrallin and TMC-264 on resin SP825L were shown in Table 3. Two equations can describe well the adsorption behaviors of botrallin and TMC-264 with their high cor-

relation coefficients (R^2). As R^2 value of Langmuir equation was higher than that of Freundlich equation at 25 ℃, Langmuir isotherm model was considered to be fitted better for the absorption equilibrium of botrallin and TMC-264 on resin SP825L.

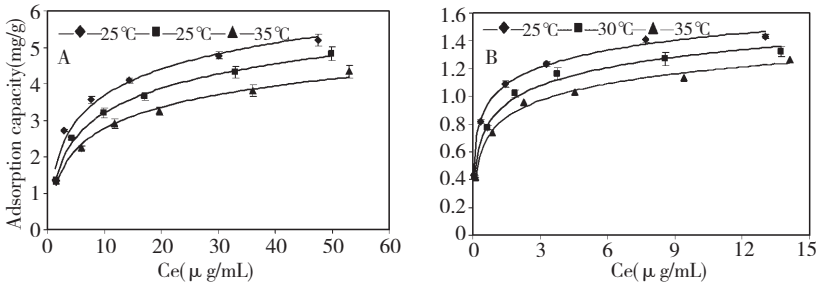

Fig. 3 Adsorption isotherms of resin SP825L for botrallin (A) and TMC-264 (B) at 25, 30 and 35 ℃, respectively

Table 3 Langmuir and Freundlich equation parameters of botrallin and TMC-264 on resin SP825L

Adsorbate	Temperature (℃)	Langmuir equation			Freundlich equation		
		Q_{max}	K_L	R^2	K_F	$1/n$	R^2
Botrallin	25	5000.00	1250.00	0.9732	1535.68	0.34	0.8876
	30	5000.00	1250.00	0.9950	1322.51	0.35	0.9548
	35	3333.33	1250.00	0.9683	1203.10	0.33	0.9898
TMC-264	25	1250.00	20000.00	0.9482	933.68	0.21	0.9714
	30	1111.11	5000.00	0.9652	798.18	0.23	0.9564
	35	1111.11	5000.00	0.9588	719.12	0.23	0.9717

Effects of pH values of sample solution on adsorption capacities of resin SP825L.

The initial pH of adsorption solution is one of the most important parameters influencing the adsorption capacity [26]. As shown in Table 4, the adsorption capacity for botrallin and TMC-264 decreased with the increase of pH values from 5.0 to 10.0. The highest adsorption capacities for botrallin and TMC-264 were observed as 4.37 and 1.30 mg/g, respectively at the pH value of 5.0. Therefore, the pH value of the sample solution was adjusted to 5.0 for all subsequent experiments.

Table 4 Effects of pH values of sample solutions on the adsorption capacities of resin SP825L

pH value	Adsorption capacity of SP825L (mg/g)	
	Botrallin	TMC-264
3.0	4.19 ± 0.11^{a}	1.29 ± 0.01^{a}
4.0	4.24 ± 0.14^{a}	1.30 ± 0.01^{a}
5.0	4.37 ± 0.16^{a}	1.30 ± 0.03^{a}
6.0	3.73 ± 0.21^{b}	1.19 ± 0.06^{ab}
7.0	3.40 ± 0.14^{b}	1.13 ± 0.04^{b}
8.0	3.12 ± 0.12^{bc}	1.11 ± 0.04^{b}
9.0	3.24 ± 0.19^{b}	1.10 ± 0.06^{b}
10.0	2.93 ± 0.21^{c}	1.08 ± 0.03^{b}

Note: The values are expressed as means ± standard deviations ($n = 3$). Different letters indicate significant differences among the treatments in each column at $P \leqslant 0.05$.

Dynamic breakthrough curves on resin SP825L for botrallin and TMC-264

In general, the breakthrough point can be defined as 10% of the ratio of the exit solute concentration to the inlet solute concentration [18]. According to this standard, the corresponding breakthrough volumes of botrallin and TMC-264 were 24 and 28 BV, respectively. If the feed volume of sample solution was selected according to botrallin, TMC-264 would not reach adsorption saturation. Therefore, in consideration of both botrallin and TMC-264, the feed volume of sample solution on resin SP825L was determined as 392 mL (28 BV).

The initial concentration of botrallin and TMC-264 in this test was 0.087 mg/mL and 0.026 mg/mL, respectively, and the flow rate investigated was 2, 3 and 4

BV/h, respectively. As shown in Fig. 4, at a flow rate of 3 BV/h, best adsorption performance of the two compounds was exhibited, which was likely due to better particle diffusion in sample solutions [27]. On the other hand, at the flow rates of 2 and 4 BV/h, the breakthrough volumes for botrallin were 20 and 16 BV, respectively, and at a flow rate of 3 BV/h, breakthrough point was obtained even after 24 BV of extract was loaded on the column. Similar results for TMC-264, at the flow rates of 2 and 4 BV/h, the breakthrough volumes were 24 and 20 BV, respectively, and at a flow rate of 3 BV/h, the breakthrough volume was 24 BV. Therefore, 3 BV/h was selected as the best flow rate.

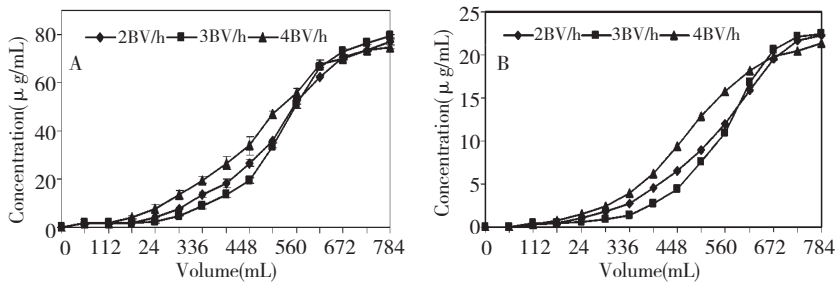

Fig. 4 Dynamic breakthrough curves of botrallin (A) and TMC-264 (B) on the column packed with resin SP825L

Dynamic desorption curves on resin SP825L for botrallin and TMC-264

Based on the data of breakthrough volume determined above, the 392 mL sample solution was fed on the column packed with 10.0 g (dry weight) of resin SP825L. After the adsorption reached equilibrium, the resin was flushed with 150 mL of distilled water for removing the high polar components such as polysaccharides and amino acids in the crude extract [28]. 50% ethanol (v/v) was used to desorb botrallin and TMC-264 from the column at a flow rate of 3, 4 and 5 BV/h, respectively, and the elution curves were obtained and shown in Fig. 5. The results showed that the best desorption performance was obtained at a flow rate of 4 BV/h. At this flow rate, the maximum concentration of the eluent was around 56 (4 BV) and 42 mL (3 BV) for botrallin and TMC-264, respectively, then decreased rapidly, and TMC-264 could be desorbed completely using approximate 6 BV of 50% aqueous ethanol. Subsequently, about another 1 BV of 50% ethanol could desorb botrallin completely. Therefore, eluent volume was selected to be 7 BV to elute botrallin and TMC-264. The low polar impurities bonded to resin SP825L could be eluted using absolute ethanol during regeneration process.

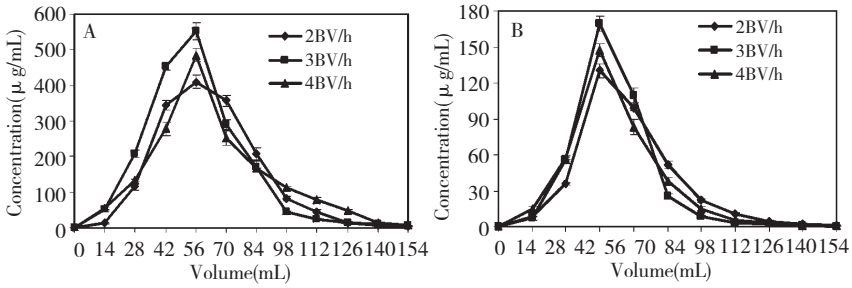

Fig. 5 Dynamic desorption curves of botrallin (A) and TMC-264 (B) on the column packed with resin SP825L

The final optimum parameters for the preparative separation of botrallin and TMC-264 on resin SP825L were determined as follows. For adsorption, the concentrations of botrallin and TMC-264 in the sample solution were 0.087 mg/mL and 0.026 mg/mL, respectively; processing volume as 28 BV; flow rate as 3 BV/h; pH value as 5.0; temperature as 25 ℃. For desorption, the ratio of ethanol – water in the elution system was 50:

50 (v/v); eluent volume as 7 BV; flow rate as 4 BV/h. Through only one cycle treatment by resin SP825L, the contents of botrallin and TMC-264 were increased from 2.18% and 0.65% in the crude extract to 20.66% and 6.58% in the final product, respectively. About 150 mg of the final product was obtained from 1600 mg of crude ethyl acetate extract of *Hyalodendriella* sp. Ponipodef12. The recoveries for botrallin and TMC-264 were 88.85% and 94.90%, respectively.

Conclusion

In this study, the performance and adsorption characteristics of six widely used nonionic macroporous resins have been critically evaluated for the enrichment and recovery of botrallin and TMC-264 from the endophytic fungus *Hyalodendriella* sp. Ponipodef12. Among them, resin SP825L was found to be the most suitable for the enrichment and recovery of botrallin and TMC-264. Resin SP825L offered the highest adsorption capacity for dibenzo-α-pyrones for its higher surface area, optimum pore diameter and appropriate surface functional polarity. The absorption equilibrium of resin SP825L was fitted well to the Langmuir isotherm model. Botrallin and TMC-264 can be obtained from the dynamic absorption and desorption experiments on resin SP825L column chromatography. The contents of botrallin and TMC-264 were increased from 2.18% and 0.65% in the crude extract to 20.66% and 6.58% in the final product with recovery yields of 88.85% and 94.90%, respectively, after a single run on resin SP825L. To the best of our knowledge, this is the first report that macroporous resins were investigated for the simultaneous enrichment and recovery of botrallin and TMC-264 from the crude extract. The achieved enrichment and purification of botrallin and TMC-264 from *Hyalodendriella* sp. Ponipodef12 will be in favor of their development and application as pharmaceuticals. Further investigations are required with regarded to the potential to produce botrallin and TMC-264 by resin adsorption at manufacturing scale.

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