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Construction of hybrid cell with *Phanerochaete chrysosporium* to degrade terephthalic acid wastewater——(I) phenotype evidence

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Abstract: The fungi *Phanerochaete chrysosporium* (PC) and *Saccharomyces cerevisiae* Y99 and the native bacterium YZ1 were the three parental strains for construction of hybrid cells through protoplast fusion to degrade terephthalic acid (TPA) wastewater. The results showed that the native bacterium YZ1 protoplasm could integrate with that of PC to form the hybrid cell Fhh and the fungus Y99 protoplasm also could integrate with that of Fhh to form the hybrid cell Fhhh. The protoplasts of YZ1 and Y99 could change the morphology of PC spore and mycelium for two times. The hybrid cell Fhhh got the best growth and degradation abilities in the wastewater. It suggested that the hybrid strains obtained from the inter-kingdom protoplast fusion of the three parental strains could create potential for the purification of TPA wastewater.

Key words: *Phanerochaete chrysosporium*; YZ1 bacterium; *Saccharomyces cerevisiae*; protoplast fusion; terephthalic acid wastewater

Introduction

Phanerochaete chrysosporium (PC) is one of the unusual white rot fungi that it is able to mineralize the compound of native lignin. And it also can degrade almost all hazardous organic pollutants including genetic mutagenic agents such as chlorinated organic compounds, polycyclic aromatic hydrocarbons, dyes, lignocellulosic materials, nitro-substituted compounds, modified polymers and other organic compounds. PC can produce an unusual enzyme system, which shows a high degree of nonspecificity and oxidizes a very variety of organic pollutants. PC has been studied in great detail with regard to the degradation of anthropogenic chemicals. It has been widely promoted as a bioremediation agent (Paszczynski, 1995; Bumpus, 1989; Yateem, 1998; Cancel, 1993). The fungus PC was used as the first parental strain for protoplast fusion in this research. We attempt that the fusant hybrid strain could get the potential from PC for the degradation for organic pollutants in TPA wastewater with high efficiency.

The second parental strain for the protoplast fusion in this study was the native bacterium YZ1, which was isolated from the activated sludge of the TPA wastewater treatment plant located in Danchang District of Nanjing City. YZ1 is a native bacterium existing in the anaerobic/oxidation (A/O) treatment system for TPA wastewater. The aim for using YZ1 as the second parental strain was to increase the growth ability for the hybrid strain Fhh in TPA wastewater. Fhh was the fusant from PC and YZ1 two parental strains. Fhhh was the fusant hybrid strain obtained through the protoplast fusion between the hybrid strain Fhh and *Saccharomyces cerevisiae* Y99. Y99 was the third parental strain. At least there were 14 flocculation genes discovered in *S. cerevisiae* (Speer, 1992; Watari, 1991). The protoplasm of the yeast Y99 integrating with that of Fhh to from Fhhh, it might enhance the flocculation of Fhhh cells in treatment system, so as to increase the purification rate and decrease the cost for remove of microbe body from effluent.

The protoplast fusion between PC (eukaryote) cell and YZ1 (prokaryote) cell for construction

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of Fhh fusant hybrid cell, and the protoplast fusion between the fusant hybrid Fhh cell and Y99 (eukaryote) cell for construction of Fhhh fusant hybrid cell were all conducted in this research. The result showed that they all could happen, which might construct useful hybrid strain with potential as TPA wastewater treatment tool.

1 Materials and methods

1.1 Strain

The strain of *Phanerochete chrysosporium* (PC) (Sm^rNt^s) was provided by Professor Wang C. H. The gram negative native bacterium YZ1 (Sm^sNt^r) was isolated from the activated sludge of the TPA wastewater treatment plant located in Danchang District of Nanjing City. The strain of the yeast *Saccharomyces cerevisiae* Y99 (Sm^rNt^s) was the production of Dongguan Sugar Mill in Guangdong Province.

The fusant hybrid strain Fhh (Sm^rNt^s) from PC and YZ1, and the fusant hybrid strain Fhhh (Sm^rNt^r) from Fhh and Y99 were constructed and identified in this research.

1.2 Methods

The protoplast fusion between PC and YZ1 to form Fhh and the protoplast fusion between Fhh and Y99 to form Fhhh were conducted according to Wang (Wang, 1996; Table 1).

Table 1 The parameters for the protoplast fusion to construct the fusants of Fhh and Fhhh

Item	Making protoplast				Item	Protoplast fusion	
	YZ1	PC	Y99	Fhh		PC + YZ1	Fhh + Y99
Enzyme	0.5 mg/ml LZ	1% SE	1% SE	1% SE	PEG	30%	30%
Buffer	Ht	HP	HP	HP	Buffer	HS	HS
Agent	2% EDTA	2% SA	2% SA	2% SA	$CaCl_2$	50 mmol/L	50 mmol/L
T, ℃	35	35	35	35	T, ℃	30	30
t, min	40	40	40	40	t, min	10	10
Product	Protoplast of YZ1	Protoplast of PC	Protoplast of Y99	Protoplast of Fhh	Product	Hybrid Fhh	Hybrid Fhhh

Notes: T. reaction temperature; LZ. lysozyme; SE. snail enzyme; HP buffer = P buffer + 20% sucrose (P buffer: $Na_2HPO_4 \cdot 12H_2O$ 29.5g, and lemon acid 1.85g dissolved in distilled water to 500 ml, pH 7.0); HT buffer = T buffer + 20% sucrose (T buffer: 25 ml 0.2 mol/L Tris and 27.5 ml 0.1 mol/L HCl dissolved in distilled water to 100 ml, pH 8.0); HS buffer. 0.5 mol/L sucrose, 20 mmol/L $MgCl_2$ and butene diacid 20 mmol/L, pH 6.5; SA. sulfhydryl alcohol; EDTA: ethylene diamine tetraacetic acid; PEG. polyethyleneglycol; MW = 6000 (Wang, 1996)

2 Results and discussion

All the cells or mycelium or spores of PC, YZ1, Y99, Fhh and Fhhh were stained with Gienmsa method (NJVEB, 1989), then magnified 303 fold with photomicrography. The parental strain PC and the two hybrid strains Fhh and Fhhh all could produce mycelium, the original mycelium of PC had no gaps, but the mycelium of Fhh and Fhhh had gaps. The mycelium diameter for PC was 3.33 μm, higher than that of Fhh with 1.66 μm and Fhhh with 1.33 μm (Table 2). The parental strains of YZ1 and Y99 had no abilities to produce mycedium and spore, but the Fhh hybrid strain from YZ1 and PC, and the Fhhh hybrid strain from Fhh and Y99 all could produce spore and mycelium. It might demonstrate that the genesis ability of PC producing spore and mycelium could dominate in the cells of the hybrid strain Fhh and Fhhh.

The sizes for the spores of PC, Fhh and Fhhh and the sizes for the cells of YZ1 and Y99 were measured. Table 2 shows that the volume of the native bacterium YZ1 cell was the smallest, it was only 0.60 μm^3 about 1/48 of Y99 cell. The volume of PC spore was 38.68 μm^3, however, after its protoplasm integrated with that of YZ1 to form Fhh, the Fhh spore volume decreased to 10.71 μm^3. It was clear that the YZ1 protoplasm affected PC spore size generation through its protoplasm integration with that of PC.

Table 2 Comparison of the sizes of spores or cells of each strains

Strain	YZ1	Y99	PC	Fhh	Fhhh
Cell or spore					
Long axle (a, μm)	1.66(cell)	5.00(cell)	6.67(spore)	7.71(spore)	5.58(spore)
Short axle (b, μm)	0.83(cell)	3.34(cell)	3.33(spore)	1.63(spore)	2.33(spore)
Volume (V, μm^3)	0.60(cell)	29.02(cell)	38.68(spore)	10.71(spore)	16.55(spore)
b/a	0.50(cell)	0.67(cell)	0.50(spore)	0.21(spore)	0.40(spore)
Mycelium					
Diameter, μm	–	–	3.33	1.66	1.33

Note: Each value in Table 2 is the mean of 10 samples; $V = (4\pi/3)(a/2)(b/2)^2$

With the same reason, the Y99 protoplast also affected Fhh spore size through integrating with that of Fhh to form Fhhh, the Fhhh spore volume was 16.55 μm^3, higher than that of Fhh but lower than that of PC (Table 2).

The volumes of both Fhh and Fhhh spore were different from each other and from that of PC, and that of Fhh was different from that of Fhhh. It might illustrated that the protoplasm of the native bacterium YZ1 and the fungus Y99 as two parental strains changed the another parental strain PC genesis feature in spore production for two times.

Ten ml of cell liquor of each strain was transferred into 150 ml of TPA wastewater with COD_{Cr}(chemical oxygen demand) from 8170 mg/L to 9400 mg/L and pH 4.9, then reacted for 9h at 30℃ with shaking at a speed of 200 r/min. The specific growth rate μh^{-1} and specific degradation rate $q h^{-1}$ were measured and are listed in Table 3.

Table 3 The kinetic parameter values of μ and q for 5 strains in TPA wastewater

Strain	PC	YZ1	Y99	Fhh	Fhhh
μ value					
0—3h, μh^{-1}	0.0246*	0.0122*	ND	0.0916*	0.0428
3—6h, μh^{-1}	0.0229	0.0118	ND	0.0726	0.1265*
6—9h, μh^{-1}	0.0035	0.0033	ND	0.0087	0.0042
q value					
0—3h, $1h^{-1}$	0.0297*	0.0175*	ND	0.2163\ + *	0.1128
3—6h, $q h^{-1}$	0.0262	0.0157	ND	0.1549	0.2238*
6—9h, $q h^{-1}$	0.0059	0.0074	ND	0.0126	0.0113

* the highest value for each kinetic parameter of each strain during 9h reaction; ND. no detection, $\mu(\ln Xn - \ln X0)/t$, Xn is the biomass concentration at the end of the reaction, $X0$ is that of at the starting of the reaction, t is the reaction time; $q = \mu(S0 - Sn)/[X0(e^{\mu t} - 1)]$, here $S0$ is the organic pollutant concentration of COD_{Cr} at the reaction starting, Sn is that at the end of the reaction, μ, $X0$ and t are the same as those in the formula for calculating μ value

The higher the specific growth rate, the better the generation ability for a strain. Table 3 shows that the highest μ value for strains occurred in the first 3h reaction in TPA wastewater, except Fhhh and Y99. Fhhh highest μ value occurred in the period of 3—6h reaction and Y99 could not grow in the wastewater. It means that Fhhh strain needed longer time to suit TPA wastewater than the other 3 strains. After 6h of reaction, the μ values for strains dropped down obviously. The pollutants in TPA wastewater or/and other conditions could not maintain good enough for strains to grow. The order of the highest μ for 5 strains during the period of 9h reaction was: Fhhh($0.1265h^{-1}$) > Fhh($0.0916h^{-1}$) > PC($0.0246h^{-1}$) > YZ1($0.0122h^{-1}$) > Y99 ($0h^{-1}$). It was very clear that the generation ability for the hybrid strains Fhhh and Fhh were better than their three parental strains.

The higher the specific degradation rate q, the higher the ability for the degradation of the organic pollutant. The order of the highest q for strains in TPA wastewater was Fhhh ($0.2238h^{-1}$) > Fhh($0.2163h^{-1}$) > PC($0.0297h^{-1}$) > YZ1($0.0175h^{-1}$) > Y99($0h^{-1}$) as shown in Table 3. It illustrated that the degradation ability of Fhhh and Fhh was higher than that of the parental strains. The highest q value for Fhhh occurred in the 3—6h, but that for the other three strains occurred in the first 3h. It might suggest that Fhhh strain needed longer time to suit TPA

wastewater than the other strains. After 6h, the q values of the strains decreased obviously. The concentration of the easy degraded pollutant might decrease to limit the rise of q value for each strain.

There was a very important change happened, the q value of the native bacterium YZ1 was $0.0074h^{-1}$ in the 6—9h reaction, it was the first time to be higher than that of PC with $0.0059h^{-1}$. It might mean that the suitability and persistence ability of YZ1 in TPA wastewater was better than those of PC, in spite of PC had higher q value in the first 6h. However the q value of YZ1 in 6—9h reaction was still lower than those of Fhhh and Fhh which were $0.0113h^{-1}$ and $0.0126h^{-1}$ respectively.

TPA wastewater had the acute toxicity on Y99 cell, so the values of μ and q for Y99 could not be detected. Although Y99 could not grow in TPA wastewater to degrade the pollutants, its protoplasm could integrate with that of Fhh to form Fhhh, and promoted Fhhh obtaining the best ability of growth and degradation. It was reported that in UASB processes of TPA wastewater treatment, the q value of the anaerobic bacteria was from $0.0035h^{-1}$ to $0.0406h^{-1}$(Cheng, 1997; Kleerebezem, 1997; Kuang, 1994). They were all lower than those of Fhh and Fhhh in the first 6h reaction time. It suggested that the hybrid cells, Fhh and Fhhh, had the potential for the TPA wastewater treatment, better than the anaerobic bacteria reported and their parental strains.

3 Conclusion

Fhh hybrid strain could be constructed through the inter-kingdom protoplast fusion of eukaryote-prokaryote cells and Fhhh could be that of eukaryote-prokaryote-eukaryote cells. Both Fhh and Fhhh could produce spores and mycelium as those of the parental strain PC.

The order of spore volumes was PC ($38.68\ \mu m^3$) > Fhhh($16.65\ \mu m^3$) > Fhh($10.71\ \mu m^3$). The mycelium for Fhh and Fhhh had gaps and PC had not. The protoplasm of YZ1 and Y99 affected the mycelium and spore morphology of PC through the process of protoplasm integration.

Fhh and Fhhh could grow on the medium with streptomycin and nystatin, but the parental strain could only grow on the medium with one of the antibiotics. The resistance of Fhh and Fhhh could be used for isolating the hybrid strains and for observing their genetic stability.

TPA wastewater had acute toxicity to Y99, but Y99 protoplast also could fuse with that of Fhh to form Fhhh and promote Fhhh abilities of growth and degradation in the wastewater. Fhh and Fhhh had the potential for increasing treatment efficiency and decreasing operation costs.

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References:

Bumpus J A, 1989. Appl Environ Microbiol [J], 55: 154—158.

Cancel A M, Orth A B, Tien M, 1993. Appl Environ Microbiol [J], 59:2909—2913.

Cheng S P, Cui Y B, 1995. Biomedi Lett [J], 52:101—112.

Cheng S S, Ho C Y, Wu J H, 1997. Pilot study of UASB process treating PTA manufacturing wastewater [C]. Anaerobic Digestion Ⅷ 8, IAWQ international conference on anaerobic digestion, Sendai Japan . 25—29.

Deng L W, Cheng S P, 1996. J Shanghai Jiaotong Univ [J], 30:60—65.

Kleerebezem R, Mortier J, Huishoffpol L W *et al.*, 1997. Wat Sci Technol [J], 2—3: 237—248.

Kuang X, Wang J, 1994. J Environ Sci [J], 2: 180—188.

NJVEB, 1989. The techniques and methods for environmental biological examination [M]. Nanjing: Nanjing University Press. 190—196.

Paszcyzynski A, Grawford R L, 1995. Biotechnol Prog [J], 11:368— 379.

Speer R A, Tung M A, Durance D T, 1992. J Inst Brew [J], 98:293— 300.

Wang Y S, Zou G H, Cui Y B *et al.*, 1996. J Biotechnol [J], 12:53— 57.

Watari J, Takata Y, Ogawa M, 1991. Agri Bio Chem [J], 6:1547—1552.

Yateem A, 1998. Environ Int [J], 24:181—187.

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