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NOTE

Enhancement of 6-pentyl-α-pyrone fermentation activity in an extractive liquid-surface immobilization (Ext-LSI) system by mixing anion-exchange resin microparticles

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The addition of anion-exchange resin microparticles into a polyacrylonitrile (PAN) ballooned microsphere layer drastically enhanced the fermentative activity of *Trichoderma atroviride* AG2755-5NM398 in an extractive liquid-surface immobilization (Ext-LSI) system. The production of 6-pentyl-α-pyrone (6PP), a fungicidal secondary metabolite, was 1.92-fold higher than the control (PAN alone).

[**Key words:** Interface bioreactor; Liquid-surface immobilization; Ion-exchange resin; *Trichoderma atroviride*; 6-Pentyl-α-pyrone]

Filamentous fungi have various important applications in many industries, such as traditional food and brewery industries; the production of metabolites, enzymes and pharmaceutical intermediates; and the biodegradation of harmful pollutants. In many cases, fungi are grown in submerged cultivation (SmC) systems to form pelleted, filamentous, or clumped forms. In general, a pelleted fungal form measuring 1–2 mm in diameter is the ideal morphology for fungi grown in SmC because this form maintains a low viscous broth (1). However, a large number of factors must be optimized for fungal cultivation, such as inoculum type and size, medium composition and pH, agitation rate, incubation temperature, and a number of additives (surfactant, polymer, and chelator) must be optimized for the formation of fungal pellets (2). Therefore, the control of the fungal morphology is important; however, this process is troublesome to implement.

Recently, fungal cultivation systems have been freed from the problem of morphology control, which has allowed us to develop three unique fungal cultivation and application systems: a liquid-surface immobilization (LSI) system (3), an extractive liquid-surface immobilization (Ext-LSI) system (4,5), and a liquid–liquid interface bioreactor (L–L IBR) system (3,6–9). In these systems, a number of unique polymeric materials, ballooned polyacrylonitrile (PAN) microspheres (MSs), are used for the immobilization of the fungal cells on the surface of a liquid medium. Therefore, the fungal cells are immobilized and morphologically differentiate on the surface of a liquid medium (LSI) or on the interface between a hydrophobic organic solvent and a liquid medium (Ext-LSI and L–L IBR).

In these systems, the fungal cells are in an almost electrically neutral PAN–MS layer. It is known that the electric charge of carriers drastically affects the physical and biochemical activity of microorganisms. For example, various anion-exchange resin particles significantly enhance the *p*-xylene-oxidation activity of *Nocardia* (10), the growth rate of *Escherichia coli* (11), and the spore-formation of *Bacillus subtilis* (12). Furthermore, a number of cationic solids exhibit antimicrobial activity against gram-negative bacteria (13,14). Therefore, the interaction between microbial activity and the electric charge of the solid surface is interesting. In this report, the electric charge of an MS layer in the Ext-LSI system was modified by mixing anion- or cation-exchange microparticles or chelating resin microparticles. The novel Ext-LSI system was applied to the production of 6-pentyl-α-pyrone (6PP), a fungicidal secondary metabolite, using *Trichoderma atroviride* AG2755-5NM398 in the Ext-LSI system (4).

T. atroviride AG2755-5NM398 was restored on potato-dextrose agar and was cultivated in F-1 medium containing 20.0 g of potato starch, 10.0 g of glucose, 20.0 g of soy protein (Soypro™; Inui Co., Ltd., Osaka), 1.0 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, and 1.0 L of reverse osmosis water, pH 6.0, for the preparation of the seed broth. A natural medium (6PP-medium) consisting of 40.0 g of fructose, 15.0 g of malt extract, and 1.0 L of reverse osmosis water, pH 7.5, was used for the production of the 6PP.

MMF-DE-1 (former MFL-80SDE; non-coated type; diameter, 30 μm; density, 0.06) (Matsumoto Yushi-Seiyaku Co., Ltd., Osaka) was used as the MS in the Ext-LSI. The MSs were sterilized in a closed bottle in an autoclave (121°C, 20 min). The excess steam

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TABLE 1. Ion-exchange resins used in this study.

	Resin type	Functional group	Total capacity
IRA98	Anion-exchange (strongest)	$-N^+(CH_3)_2$	≥1.35 meq/g
IRA910CT	Anion-exchange (strong)	$-N^+(C_2H_4OH)=(CH_3)X$	≥1.00
IRA958	Anion-exchange (middle)	$-N^+\equiv(CH_3)X$	≥0.80
IRA904	Anion-exchange (weak)	$-N^+\equiv(CH_3)X$	≥0.65
IRC76	Cation-exchange (strong)	$-COO^-M$	≥3.90
252	Cation-exchange (weak)	$-SO_3^-M$	≥1.80
IRC748	Chelation	$-N^+=(CH_2COO^-)_2M$	≥1.35

X, halogen ion (Cl^-); M, metal ion (Na^+).

caused the MS microparticles to shrink. Different anion-exchange resins (IRA98, IRA910CT, IRA958, and IRA904), two cation-exchange resins (IRC76 and IRC252), and a chelating resin (IRC748) were used as the ion-exchange resins (IERs, Table 1). The IER particles were soaked overnight in reverse osmosis water. Next, the anion-exchange and the cation-exchange resin particles were soaked and stirred in a 1 N HCl and 1 N NaOH solution, respectively, at 700 rpm for 4 h and were washed repeatedly using reverse osmosis water (700 rpm, 2–3 h, 20 times). After removing the moisture at 105°C, the IER particles were crushed using an auto-mortar and the resin shards were fractionated using a 25, 50, or 100 μm-mesh.

Fifteen ml of a mixture of 75 ml of 6PP-medium, 150 or 225 mg of the control of MS (MMF-DE-1), 375, 750, or 1125 mg of the IER microparticles, and 1.125 ml of a 3-day-old culture of *T. atroviride* AG2755-5NM398 was poured into a glass vessel (diameter, 30 mm; volume, 50 ml). Four duplicates were prepared in each mixture. After precultivation at 25°C by allowing the vessels to stand for 3 days, 3 ml of low viscous dimethyl silicone oil (KF-96L-1CS; 1 cP) (Shin-Etsu Chemical Co., Ltd., Tokyo) was added to the fungus-MS/IER mat. The still cultivation was continued for 3 weeks, and the organic phase was analyzed directly by gas chromatography using a column (0.25 mm i.d. × 60 m) containing Equity-5 (Supelco Co., Ltd., Bellefonte, PA, USA). The column temperature was 230°C

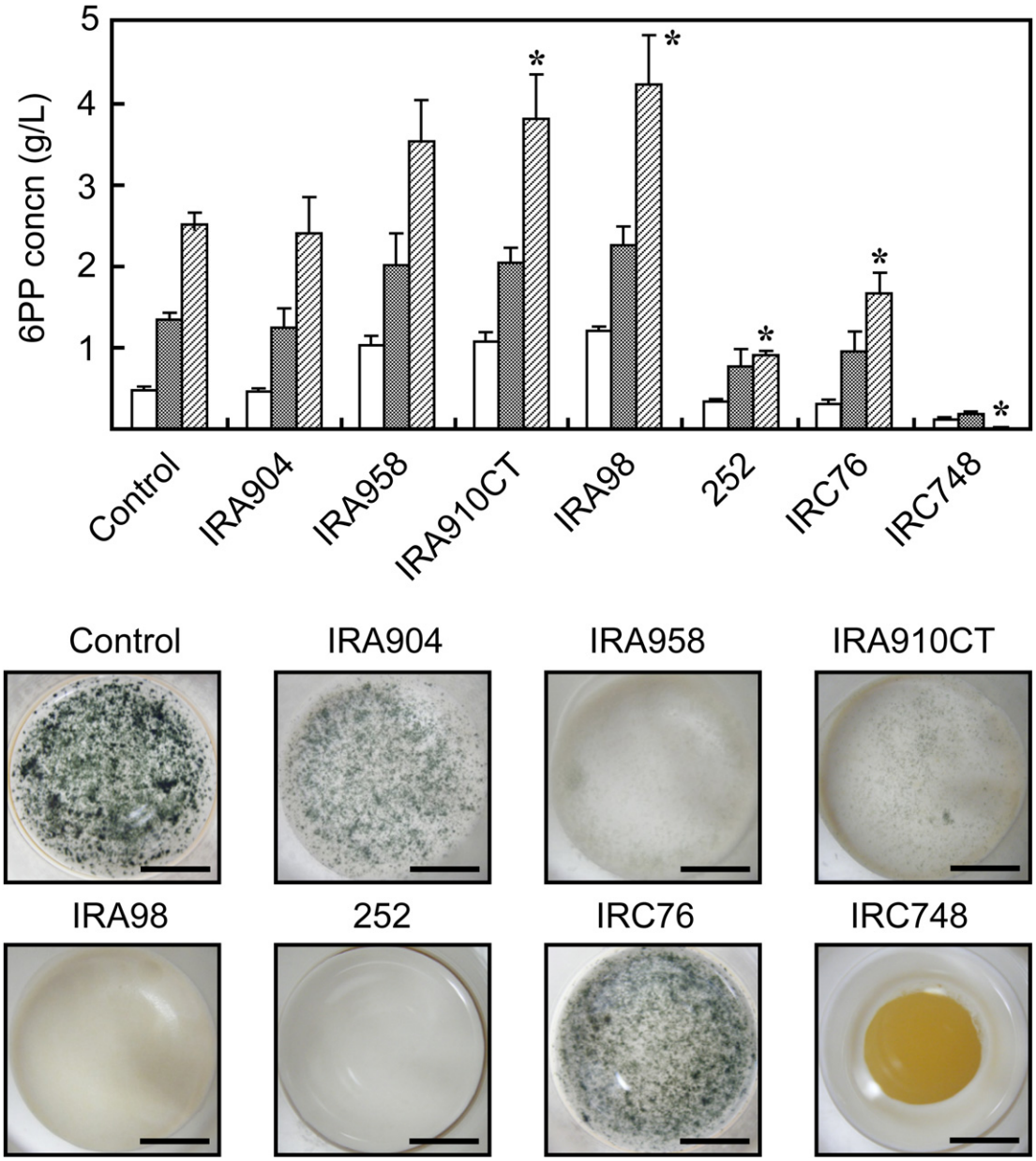

FIG. 1. Effect of the addition of ion-exchange resins (IERs) into a polyacrylonitrile (PAN)-microsphere (MS) layer on the production of 6PP and spores. Each IER microparticle (IER, 50 μm-passed) was mixed with MSs at the ratio of IER:MS = 5:1. *Significant differences compared to the control (only PAN) at $p < 0.05$ in a *t*-test after 3 weeks of cultivation ($n = 4$). The scale bar in each image represents 10 mm.