Synthetic and Systems Biotechnology 6 (2021) 127–133

Contents lists available at ScienceDirect

Synthetic and Systems Biotechnology

journal homepage: www.sciencedirect.com/journal/synthetic-and-systems-biotechnology

An efficient marker recycling system for sequential gene deletion in a deep sea-derived fungus *Acremonium* sp. HDN16-126

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ARTICLE INFO

Keywords:
Acremonium sp.
Deep sea-derived
Homologous recombinant
Marker recycling system
Markerless deletion

ABSTRACT

Acremonium species are prolific producers of therapeutic molecules which include the widely used beta-lactam antibiotic, cephalosporin. In light of their significant medical value, an efficient gene disruption method is required for the physiological and biochemical studies on this genus of fungi. However, the number of selection markers that can be used for gene targeting is limited, which constrain the genetic analysis of multiple functional genes. In this study, we established a uridine auxotrophy based marker recycling system which achieves scarless gene deletion, and allows the use of the same selection marker in successive transformations in a deep sea-derived fungus *Acremonium* sp. HDN16-126. We identified one homologue of *Acremonium chrysogenum pyrG* (also as a homologous gene of the yeast *URA3*) from HDN16-126, designated as *pyrG-A1*, which can be used as a selection marker on uridine free medium. We then removed *pyrG-A1* from HDN16-126 genome via homologous recombination (HR) on MM medium with 5-fluoroortic acid (5-FOA), a chemical that can be converted into a toxin of 5-flurouracil by *pyrG-A1* activity, thus generating the HDN16-126-△*pyrG* mutant strain which showed auxotrophy for uridine but insensitivity to 5-FOA and enabled the use of exogenous *pyrG* gene as both positive and negative selection marker to achieve the scarless deletion of target DNA fragments. We further applied this marker recycling system to successfully disrupt two target genes *pepL* (encodes a putative 2OG-Fe (II) dioxygenase) and *pepM* (encodes a putative aldolase) identified from HDN16-126 genome, which are proposed to be functional genes related to 2-aminoisobutyric acid metabolism in fungi. This work is the first application of uridine auxotrophy based scarless gene deletion method in *Acremonium* species and shows promising potential in assisting sequential genetic analysis of filamentous fungi.

1. Introduction

Acremonium sp. is a group of filamentous fungi widely distributed in soil, sea water and on decaying plant material in nature [1,2]. As the marked [beta]-lactam antibiotic of cephalosporin C was first discovered from *Acremonium* species, this genus has been regarded as one of the most pharmaceutically important filamentous fungi following *Penicillium* species [3–5].

Chemical and pharmaceutical studies on *Acremonium* spp. have proven that they are also prolific producers of other secondary metabolites including steroids, terpenoids, metroterprnoids, polyketides, alkaloids and peptides [6–8]. However, the vast majority of the biosynthetic machinery of those metabolites as well as physiological and biochemical effectors of this genus are still obscure. This is partially due to the lack of efficient genetic manipulation system and the limited availability of selection markers for transformation.

As so far, marker recycling system and scarless deletion methods are widely adopted to circumvent the limitation of selection markers [9,10], among which, the *pyrG*-based marker recycling system is often used in eukaryotic microorganism [11]. *PyrG/URA3* orthologues are genes that

Peer review under responsibility of KeAi Communications Co., Ltd.
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https://doi.org/10.1016/j.synbio.2021.05.001
Received 26 February 2021; Received in revised form 11 May 2021; Accepted 17 May 2021

encode orotidine-5′-monophosphate decarboxylases (OMPdecase) or orotidylate decarboxylase that catalyze the decarboxylation of orotidine 5'-monophosphate to form uridine monophosphate [12,13]. This family of enzymes also could convert 5-fluoroorotic acid (5-FOA), an analogue of the uracil precursor, to 5-fluorouracil, a toxic compound that inhibits DNA and RNA synthesis [13,14]. Therefore, a *pyrG* or *URA3* gene could be used as a feasible counter-selection marker when it is positioned between homologous sequences, excision of *pyrG/URA3* cassette by homologous recombination can be selected with 5-FOA added in medium. Indeed, the *pyrG/URA3*-based counter-selection system has been successfully applied in *Saccharomyces cerevisiae* [15], *Aspergillus* spp. [16,17], *Candida albicans* [18], *Neurospora crassa* [19], *Talaromyces versatilis* [20], *Mucor circinelloides* [21] and *Colletotrichum orbiculare* [22]. However, this system has never been applied to the genus of *Acremonium* species.

During our continued work in searching pharmaceutical fungal strains from Deep-Sea environments, one fungal strain *Acremonium* sp. HDN16-126 was obtained from surface sediment sample collected from Mariana Trench. Chemical studies on this strain afforded several antibacterial structures including prenylphenols, macrolides, diketopiperazines and peptaibols (data not published), which indicated talented metabolic capacity of HDN16-126. To facilitate further biochemistry studies on the strain HDN16-126, we established a uridine auxotrophy based scarless gene deletion system. In this method, the native *pyrG* (*pyrG-A1*) in HDN16-126 was first deleted from chromosome using a *hyg* cassette to generate the △*pyrG* strain, which enables the employment of *pyrG-A1* gene as a counter-selection marker. This △*pyrG* strain was then subjected to take up a gene knock out cassette carrying *pyrG-A1* flanked by three homologous arm fragments. After two rounds of screening on uridine free medium (*pyrG-A1* worked as complementary for the uridine auxotrophy) and MM medium supplemented with 5-FOA (*pyrG-A1* worked as counter-selection marker), the cassette was first knocked-into and then knocked-out from the chromosome, resulting in the recycling of the *pyrG-A1* marker and scarless deletion of target genes. Here we will describe the details of the marker recycling system together with its successful application in disrupting two target genes *pepL* and *pepM*, which are proposed to be related to 2-aminoisobutyric acid metabolism in fungi.

2. Materials and methods

2.1. Strains and culture conditions

The fungal strain HDN16-126 was isolated from Mariana Trench sediment sample (−8000 m) and identified as *Acremonium* sp. based on the internal transcribed spacer (ITS) region of rDNA sequence (GenBank no. MK543173). The strain was cultured on PDA solid medium (BD/Difco), the spores were collected and stored at −80 °C in 20% glycerol.

Saccharomyces cerevisiae BJ5464 [23] was used for yeast homologous recombinant, *Escherichia coli* DH10B was used for standard cloning (purcased from Solarbio, Beijing) (Table 1).

2.2. Bioinformatic analysis of target genes

The genome of *Acremonium* sp. HDN16-126 was sequenced at Beijing Genomics Institute (BGI) Co. Ltd (Qingdao, China). OMPdecase (GenBank: P14017.1) from the strain *Acremonium chrysogenum* [24] was selected as a query to identify *pyrG* gene from the genome of *Acremonium* sp. HDN16-126 using NCBI local blastp tool (https://www.ncbi.nlm.nih.gov/books/NBK52637/). Similarly, two target genes *pepL* and *pepM* which are postulated to be 2-aminoisobutyric acid metabolism relating genes were identified through local blastp tool by using the amino acid sequence of TqaL and TqaM as queries from *Penicillium aethiopicum* [25].

The sequences relationship of those genes with their orthologues were further predicted and analyzed by ClustalW alignments [26] and cytoscape software [27,28].

2.3. Hygromycin and 5-FOA sensitivity assay and uridine complementation assay

Agar dilution method was adopted to identify the lethal doses and proper working concentrations of hygromycin and 5-FOA. Spores of HDN16-126 were inoculated on PDA medium supplemented with hygromycin at gradient concentrations (0, 25, 50, 100, 200, 300 μg/mL) and on MM solid medium (1% glucose, 5% nitrate salts, 0.1% trace elements) supplemented with 5-FOA at gradient concentrations of (0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/mL), respectively. To confirm the proper dose of uridine for the auxotrophic mutants required to restore their growth, the △*pyrG* mutant type (MT) strains were inoculated on MM medium with 5-FOA and different concentrations of uridine (0, 1, 2.5, 5, 10 mM) in the second-round selection process. Each culture inoculated with equivalent spores and incubated at 28 °C for 4 days, all experiments were conducted in triplicate and cultures with the same volume of solvent worked as controls.

2.4. Preparation the protoplast of HDN16-126

HDN16-126 was cultured in PDA solid medium at 28 °C for 5 days, and the spores were collected and stored at −20 °C in 20% glycerol (4×10^5/mL). 1 mL spores were then inoculated into 40 mL PDB + YE liquid medium and incubated at 28 °C, 180 rpm for about 10 h. Mycelia was harvested by centrifugation at 4000 rpm for 15 min at 4 °C. The supernatant was removed and the mycelia was washed with 15 mL Osmotic buffer (1.2 M $MgSO_4 \cdot 7H_2O$, 10 mM sodium phosphate, pH 5.8) and centrifuge at 4000 rpm for 15 min at 4 °C. Then the sediment was re-suspended with 10 mL Osmotic buffer containing 0.04 g Yatalase and

Table 1
Strains and plasmids used in this study.

Strains or plasmids	Characteristics	Sources
Strains		
Acremonium sp. HDN16-126	Isolated from sediment sample collected from Mariana Trench	Preserved in our laboratory
Saccharomyces cerevisiae BJ5464	Recombination host of the knock out fragments	Preserved in our laboratory
Escherichia coli DH10B	Cloning host	Solarbio life sciences
Plasmids		
pYEU	Recombination vector of the knock out fragments	Preserved in our laboratory
pHyg	Hygromycin resistance gene carrier	Preserved in our laboratory
pYEU-*pyrG*	pYEU carring *pyrG*-up, *pyrG*-dn, *hyg*, *pyrG* gene fragments	This study
pYEU-*pepL*	pYEU carring *pepL*-up, *pepL*-dn, *pyrG*, *pepL* gene fragments	This study
pYEU-*pepM*	pYEU carring *pepM*-up, *pepM*-dn, *pyrG*, *pepM* gene fragments	This study

0.06 g Lysing Enzymes from *Trichoderma* (Takara Bio). The resulting mixture was transferred in a 50 mL sterilized conical flask. The flask was incubated at 28 °C, 80 rpm overnight, then the culture liquid was transferred into 50 mL sterile centrifuge tube and added isovolumetric trapping buffer (0.6 M sorbitol, 0.1 M Tris-HCl, pH 7.0) softly, the mixture was centrifuged at 4000 rpm at 4 °C for 15 min. Then the protoplast layer was obtained and re-suspended in STC buffer (1.2 M sorbitol, 10 mM $CaCl_2$, 10 mM Tris-HCl, pH 7.5) with 2x volume, followed by centrifuge at 4000 rpm at 4 °C for 15 min. The supernatant was discarded and the protoplast was harvested and stored in 1 mL STC buffer for transformation.

2.5. Generation of pyrG-A1 deletion mutant

Yeast homologous recombination [29] was used for the construction of *pyrG* knock out cassette (Fig. S2). The cassette was designed to carry four fragments: upstream (left flank sequence of *pyrG-A1* gene's promoter), downstream (right flank sequence of *pyrG-A1* gene's terminator), *hyg* (hygromycin resistance gene) and *pyrG-A1* (partial sequence of *pyrG-A1* expression cassette). For the construction, *hyg* gene fragments with its promoter and terminator were PCR amplified from the pHyg plasmid using primer pair of hyg-F/hyg-R and *pyrG-A1* fragments together with its upstream and downstream flanking sequences amplified from genomic DNA of HDN16-126 using primer pairs of pyrg-F/pyrg-R, pyrg-up-F/pyrg-up-R, pyrg-dn-F/pyrg-dn-R (Table S1), respectively. To facilitate yeast homologous recombination and assemble these DNA fragments into one construct, 30-bp homology regions were added to primers thus generating short overlap sequences between these fragments and the cloning vector pYEU (Fig. S1). The cloning vector pYEU was linearized by SpeI/PmlI and co-transformed together with above four fragments into *S. cerevisiae* BJ5464 by using Frozen-EZ Yeast Transformation II Kit (Solarbio, Beijing). Transformants were selected on semisynthetic medium (dropout uridine) and the constructed plasmid pYEU-*pyrG* cassette was rescued from yeast cells by Zymoprep™ II-Yeast Plasmid Miniprep and re-transformed into *E.coli* DH10B by $CaCl_2$-transformation method. The propagated plasmids were finally extracted from *E.coli* and verified by PCR and sequencing.

The *pyrG-A1* knock out cassette was PCR amplified from plasmid pYEU-*pyrG* using primers of kz-pyrg-F/kz-pyrg-R and transformed into the protoplast by the polyethylene glycol (PEG)-$CaCl_2$ method [30]. 20 μL PCR product of *pyrG-A1* knock out cassette was added into 100 μL protoplast, mixed gently and placed on ice for 1 h, then 600 μL PEG was added and the mixture was incubated at room temperature for 30 min to accomplish the first round of homologous recombination. The reaction product was cultivated on PSA plates (PDA plates supplemented with 218.6 mg/mL sorbital) supplemented with hygromycin at 28 °C for 3–4 days, and recombinants were picked up and verified by PCR using primer yz-hygMT-F/yz-hygMT-R. The correct recombinants were further screened on MM medium plus uridine and 5-FOA, where the uridine auxotrophy mutants (HDN16-126-△*pyrG*) were picked up, followed by PCR verification using the primer pair of yz-pyrgup-F1/yz-pyrgdn-R1 and yz-pyrgup-F2/yz-pyrgdn-R2 (Fig. 2C).

2.6. Generation of pepL and pepM deletion mutant

To tested whether uridine auxotrophy could be applied to scarless gene deletion, *pepL* and *pepM* were selected as target genes for disruption. The *pepL* disruption cassette was designed to contain four fragments: upstream and downstream of *pepL* amplified by the primer L-UP-F/L-UP-R and L-DN-F/L-DN-R; the *pepL* gene fragment amplified by the primer pair of L-F/L-R; and the *pyrG-A1* together with promoter (~1.5 kb) and terminator (~800 bp) amplified by primer pair of PYRG-F/PYRG-R (Table S1). Four fragments were integrated into the plasmid pYEU using yeast homologous recombination method as described in this paper with the order as upstream-downstream-*pyrG-pepL*, thus generating pYEU-*pepL* (Fig. S1). The *pepL* knock out cassette was PCR amplified from plasmid pYEU-*pepL* using primers kz-L-F/kz-L-R and transformed into the protoplast of HDN16-126-△*pyrG* by the polyethylene glycol (PEG)-$CaCl_2$ method as described before for homologous recombination. After one round of screening on uridine free medium and one round of screening on medium with 5-FOA, the mutant strain with scarless deletion of *pepL* (△*pepL*) was obtained. The △*pepM* mutant was generated by the same method as above, both mutants were verified by PCR primers (yz-L-F/yz-L-R for △*pepL* and yz-M-F/yz-M-R for △*pepM*).

3. Results

3.1. Bioinformatic analysis of pyrG, pepL and pepM

PyrG, pepL and pepM homologous sequences were identified by NCBI local blastp program. The 1104 bp open reading frame encoding a protein of 368 amino acids was identified from HDN16-126 and designated as *pyrG-A1* based on the high similarity with OMPdecase from *Acremonium chrysogenum* (81.71% identity). Protein interaction analysis of OMPdecase was conducted using *A. chrysogenum*, *Trichoderma* sp., *Fusarium* sp. and other fungal species as references. As shown in Fig. 1A, OMPdecase-HDN16-126 clustered with homologues from *A. chrysogenum* and several other species, which showed a high possibility of the similarity in functions among those genes (E value: 50).

The gene of *pepL* encoding a putative 2OG-Fe (II) dioxygenase and *pepM* encoding a putative aldolase were identified based on their similarities to *Penicillium aethiopicum* derived TqaL (57.95%) and TqaM (67.32%), respectively. The protein interaction network as shown in Fig. 1B and C denoted their functional similarities with the homologous genes from reference filamentous strains (E value: 50).

3.2. Antibiotic sensitivity of Acremonium sp.

Wild type *Acremonium* sp. (WT) was subjected to agar dilution test with hygromycin (0–300 μg/mL) and 5-FOA (0–2.5 mg/mL). Growth of WT could be totally inhibited on PDA medium with 50 μg/mL of hygromycin and MM medium with 0.5 mg/mL of 5-FOA, which determined the working concentration of 50 μg/mL for hygromycin and 0.5 mg/mL for 5-FOA in transformants and mutants selection (Fig. S6A).

3.3. Generation of pyrG-A1 mutant

A 6732 bp *pyrG-A1* knock out cassette was amplified from plasmid pYEU-*pyrG* (Fig. S4) and dissolved in 20 μL STC buffer with final concentration of 400 ng/μL. Protoplast harvested from 5-day culture of HDN16-126 was transformed with above *pyrG-A1* knock out cassette solution. After the first round of recombination (Fig. 2B), 12 mutants were picked up from the PSA plus 50 μg/mL hygromycin selective medium and all the mutants were verified by PCR to be the correct clones with the *hyg* gene cassette integrated into the correct locus by homologous recombination (Fig. S7). Strikingly, the homologous recombination rate in this step was much higher than literature reports for other filamentous fungi where a big portion of wrong insertions were generated by non-homologous recombination [31]. Then the 12 mutants were further selected on MM plates plus uridine and 5-FOA (0.5 mg/mL), where the *hyg* gene and *pyrG-A1* gene would be removed from the genome with 5-FOA working as a counter selection marker. After this second round of selection, one *pyrG-A1* deficient strain HDN16-126-△*pyrG* was obtained and verified by PCR test. As shown in Fig. 2C, the length of the PCR product for the mutant strain was about 0.7 kb and for the wild type strain was about 1.8 kb, which confirmed the successful removal of *pyrG-A1* from HDN16-126 chromosome.

The HDN16-126-△*pyrG* strain was unable to grow on MM, but grow on MM supplemented with 10 mM uridine (Fig. 2D). This strain also showed low sensitivity to 5-FOA treatment, which is in consistent with the absence of the *pyrG-A1* cassette. In addition, the morphology of mycelium showed no significant difference compared with that of the WT except for a slightly darker pigmentation on the mycelium (Fig. 2D).

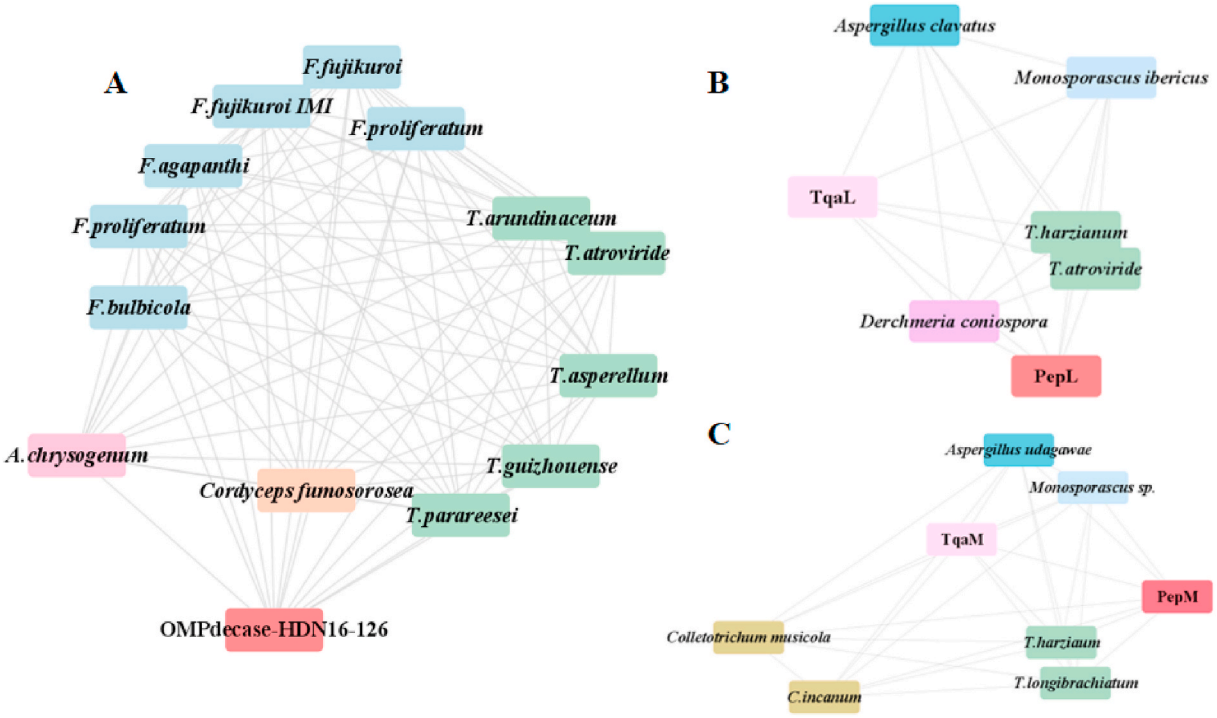

Fig. 1. Protein interaction network of *pyrG-A1* (A), *pepL* (B) and *pepM* (C).

Together, we concluded that by two rounds of selection with hygromycin and 5-FOA treatments, the *pyrG-A1* gene could be scarlessly knocked out from HDN16-126 genome.

3.4. Generation of pepL and pepM mutant

With the uridine auxotroph mutant HDN16-126-△*pyrG* in hand, we continued to test whether the marker recycling system could be used for sequential disruption of multiple genes. Two genes of *pepL* and *pepM* which are proposed to be 2-aminoisobutyric acid metabolism relating genes were selected as targets for disruption. The *pepL* and *pepM* disruption cassettes were designed as shown in Fig. 3A. After first round of selection on MM-sorbital dropout uridine medium, the cassettes with *pyrG-A1* gene flanked by three homologous fragments were integrated into the upstream next to *pepL* and *pepM* respectively. Each 12 single clones were picked up from *pepL* and *pepM* disruption mutants, within which the expression of *pyrG-A1* from the cassette could complement the uridine auxotrophy and restore the cell growth. Then all the clones were transferred to MM plus uridine and 5-FOA medium for dropping out of *pyrG-A1*. 10 out of 12 clones for △*pepL* and 8 out of 12 clones for △*pepM* were grown. All the mutants were verified to be correct by PCR using primer yz-L-F/yz-L-R or yz-M-F/yz-M-R (Fig. 3B), where the length of PCR products from △*pepL* and △*pepM* mutants were 1105 bp and 1078 bp respectively, while as control, the PCR products from WT were 2460 bp and 2399 bp in correlation with *pepL* and *pepM*. The △*pepL*, △*pepM* mutants were then tested for their growth on MM with or without uridine (10 mM) and results showed they could only grow on uridine supplied MM medium and compared with WT strain, both △*pepL* and △*pepM* mutants showed less spore production and much darker pigmentation on mycelium (pink for mutants and pale for WT, Fig. 3C). The disruption efficiency for gene *pepL* and *pepM* were 83.3% and 66.7%, respectively, and these data demonstrate that the marker recycling system can be applied to genetic manipulation of *Acremonium* species for generating scarless gene deletion and replacement mutations.

4. Discussion

In general, traditional homologous recombination techniques are commonly used to generate gene disruption and replacement [32] in fungi. During this process, resistant marker will be retained on the chromosome after one round of recombination, making it impossible to use the same selection marker during the next cycle of gene manipulation, which means increasingly more selection markers are required for completing multiple gene disruptions. Consequently, the physiological and biochemical studies of many fungal strains are limited by the availability of suitable selection markers.

In this study, we report the establishment of a uridine auxotrophy based scarless gene deletion method in *Acremonium* sp. HDN16-126, a fungus collected from the Mariana Trench (−8000 m). Importantly, the *pyrG-A1* marker was successfully recycled from the chromosome, which allows the use of the same selection marker in successive transformations. The *pyrG-A1*-based marker recycling system showed several advantages over other gene deletion systems, such as sit-directed recombination methods including Flp-FRT and Cre-loxP recombinations, which have been widely used in prokaryotes and eukaryotes [22]. Those tools could easily conduct the excision of target DNA between two repeated specific sites, while for a legacy, one of the repeated sites is left on the chromosome, which makes it an unstable factor as the site specific sequences accumulated in the genome. Compared to above methods, the *pyrG-A1*-based marker recycling system could create much more stable multiple knock-out mutants.

Using this *pyrG-A1* based marker recycling system, sequential disruption of two target genes *pepL* and *pepM* were achieved in HDN16-126-△*pyrG* strain. To our best knowledge, this is the first report of the application of uridine auxotrophy based marker recycling system in deep sea-derived *Acremonium* species. In addition, the reintroduction of *pyrG-A1* could restore the growth of HDN16-126-△*pyrG* mutant to wild-type levels, which indicated the potential of this system to access disruption and function analysis of any gene of interest in filamentous fungi. With the help of this efficient gene manipulation system, more in depth physiological and biochemical investigations including the *pepL* and *pepM*

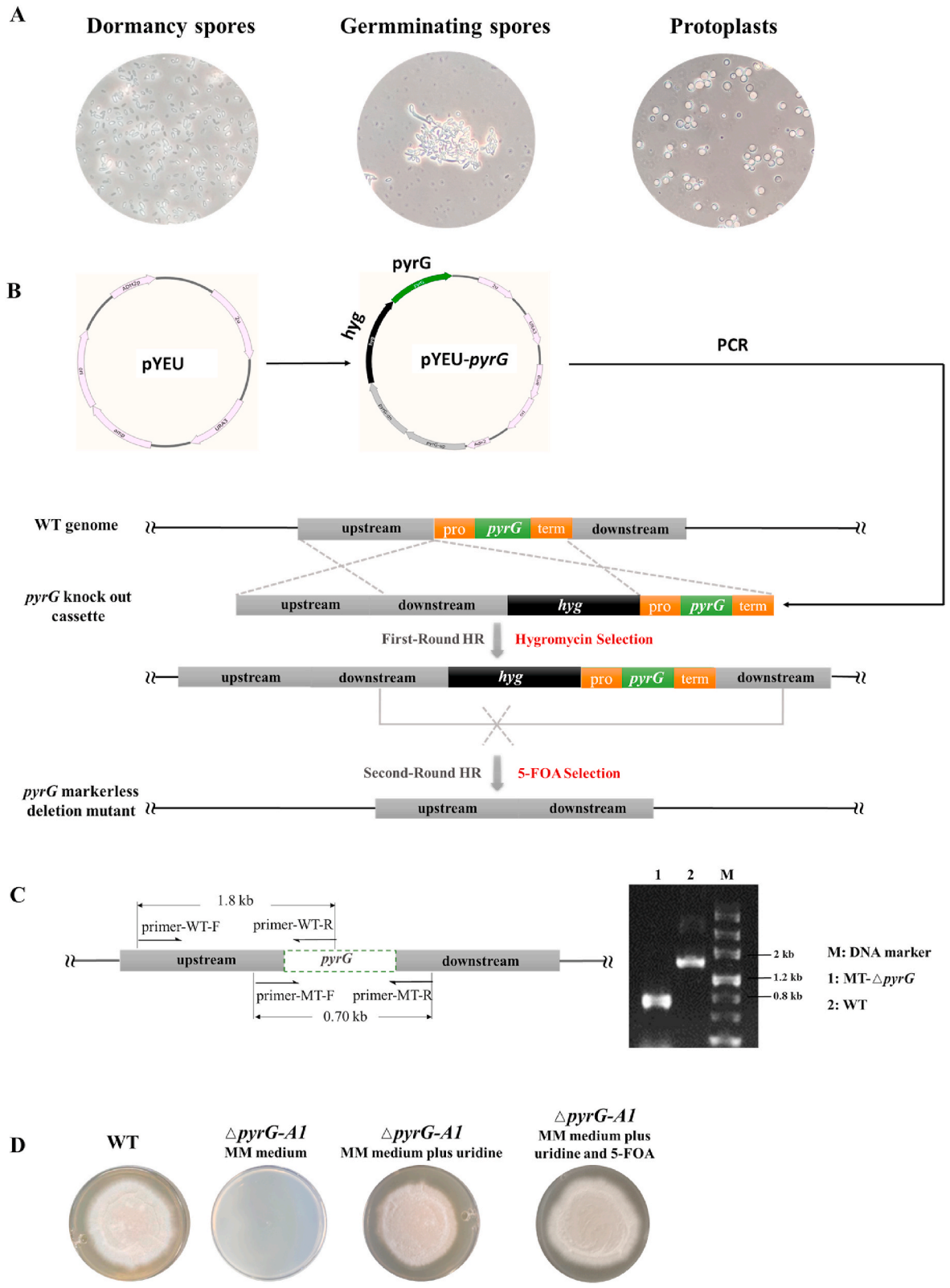

Fig. 2. Construction of markerless △*pyrG* mutant. (A) Morphology of the WT spores with spores in dormancy period, spores in germination and protoplasts of the WT; (B) Flow chart of construction procedure of △*pyrG* mutant; (C) PCR verification of the MT, the length of PCR product from MT was about 0.7 kb, and the length of PCR product from WT was about 1.8 kb; (D) Growth of △*pyrG* on MM medium, MM plus uridine (10 mM) medium and MM plus 5-FOA and uridine medium, the WT on MM medium was used as a control.

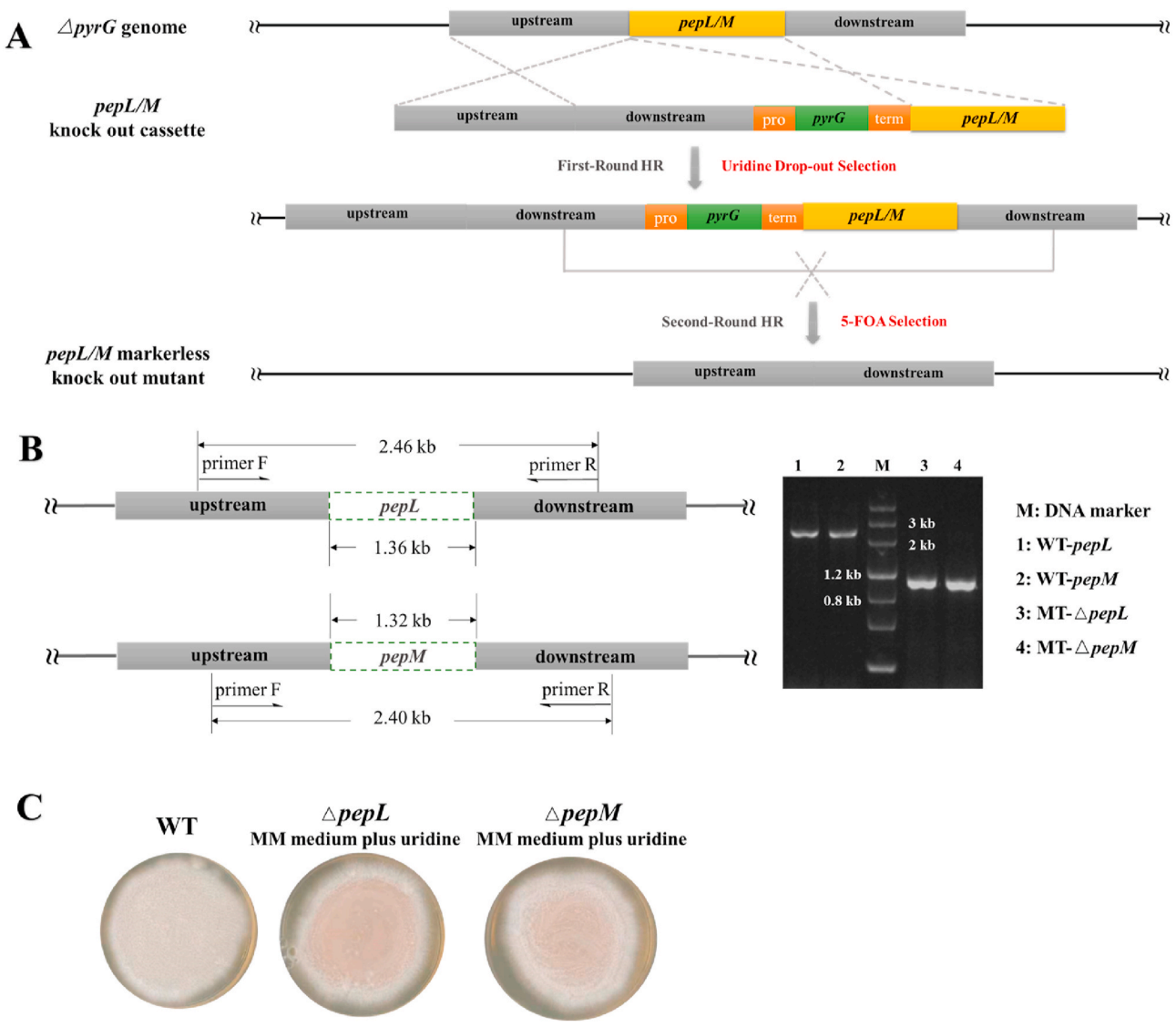

Fig. 3. Construction of *pepL* and *pepM* scarless deletion mutants. (A) Two rounds of homologous recombination and selection on MM-sorbital dropout uridine medium and MM with 5-FOA medium; (B) PCR verification of △*pepL* and △*pepM* mutant; (C) Growth of △*pepL*, △*pepM* on MM plus uridine (10 mM) medium compared with WT strain.

functions in amino acids metabolism are at the right time in progress.

CRediT authorship contribution statement

Ruonan Sun: Experiment operation, Writing – original draft. **Hengyi Xu:** Experiment operation. **Yanyan Feng:** and. **Xuewen Hou:** Formal analysis, and chemical study of strains. **Tianjiao Zhu:** and. **Qian Che:** and. **Blaine Pfeifer:** Checking and confirming all procedures. **Guojian Zhang:** and. **Dehai Li:** Designed the study, supervised the laboratory work, and, contributed to the critical reading of the manuscript.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Acknowledgement

This work was funded by the National Key R&D Program of China (2019YFC0312504), the National Natural Science Foundation of China (41976105, 81991522), Major Basic Research Programs of Natural Science Foundation of Shandong Province (ZR2019ZD18), the Fundamental Research Funds for the Central Universities (201941001), the NSFC-Shandong Joint Fund (U1906212), the Marine S&T Fund of Shandong Province for Pilot National Laboratory for Marine Science and Technology (Qingdao) (No. 2018SDKJ0401-2), the Youth Innovation Plan of Shandong province (2019KJM004) and the Taishan Scholar Youth Expert Program in Shandong Province (tsqn201812021).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.synbio.2021.05.001.

References

[1] Abraham EP, Newton GGF, Hale CW. Purification and some properties of cephalosporin N, a new penicillin. Biochem J 1954;58(1):94–102. https://doi.org/10.1016/0003-9861(54)90367-7.
[2] Holten KB. Appropriate prescribing of oral beta-lactam antibiotics. Am Fam Physician 2000;62(3):611–20. https://doi.org/10.1159/000050607.
[3] Hu Y, Zhu B. Study on genetic engineering of *Acremonium chrysogenum*, the cephalosporin C producer. Synth. Syst. Biotechnol 2016;1(3):143–9. https://doi.org/10.1016/j.synbio.2016.09.002.

[4] Howard KC, Dennis EK, Watt DS, Garneau-Tsodikova S. A comprehensive overview of the medicinal chemistry of antifungal drugs: perspectives and promise. Chem Soc Rev 2020;49:2426–80. https://doi.org/10.1039/C9CS00556K.
[5] Izumikawa M, Khan ST, Komaki H, Nagai A, Inaba S, Takagi M, et al. JBIR-37 and -38, novel glycosyl benzenediols, isolated from the sponge-derived fungus, *Acremonium* sp. SpF080624G1f01. Biosci Biotechnol Biochem 2009;73(9): 2138–40. https://doi.org/10.1271/bbb.90346.
[6] Todaro AR, Nascimento VX, Souza NCC, Silva PP, Santos JM, Ramalho EAVF, Melo TVC, Silva ISM, Júnior GSL, Bastos MLA, Farias DF, Souza PCV, Carvalho AFU, Silva MCS, Almeida RMRG, Neto ER. Genetic variability in the ITS and IGS regions of the ribosomal DNA of *Acremonium cavaraeanum* exhibiting antimicrobial activity. Genet Mol Res 2013;12(4):6983–95. https://doi.org/10.4238/2013.december.20.1.
[7] Tian J, Lai D, Zhou L. Secondary metabolites from *Acremonium* fungi: diverse structures and bioactivities. Mini Rev Med Chem 2017;17(7):603–32. https://doi.org/10.2174/1389557516666160914194134.
[8] Twaruschek K, Spörhase P, Michlmayr H, Wiesenberger G, Adam G. New plasmids for Fusarium transformation allowing positive-negative selection and efficient cre-loxP mediated marker recycling. Front Microbiol 2018;9:1954. https://doi.org/10.3389/fmicb.2018.01954.
[9] Nan Y, Ouyang L, Chu J. In vitro CRISPR/Cas9 system for genome editing of *Aspergillus niger* based on removable bidirectional selection marker *AmdS*. Biotechnol Appl Biochem 2020:1–7. https://doi.org/10.1002/bab.1996.
[10] Yang F, Zhang S, Tang W, Zhao ZK. Identification of the orotidine-5′-monophosphate decarboxylase gene of the oleaginous yeast *Rhodosporidium toruloides*. Yeast 2008;25(9):623–30. https://doi.org/10.1002/yea.1607.
[11] Wilson LJ, Carmona CL, Ward M. Sequence of the *Aspergillus niger pyrG* gene. Nucleic Acids Res 1988;16(5):2339. https://doi.org/10.1093/nar/16.5.2339.
[12] Kyotaro H, Tomoko I, Kaoru T, Yuko GH, Hideki T. A simple and effective chromosome modification method for large-scale deletion of genome sequences and identification of essential genes in fission yeast. Nucleic Acids Res 2006;34(2): e11. https://doi.org/10.1093/nar/gnj011.
[13] Flynn PJ, Reece RJ. Activation of transcription by metabolic intermediates of the pyrimidine biosynthetic pathway. Mol Cell Biol 1999;19(1):882–8. https://doi.org/10.1128/MCB.19.1.882.
[14] Alani E, Cao L, Kleckner N. A method for gene disruption that allows repeated use of *URA3* selection in the construction of multiply disrupted yeast strains. Genetics 1987;116(4):541–5. https://doi.org/10.1534/genetics.112.541.test.
[16] D'Enfert C, Diaquin M, Delit A, Wuscher N, Latge JP. Attenuated virulence of uridine-uracil auxotrophs of *Aspergillus fumigatus*. Infect Immun 1996;64(10): 4401–5. https://doi.org/10.1016/0928-8244(96)00063-6.
[17] Fonzi WA, Irwin MY. Isogenic strain construction and gene mapping in *Candida albicans*. Genetics 1993;134(3):717–28. https://doi.org/10.1101/gad.7.7b.1459.
[18] Turner GE, Jimenez TJ, Chae SK. Utilization of the *Aspergillus nidulans pyrG* gene as a selectable marker for transformation and electroporation of *Neurospora crassa*. Fungal Genet Biol 1997;44(1):57–9. https://doi.org/10.4148/1941-4765.1289.
[19] Delmas S, Llanos A, Parrou JL, Kokolski M, Pullan ST, Shunburne L, Archer DB. Development of an unmarked gene deletion system for the filamentous fungi *Aspergillus niger* and *Talaromyces versatilis*. Appl Environ Microbiol 2014;80(11): 3484–7. https://doi.org/10.1128/AEM.00625-14.
[20] Garcia A, Adedoyin G, Heitman J, Lee SC. Construction of a recyclable genetic marker and serial gene deletions in the human pathogenic mucorales *Mucor circinelloides*. G3-Genes Genom Genet 2017:2047–54. https://doi.org/10.1534/g3.117.041095.
[22] Komen SV, Macris M, Sehorn MG, Sung P. Purification and assays of *Saccharomyces cerevisiae* homologous recombination proteins. Methods Enzymol 2006;408: 445–63. https://doi.org/10.1016/s0076-6879(06)08028-1.
[23] Vian A, Peñlva MA, Vilan A. Nucleotide sequence of the *Cephalosporium acremonium pyr4* gene. Nucleic Acids Res 1989;17(21):8874. https://doi.org/10.1093/nar/17.21.8874.
[24] Gao X, Chooi Y-H, Ames BD, Wang P, Walsh CT, Tang Y. Fungal indole alkaloid biosynthesis: genetic and biochemical investigation of the tryptoquialanine pathway in *Penicillium aethiopicum*. J Am Chem Soc 2011;133(8):2729–41. https://doi.org/10.1021/ja1101085.
[25] Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 2011;7(1):539. https://doi.org/10.1038/msb.2011.75.
[26] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13(11):2498–504. https://doi.org/10.1101/gr.1239303.
[27] Otasek D, Morris JH, Bouças J, Pico AR, Demchak B. Cytoscape Automation: empowering workflow-based network analysis. Genome Biol 2019;20(1):1–15. https://doi.org/10.1186/s13059-019-1758-4.
[28] Orr-Weaver TL, Szostak JW, Rothstein RJ. Yeast transformation: a model system for the study of recombination. Proc Natl Acad Sci Unit States Am 1981;78(10): 6354–8. https://doi.org/10.1016/0003-9861(54)90367-7.
[29] Mathur J, Koncz C. PEG-mediated protoplast transformation with naked DNA. Methods Mol Biol 1998;82:267–76. https://doi.org/10.1385/0-89603-391–0:267.
[30] Krappmann S, Sasse C, Braus GH. Gene targeting in *Aspergillus fumigatus* by homologous recombination is facilitated in a nonhomologous end-joining-deficient genetic background. Eukaryot Cell 2006;5(1):212–5. https://doi.org/10.1128/ec.5.1.212-215.2006.
[31] Bloemendal S, Löper D, Terfehr D, Kopke K, Kluge J, Teichert I, Kück U. Tools for advanced and targeted genetic manipulation of the β-lactam antibiotic producer *Acremonium chrysogenum*. J Biotechnol 2014;169:51–62. https://doi.org/10.1016/j.jbiotec.2013.10.036.
[33] Kumakura N, Ueno A, Shirasu K. Establishment of a selection marker recycling system for sequential transformation of the plant-pathogenic fungus *Colletotrichum orbiculare*. Mol Plant Pathol 2019;20(3):447–59. https://doi.org/10.1111/mpp.12766.
[34] Guarro J, Gams W, Pujol I, Gené J. *Acremonium* species: new emerging fungal opportunists-in vitro antifungal susceptibilities and review. Clin. Infect. Dis 1997; 25(5):1222–9. https://doi.org/10.1086/516098.
[35] Oakley BR, Rinehart JE, Mitchell BL, Oakley CE, Carmona C, Gray GL, et al. Cloning, mapping and molecular analysis of the *pyrG* (orotidine-5'-phosphate decarboxylase) gene of *Aspergillus nidulans*. Gene 1987;61(3):385–99. https://doi.org/10.1016/0378-1119(87)90201-0.