Received: 6 June 2022 | Accepted: 10 November 2022

DOI: 10.1111/1751-7915.14176

MICROBIAL BIOTECHNOLOGY Applied Microbiology International

RESEARCH ARTICLE

New application of *Aspergillus versicolor* in promoting plant growth after suppressing sterigmatocystin production via genome mining and engineering

Siqi Ma[1] | **Donglin Zhao**[1] | **Xiaobin Han**[2] | **Yulong Peng**[2] | **Tingting Ren**[1] | **Mei Wang**[1] | **Jun Wan**[2] | **Jilin Ding**[2] | **Xiuchun Du**[3] | **Fubin Zhao**[4] | **Yiqiang Li**[1] | **Chengsheng Zhang**[1]

[1]Marine Agriculture Research Center, Tobacco Research Institute of Chinese Academy of Agricultural Sciences, Qingdao, China

[2]Zunyi Branch, Guizhou Tobacco Company, Zunyi, China

[3]Jiaozhou Branch of Qingdao Tobacco Co. Ltd., Qingdao, China

[4]Huangdao Branch of Qingdao Tobacco Co. Ltd., Qingdao, China

Correspondence
Yiqiang Li and Chengsheng Zhang, Marine Agriculture Research Center, Tobacco Research Institute of Chinese Academy of Agricultural Sciences, Qingdao 266101, China.
Email: liyiqiang@caas.cn and zhangchengsheng@caas.cn

Funding information
Fundamental Research Funds for Central Non-profit Scientific Institution, Grant/Award Number: 1610232021007; the Agricultural Science and Technology Innovation Program of China, Grant/Award Number: ASTIP-TRIC06; the Agricultural Science and Technology Project of Guizhou Province, Grant/Award Number: 2021XM10 and 201803; the Agricultural Science and Technology Project of Qingdao, Grant/Award Number: 2022370200270151 and 2022370200270187

Abstract

Aspergillus genus is a key component in fermentation and food processing. However, sterigmatocystin (STE)—a mycotoxin produced by several species of *Aspergillus*—limits the use of some *Aspergillus* species (such as *Aspergillus versicolor*, *Aspergillus inflatus*, and *Aspergillus parasiticus*) because of its toxicity and carcinogenicity. Here, we engineered an STE-free *Aspergillus versicolor* strain based on genome mining techniques. We sequenced and assembled the *Aspergillus versicolor* D5 genome (34.52 Mb), in which we identified 16 scaffolds and 54 biosynthetic gene clusters (BGCs). We silenced cytochrome P450 coding genes *STC17* and *STC27* by insertional inactivation. The production of STE in the *Δstc17* mutant strain was increased by 282% but no STE was detected in the *Δstc27* mutant. Metabolites of *Δstc27* mutant exhibited growth-promoting effect on plants. Our study makes significant progress in improving the application of some *Aspergillus* strains by restricting their production of toxic and carcinogenic compounds.

INTRODUCTION

Sterigmatocystin (STE) is a common toxic, carcinogenic mycotoxin and can be isolated from *Aspergillus versicolor* cultures (Brown et al., 1996; Jurjevic et al., 2013; Samson et al., 2014). Other strains that can also produce high yield of STE include *Aspergillus aflatus, Aspergillus parasiticus* and *Aspergillus nidulans* (Gruber-Dorninger et al., 2017; Rank et al., 2011). STE is mainly produced in mouldy cereals, nuts, cheese and

Siqi Ma, Donglin Zhao and Xiaobin Han contributed equally to this work.

other foods (Flores-Flores et al., 2015; Ji et al., 2016); all unprocessed European rice samples are prone to STE (Versilovskis & De Saeger, 2010).

Sterigmatocystin is as toxic as aflatoxin B1 (AFB_1) because of the similar chemical structure (Nieto et al., 2018) and therefore is a relevant mycotoxin (Yang et al., 2020, 2022). STE has been found to induce malignant tumours in many animal species and promote apoptosis in HepG2 cells (Pfeiffer et al., 2014). STE could induce DNA damage and cell cycle arrest in a human immortalized bronchial epithelial cell line and human lung cancer cell line in vitro (Cao et al., 2018; Zingales et al., 2020).

The biosynthetic pathway of STE is well characterized (Kelkar et al., 1997; Keller et al., 2000; Yabe & Nakajima, 2004), which starts from acetate and malonyl-CoA. At least 18 enzyme steps are required to obtain the final product aflatoxin/STE. The biosynthesis of STE requires some conserved enzymes, for instance, the oxidation of the desaturated bisfuran moiety needs cytochrome P-450 monooxygenase.

Fungi *Aspergillus* produce a wide variety of secondary metabolites with a wide range of biological activity. Some alkaloids and anthraquinones made by *Aspergillus versicolor* have antimicrobial activity including the plant pathogen *Ralstonia* (Zhao et al., 2020). The *Aspergillus versicolor* has great potential for application, but limits by the STE toxicity. Here, we analysed and engineered the gene cluster of STE based on the genome of *Aspergillus versicolor* D5, and obtained an STE-free mutant strain which has the function of promoting plant growth. The STE-free mutant strain could be used for mining more beneficial secondary metabolites and extensively used in more areas of interest.

EXPERIMENTAL PROCEDURES

Fungal strains and culture conditions

The fungal strain *Aspergillus versicolor* D5 was isolated and identified in our laboratory (Zhao et al., 2020). *Aspergillus versicolor* D5 and its mutant lines were grown in potato dextrose agar (PDA) plates and rice cultures. *Aspergillus versicolor* D5 and its mutant lines were cultivated in rice culture medium at 28°C for 30 days without shaking.

Sequencing and genome assembly

Whole genomic DNA of *Aspergillus versicolor* D5 was extracted using the method in Lim et al. (2016) and sequenced using PacBio Sequel platform and Illumina NovaSeq PE150 at the Beijing Novogene Bioinformatics Technology Co., Ltd. We used the SMRT link software (v5.0.1) for the preliminary genome assembly and the arrow algorithm to correct and count the variant sites. Functions of predicted genes were annotated using GeneWise and Augustus. We annotated genes with function information using GO (Gene Ontology) database, KEGG (Kyoto Encyclopedia of Genes and Genomes), KOG (Clusters of Orthologous Groups), NR (Non-Redundant Protein Database), TCDB (Transporter Classification Database) and FCPD (The Fungal Cytochrome P450 Database), and so on.

Molecular genetic manipulations

With the genomic DNA of *Aspergillus versicolor* D5 as template, we amplified the 1–1.5 kb upstream and downstream homologous regions of *STC17* and *STC27* genes, then the amplicons were fused with the selectable marker *hygromycin B* by PCR. All primers used in this study are listed in Table S1 and Figure S1.

Transformation of *Aspergillus versicolor* D5 was performed using the protoplast-PEG method (Huang et al., 2016; Tang et al., 2022). Briefly, the mycelium was harvested after cultivation in PDA for 18–20 h at 30°C and washed with 0.8 M KCl solution. To release protoplasts, 1 g mycelium was mixed in 10 ml enzyme lysis buffer (1% Cellulase, Sigma C1184; 1% Lysing enzymes, Sigma L1412; 1% Snailase, BBI A600870) for 4 h at 30°C, 120 rpm. The protoplasts were filtered through Miracloth and collected by centrifugation at 2500 *g* for 10 min. Filtrates were then washed with 10 ml 1.2 M sorbitol and centrifugation at 2500 *g* for 10 min. Protoplasts were resuspended in 1 ml cold STC buffer (1 M sorbitol, 50 mM Tris–HCl pH 8.0, 50 mM $CaCl_2$). Then the fusion PCR products (20 ng) were incubated with 200 μl (at least 10 Versilovskis & De Saeger, 2010) protoplasts and 10 μl PSTC buffer (40% PEG8000, 1 M sorbitol, 50 mM Tris–HCl pH 8.0, 50 mM $CaCl_2$) for 25 min on ice. Then add 1 ml PSTC buffer and incubate the mixture for 10 min at room temperature. Transformants were regenerated and selected on PDASH sodium (PDA supplemented with 1.2 M sorbitol and 50 μg/ml Hygromycin B).

Fermentation analysis of the *Aspergillus versicolor* D5 mutants

The fungal strain *Aspergillus versicolor* D5 was fermented on rice medium in three Erlenmeyer flasks at 28°C for 30 days. Each flask contained 60 g of rice and 75 ml of H_2O. The rice medium was extracted once with ethyl acetate (EtOAc) and twice with dichloromethane methanol (1:1, v/v). The extract was concentrated under reduced pressure to remove organic carryover and obtain an aqueous solution. Subsequently, the aqueous solution was extracted with EtOAc three times to yield an EtOAc extract after removal of the solvent under reduced pressure.

The extract was dissolved in chromatographic methanol, and was filtered through a 0.22 μm filter membrane for HPLC analysis. HPLC fingerprints were acquired on a Waters e2695 system with a Waters 2998 detector and a Waters Sunfire C18 (5 μm, 4.5 × 250 mm) column. The mobile phases were methanol in Channel A and H_2O with 0.1% (v/v) trifluoroacetic acid in Channel B. A gradient elution program was performed at a flow rate of 1.0 ml/min: (0 to 5 min, 10% A), (5 to 20 min, 10% A to 50% A), (20 to 40 min, 50% A to 100% A), (40 to 50 min, 100% A), (50 to 51 min, 100% A to 5% A), (51 to 60 min, 100% A), with the injection volume of 10 μl.

Growth-promoting activity assay

Briefly, the seeds were germinated on 1/2 Murashige and Skoog (MS) medium for 7 days, and then transplanted to experimental plots (12 *Amaranthus retroflexus* L. each plot and three repeats; one *Nicotiana tabacum* L. 'K326' each plot and 36 repeats; four *Glycine max* 'William 82' each plot and nine repeats). The seedlings were treated with 1 mg/ml extract from wild-type and the mutant lines. After 3–7 days, shoot lengths were measured and analysed.

RESULTS

Gene prediction, functional annotation and BGC identification in *Aspergillus versicolor* D5

We determined that *Aspergillus versicolor* D5 genome size is 34,517,828 bp with a GC content of 49.95%. We identified 16 scaffolds—the largest one up to 5,021,057 bp (Table 1, Figure 1A and Table S2)—and predicted 12,702 protein-coding genes, with an average length of 1846 bp (Table 1). Moreover, we found 136 tRNA and 45 rRNA genes.

The KEGG analysis showed that the genes clustered in six groups, including metabolism, human diseases, organismal systems, genetic information processing, environmental information processing and cellular processes. More than 250 genes clustered in pathways for carbohydrate metabolism, amino acid metabolism, translation, transport and catabolism (Figure 1B). Further comparison analysis of Carbohydrate Active Enzyme Database (CAZy) found 611 genes coding CAZymes, of which 257 are glycoside hydrolases (GHs) (Table S3). The GHs are vital for the cleavage of polymerized substrates. Besides, there are 321 genes encoding cytochromes P450, which are important for oxidizing many substrates (Table S4).

The assembled genome of *Aspergillus versicolor* D5 was analysed with the antiSMASH pipeline. The results showed that *Aspergillus versicolor D5* contains 54 BGCs (Biosynthetic Gene Clusters) across disparate categories, including non-ribosomal peptide synthetase (NRPS) cluster, NRPS-like cluster, type I polyketide synthase (T1PKS) cluster, terpene cluster, beta-lactone containing protease inhibitor (beta-lactone) cluster and indole cluster (Figure 1A and Table S5). Most of the BGCs showed low or no similarity to known BGCs, further indicating that *Aspergillus versicolor D5* is a promising strain for the discovery of new metabolic pathways.

TABLE 1 Assembly statistics and general features of *Aspergillus versicolor* D5 genome.

Total number of reads-Illumina	982,618
Genome size(bp)	34,517,828
Number of scaffolds	16
Largest Scaffold length (bp)	5,021,057
Scaffold N50(bp)	2,791,892
Scaffold N90 (bp)	1,107,595
%GC	49.95
Number of predicted genes	12,702
Gene average length (bp)	1846
tRNA genes	136
rRNA genes	45

Phylogenetic analysis and comparative genomics of *Aspergillus versicolor* D5

A genome-wide phylogenetic analysis was performed to understand the relationship between *Aspergillus versicolor* D5 and other related *Aspergillus* genera in MycoCosm. The results of the phylogenetic analysis showed that they are classified into four clades. *Aspergillus versicolor* D5 was positioned in the fourth clade, separated from the others, consistent with *Aspergillus versicolor* CBS 583.65, *Aspergillus puulaauensis* and *Aspergillus sydowii* CBS 593.65 (Figure 2). Following the previous phylogenetic study, only nearly 64% amino acid identity was discovered between *A. zonatus* and *Aspergillus versicolor* (de Vries et al., 2017; Jurjevic et al., 2013; Ryngajllo et al., 2021). This observed genetic diversity indicated that *Aspergillus versicolor* contains many unique genes and has the potential to produce a diverse range of secondary metabolites.

Suppressing STE production by analysing and engineering the gene cluster

Genome mining and antiSMASH analysis showed that there is a gene cluster related to STE (Figure 3A). The genes in this cluster were named *STC1* to *STC32* based on their locations. *STC1*, *STC13* and *STC26*

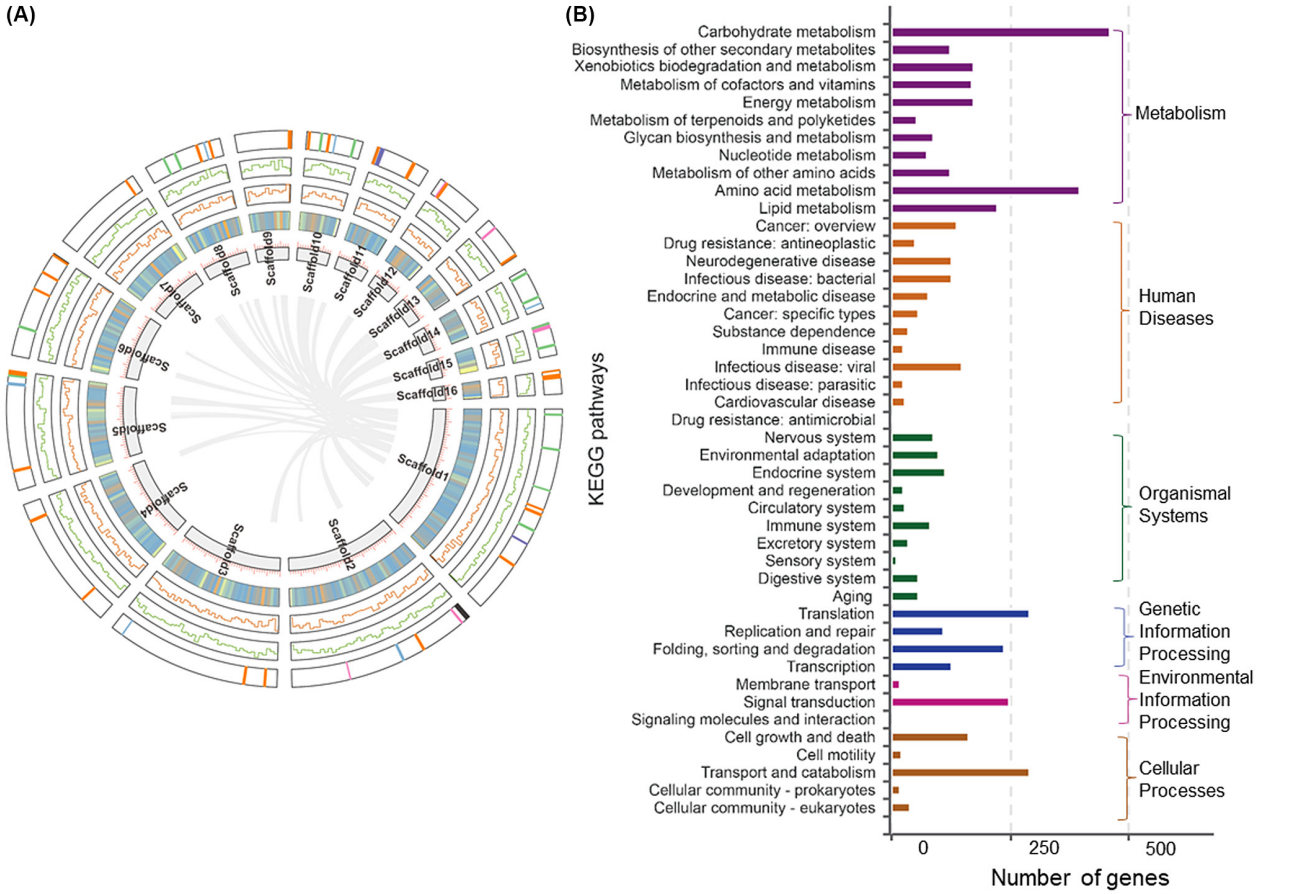

FIGURE 1 Genome analysis of *Aspergillus versicolor* D5. (A) Assembly of *Aspergillus versicolor* D5 genome. From the inside out, circle 1 showed the scaffolds of D5 genome, circle 2–4 showed all genes position, circle 5 showed the position of different types BCGs. (B) KEGG analysis of predicted genes in *Aspergillus versicolor* D5 genome.

were the core biosynthetic genes in this cluster and predicted as the NRPS and T1PKS genes. It was predicted that there are nine additional biosynthetic genes and a transporter gene. To identify the key genes or regulators in this cluster, expression levels of these genes were determined at day 1, 3, 5, 7, 9 and 13 of cultivation; results showed variability of expression levels of the genes in STE cluster (Figure 3B). It was reported that cytochromes P450 play an important role in the production of STE (Yabe & Nakajima, 2004). Two P450-coding genes (*STC17* and *STC27*, which are the genes labelled 01931 and 01941, respectively, in Table S4) were found among the nine additional biosynthetic genes. The expression patterns of *STC17* and *STC27* differed; *STC17* expression was higher at the early stage than that of *STC27* at the late stage, suggesting that both may be essential to STE biosynthesis. Thus, these genes were knocked out to determine their significance in STE biosynthesis (Figure S1).

The analysis showed no indications of disruption in cell growth. Mutants *Δstc17* and *Δstc27* differed significantly in STE production (Figure 3C). HPLC analysis showed that STE production in *Δstc17* was increased by 282% based on the peak areas (4,200,800 mv·s to 11,841,613 mv·s), whereas no STE was detected in *Δstc27*. These results indicated that both genes are essential for STE biosynthesis. The scheme of the STE metabolic pathway was modified based on the previous studies as indicated in Figure S2 (Yabe & Nakajima, 2004). There are five reactions which possibly require P450 enzymes (the red star reactions in Figure S2). To locate the reactions of *STC17* and *STC27*, the phylogenetic analysis was performed between *STC17/27* and these reported P450s (Figure S3). The results showed that *STC17* was more closely positioned with *stcU* which participates in the reaction of Versicolorin A (VA) to Demethylsterigmatocystin (DMST) or Versicolorin B (VB) to Dihydrodemethylsterigmatocystin (DHDMST). And *STC27* was closely positioned with *stcW* which participated in the conversion of averufin to hydroxyversicolorone (Brown et al., 1996; Keller et al., 2000).

STE-free strain products stimulate plant growth

Previous studies have shown that STE and DHST (dihydrosterigmatocystin) have herbicidal effects on

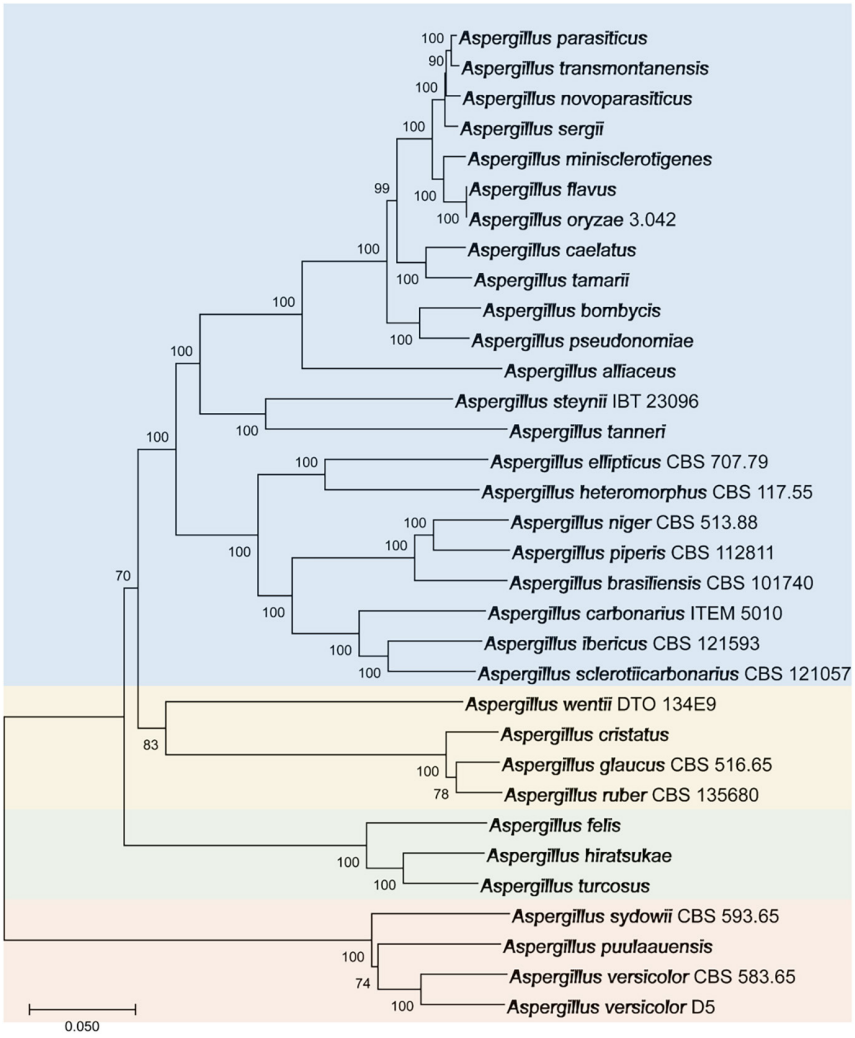

FIGURE 2 Phylogenetic tree of *Aspergillus versicolor* D5 and other related *Aspergillus* genera in MycoCosm.

Amaranthus retroflexus L (Zhao et al., 2020). To explore the potential application of this STE-free strain, we examined the activity of the products of *Δstc27*. The products of *Δstc27* mutant lost the herbicidal activity, as expected (Figure 4A). Besides, the products of *Δstc27* mutant demonstrated significant growth-promoting activity in *Amaranthus retroflexus*. In order to determine whether this plant growth stimulation is universal, we performed further analysis in *Nicotiana tabacum* and *Glycine max*. The results showed that *Δstc27* mutant products could promote seedling growth both on *Nicotiana tabacum* and *Glycine max* (Figure 4B,C). Above all, these results demonstrated that the STE-free strain we engineered by knocking out the *STC27* gene could be used to promote plant growth.

DISCUSSION

The sequencing and assembling of the *Aspergillus versicolor* D5 genome produced 16 scaffolds and predicted 54 BGCs (Figure 1). About 40% of the BGCs have homologues in other lineages of fungi, indicating that the majority of BGCs are specific or unknown to *Aspergillus versicolor* D5. The phylogenetic analysis also highlighted the considerable genomic distance between *Aspergillus versicolor* D5 and other strains (Figure 2). These findings suggest that there are genetic differences between *Aspergillus versicolor* D5 and the other *Aspergillus* strains, which reflects the potential of these microorganisms to produce a relatively wide spectrum of secondary metabolism pathway.

Sterigmatocystin (STE) is a polyketide mycotoxin produced by certain species of *Aspergillus* such as *Aspergillus versicolor* (Jurjevic et al., 2013; Samson et al., 2014), and the toxicity limits the applications of these strains. Here, we analysed the biosynthesis gene cluster of STE in *Aspergillus versicolor* D5 and identified that there are two cytochromes P450, *STC17* and *STC27*, play an important role in determining the production of STE. The STE production in the *Δstc17* mutant was increased and no STE was detected in the *Δstc27* mutant. Surprisingly, the products of the *Δstc27* mutant showed significant growth-promoting activity. This indicates that STE biosynthesis probably

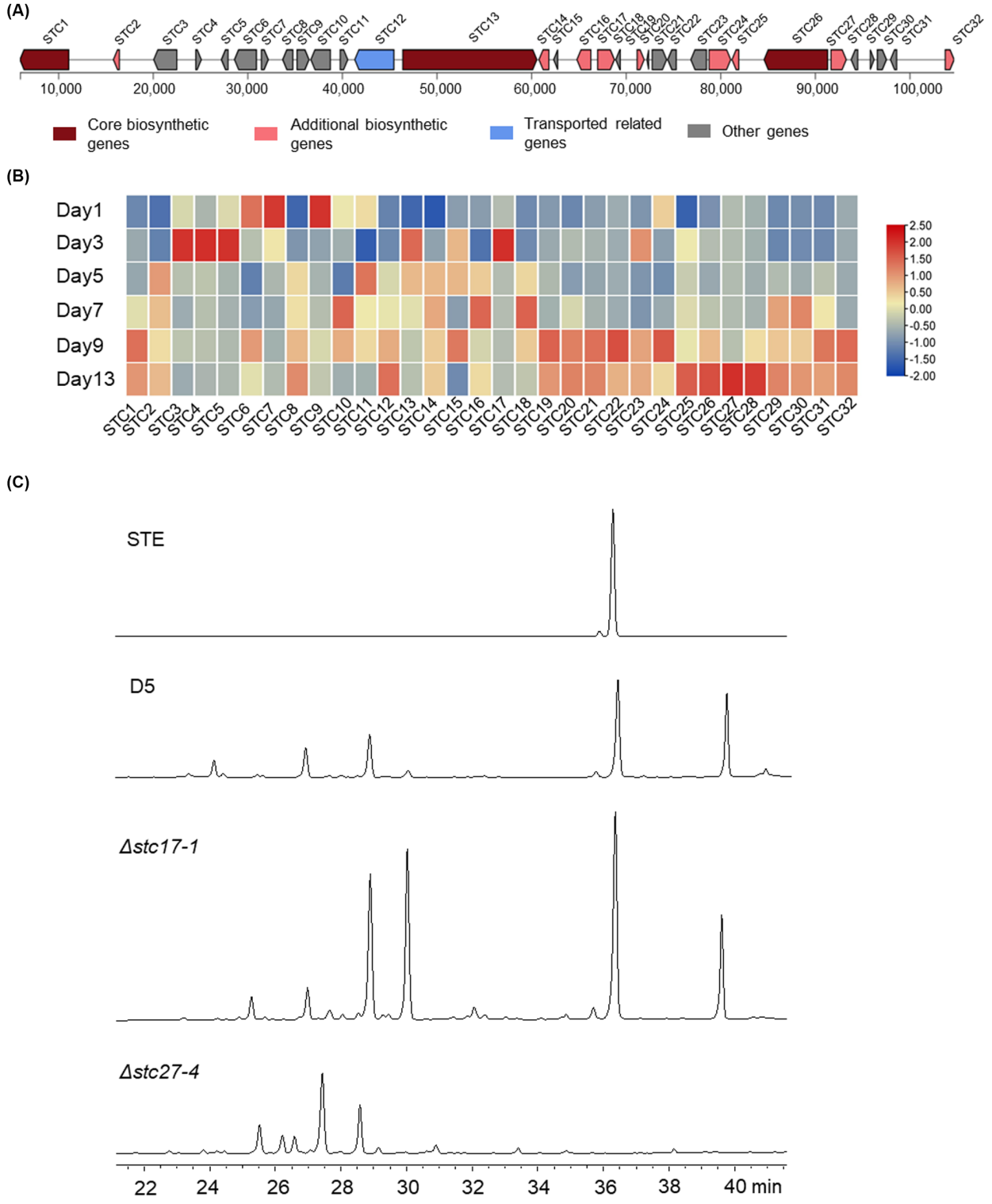

FIGURE 3 Analysis and modification of STE-related gene cluster in *Aspergillus versicolor* D5. (A) Predicted genes in STE-related gene cluster. (B) Heatmap of expression levels of the genes in STE-related gene cluster. (C) HPLC analysis of the fermentation products produced by mutant strains and wild strains. STE, STE reference standard; D5, wild strain; *Δstc17-1* and *Δstc27-4*, the mutant strain that silenced *STC17* and *STC27* respectively.

competes with some biosynthesis pathway of natural products (Min et al., 2019; Su et al., 2021).

Aspergillus versicolor D5 was found to contain many silenced gene clusters based on the analysis of the genome (Figure 1A) and the previous isolation of the products by our lab (Zhao et al., 2020). We engineered a mutant strain of *Aspergillus versicolor* D5 (*Δstc27*), which not only does not produce the toxic and carcinogenic STE but also

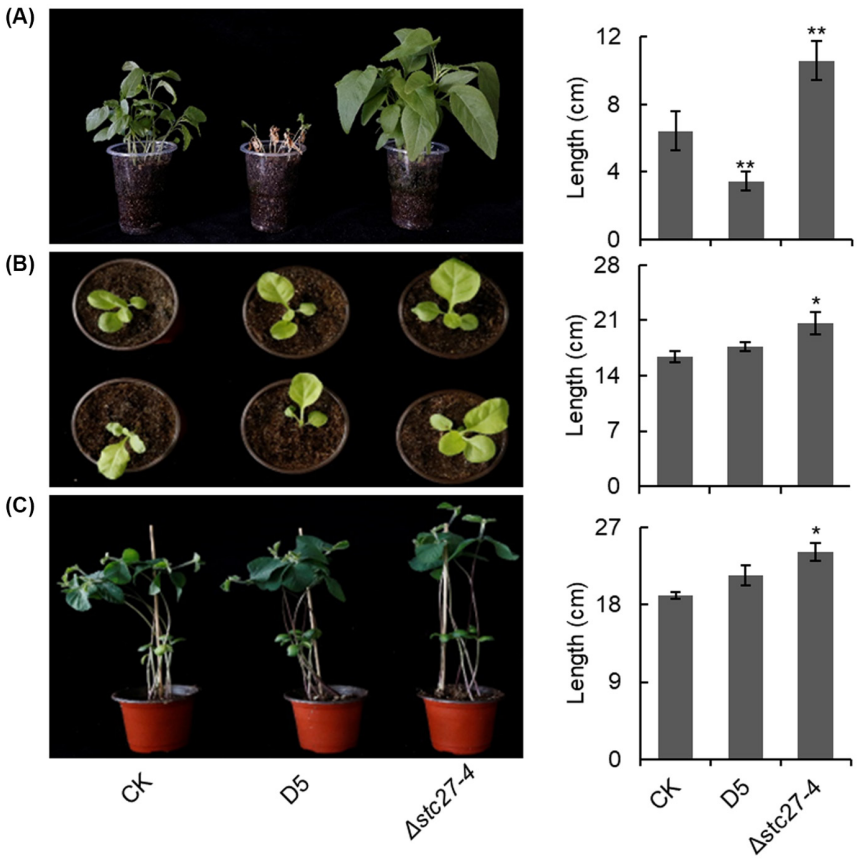

FIGURE 4 Analysis of the activity of STE-free strain products on plant growth. Error bars indicate SD ($n = 36$); the statistical significance was determined by Student's *t*-test (*$p < 0.05$, two-sided). (A) *Amaranthus retroflexus* L; (B) *Nicotiana tabacum*; (C) *Glycine max*.

promotes plant growth. This STE-free strain can be promising for future applications. Research has shown that *Aspergillus niger* could boost the growth of plants, such as lettuce, kale, scarlet eggplant, watermelon, melon, pepper and tomato (Mundim et al., 2022). Wheat plants developed significantly longer shoots and roots, biomass and chlorophyll after inoculation with *Aspergillus terreus* BTK-1, an endophytic fungus (Khan et al., 2022). Plants inoculated with *Bacillus licheniformis* and *Aspergillus violaceofuscus* improved in height, leaf area, biomass, relative water content, proline content and CAT activity (Muthuraja & Muthukumar, 2022). These indicated that *Aspergillus* has great potential in agricultural application.

CONCLUSIONS

The genome of *Aspergillus versicolor* D5 was 34.52 Mb long and consisted of 54 BGCs. We identified and silenced two genes encoding cytochrome P450. The production of STE in *Δstc17* mutant was increased by 282%, and no STE was detected in *Δstc27* mutant. The products of STE-free Δstc27 mutant could significantly stimulate plant growth.

AUTHOR CONTRIBUTIONS

Siqi Ma: Conceptualization (lead); data curation (lead); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); resources (lead); software (lead); supervision (equal); validation (equal); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Donglin Zhao:** Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); software (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Xiaobin Han:** Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); supervision (equal); writing – original draft (supporting); writing – review and editing (equal). **Yulong Peng:** Data curation (equal). **Tingting Ren:** Data curation (equal). **Mei Wang:** Data curation (equal). **Jun Wan:** Data curation (equal). **Jilin Ding:** Data curation (equal). **Xiuchun Du:** Data curation (equal). **Fubin Zhao:** Data curation (equal). **Yiqiang Li:** Data curation (equal); formal analysis (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal). **Chengsheng Zhang:** Data curation (equal); formal analysis (equal); funding acquisition (equal); writing – original draft (equal); writing – review and editing (equal).

ACKNOWLEDGEMENTS

This work was supported by the Fundamental Research Funds for Central Non-profit Scientific

Institution (1610232021007), the Agricultural Science and Technology Innovation Program of China (ASTIP-TRIC06), the Agricultural Science and Technology Project of Guizhou Province (2021XM10; 201803) and the Agricultural Science and Technology Project of Qingdao (2022370200270151;2022370200270187).

FUNDING INFORMATION

This work was supported by the Fundamental Research Funds for Central Non-profit Scientific Institution (1610232021007), the Agricultural Science and Technology Innovation Program of China (ASTIP-TRIC06), the Agricultural Science and Technology Project of Guizhou Province (2021XM10; 201803) and the Agricultural Science and Technology Project of Qingdao (2022370200270151;2022370200270187).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The genome data was deposited in the Genbank PRJNA902500.

ORCID

Siqi Ma https://orcid.org/0000-0002-1447-2987

REFERENCES

Brown, D.W., Yu, J.H., Kelkar, H.S., Fernandes, M., Nesbitt, T.C., Keller, N.P. et al. (1996) Twenty-five coregulated transcripts define a sterigmatocystin gene cluster in *Aspergillus nidulans*. *Proceedings of the National Academy of Sciences of the United States of America*, 93, 1418–1422. Available from: https://doi.org/10.1073/pnas.93.4.1418

Cao, X.Q., Li, X., Li, J., Niu, Y., Shi, L., Fang, Z. et al. (2018) Quantitative determination of carcinogenic mycotoxins in human and animal biological matrices and animal-derived foods using multi-mycotoxin and analyte-specific high performance liquid chromatography-tandem mass spectrometric methods. *Journal of Chromatography B*, 1073, 191–200. Available from: https://doi.org/10.1016/j.jchromb.2017.10.006

de Vries, R.P., Riley, R., Wiebenga, A., Aguilar-Osorio, G., Amillis, S., Uchima, C.A. et al. (2017) Comparative genomics reveals high biological diversity and specific adaptations in the industrially and medically important fungal genus *Aspergillus*. *Genome Biology*, 18, 28. Available from: https://doi.org/10.1186/s13059-017-1151-0

Flores-Flores, M.E., Lizarraga, E., de Cerain, A.L. & Gonzalez-Penas, E. (2015) Presence of mycotoxins in animal milk: a review. *Food Control*, 53, 163–176. Available from: https://doi.org/10.1016/j.foodcont.2015.01.020

Gruber-Dorninger, C., Novak, B., Nagl, V. & Berthiller, F. (2017) Emerging mycotoxins: beyond traditionally determined food contaminants. *Journal of Agricultural and Food Chemistry*, 65, 7052–7070. Available from: https://doi.org/10.1021/acs.jafc.6b03413

Huang, X., Chen, M., Li, J. & Lu, X. (2016) Establishing an efficient gene-targeting system in an itaconic-acid producing *Aspergillus terreus* strain. *Biotechnology Letters*, 38, 1603–1610. Available from: https://doi.org/10.1007/s10529-016-2143-y

Ji, C., Fan, Y. & Zhao, L.H. (2016) Review on biological degradation of mycotoxins. *Animal Nutrition*, 2, 127–133. Available from: https://doi.org/10.1016/j.aninu.2016.07.003

Jurjevic, Z., Peterson, S.W., Solfrizzo, M. & Peraica, M. (2013) Sterigmatocystin production by nine newly described *Aspergillus* species in section Versicolores grown on two different media. *Mycotoxin Research*, 29, 141–145. Available from: https://doi.org/10.1007/s12550-013-0160-4

Kelkar, H.S., Skloss, T.W., Haw, J.F., Keller, N.P. & Adams, T.H. (1997) *Aspergillus nidulans stcL* encodes a putative cytochrome P-450 monooxygenase required for bisfuran desaturation during aflatoxin/sterigmatocystin biosynthesis. *The Journal of Biological Chemistry*, 272, 1589–1594. Available from: https://doi.org/10.1074/jbc.272.3.1589

Keller, N.P., Watanabe, C.M., Kelkar, H.S., Adams, T.H. & Townsend, C.A. (2000) Requirement of monooxygenase-mediated steps for sterigmatocystin biosynthesis by *Aspergillus nidulans*. *Applied and Environmental Microbiology*, 66, 359–362. Available from: https://doi.org/10.1128/AEM.66.1.359-362.2000

Khan, M.I., Ali, N., Jan, G., Hamayun, M., Jan, F.G., Iqbal, A. et al. (2022) Salt stress alleviation in Triticum aestivum through primary and secondary metabolites modulation by *Aspergillus terreus* BTK-1. *Frontiers in Plant Science*, 13, 779623. Available from: https://doi.org/10.3389/fpls.2022.779623

Lim, H.J., Lee, E.H., Yoon, Y., Chua, B. & Son, A. (2016) Portable lysis apparatus for rapid single-step DNA extraction of *Bacillus subtilis*. *Journal of Applied Microbiology*, 120, 379–387. Available from: https://doi.org/10.1111/jam.13011

Min, T., Xiong, L., Liang, Y., Xu, R., Fa, C., Yang, S. et al. (2019) Disruption of stcA blocks sterigmatocystin biosynthesis and improves echinocandin B production in *Aspergillus delacroxii*. *World Journal of Microbiology and Biotechnology*, 35, 109. Available from: https://doi.org/10.1007/s11274-019-2687-9

Mundim, G.D.M., Maciel, G.M. & Mendes, G.D. (2022) *Aspergillus Niger* as a biological input for improving vegetable seedling production. *Microorganisms*, 10, 674. Available from: https://doi.org/10.3390/microorganisms10040674

Muthuraja, R. & Muthukumar, T. (2022) Co-inoculation of halotolerant potassium solubilizing *Bacillus licheniformis* and *Aspergillus violaceofuscus* improves tomato growth and potassium uptake in different soil types under salinity. *Chemosphere*, 294, 133718. Available from: https://doi.org/10.1016/j.chemosphere.2022.133718

Nieto, C.H.D., Granero, A.M., Zon, M.A. & Fernández, H. (2018) Sterigmatocystin: a mycotoxin to be seriously considered. *Food and Chemical Toxicology*, 118, 11–470.

Pfeiffer, E., Fleck, S.C. & Metzler, M. (2014) Catechol formation: a novel pathway in the metabolism of sterigmatocystin and 11-methoxysterigmatocystin. *Chemical Research in Toxicology*, 27, 2093–2099. Available from: https://doi.org/10.1021/tx500308k

Rank, C., Nielsen, K.F., Larsen, T.O., Varga, J., Samson, R.A. & Frisvad, J.C. (2011) Distribution of sterigmatocystin in filamentous fungi. *Fungal Biology*, 115, 406–420. Available from: https://doi.org/10.1016/j.funbio.2011.02.013

Ryngajllo, M., Boruta, T. & Bizukojc, M. (2021) Complete genome sequence of lovastatin producer *Aspergillus terreus* ATCC 20542 and evaluation of genomic diversity among *A. terreus* strains. *Applied Microbiology and Biotechnology*, 105, 1615–1627. Available from: https://doi.org/10.1007/s00253-021-11133-0

Samson, R.A., Visagie, C.M., Houbraken, J., Hong, S.B., Hubka, V., Klaassen, C.H.W. et al. (2014) Phylogeny, identification and nomenclature of the genus *Aspergillus*. *Studies in Mycology*, 78, 141–173. Available from: https://doi.org/10.1016/j.simyco.2014.07.004

Su, A., Yu, Q., Luo, Y., Yang, J., Wang, E. & Yuan, H. (2021) Metabolic engineering of microorganisms for the production of multifunctional non-protein amino acids: gamma-aminobutyric acid and delta-aminolevulinic acid. *Microbial Biotechnology*, 14, 2279–2290. Available from: https://doi.org/10.1111/1751-7915.13783

Tang, S., Men, P., Zhang, W., Li, H., Li, Z., Huang, X. et al. (2022) Identification of a polyketide biosynthesis gene cluster by

transcriptional regulator activation in *Aspergillus terreus*. *Fungal Genetics and Biology*, 160, 103690. Available from: https://doi.org/10.1016/j.fgb.2022.103690

Versilovskis, A. & De Saeger, S. (2010) Sterigmatocystin: occurrence in foodstuffs and analytical methods - an overview. *Molecular Nutrition & Food Research*, 54, 136–147. Available from: https://doi.org/10.1002/mnfr.200900345

Yabe, K. & Nakajima, H. (2004) Enzyme reactions and genes in aflatoxin biosynthesis. *Applied Microbiology and Biotechnology*, 64, 745–755. Available from: https://doi.org/10.1007/s00253-004-1566-x

Yang, G., Cao, X., Ma, G., Qin, L., Wu, Y., Lin, J. et al. (2020) MAPK pathway-related tyrosine phosphatases regulate development, secondary metabolism and pathogenicity in fungus *Aspergillus flavus*. *Environmental Microbiology*, 22, 5232–5247. Available from: https://doi.org/10.1111/1462-2920.15202

Yang, K., Tian, J. & Keller, N.P. (2022) Post-translational modifications drive secondary metabolite biosynthesis in *Aspergillus*: a review. *Environmental Microbiology*, 24, 2857–2881. Available from: https://doi.org/10.1111/1462-2920.16034

Zhao, D.L., Han, X.B., Wang, M., Zeng, Y.T., Li, Y.Q., Ma, G.Y. et al. (2020) Herbicidal and antifungal xanthone derivatives from the alga-derived fungus *Aspergillus versicolor* D5. *Journal of Agricultural and Food Chemistry*, 68, 11207–11214. Available from: https://doi.org/10.1021/acs.jafc.0c04265

Zingales, V., Fernandez-Franzon, M. & Ruiz, M.J. (2020) Sterigmatocystin: occurrence, toxicity and molecular mechanisms of action - A review. *Food and Chemical Toxicology*, 146, 111802. Available from: https://doi.org/10.1016/j.fct.2020.111802

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Ma, S., Zhao, D., Han, X., Peng, Y., Ren, T., Wang, M. et al. (2023) New application of *Aspergillus versicolor* in promoting plant growth after suppressing sterigmatocystin production via genome mining and engineering. *Microbial Biotechnology*, 16, 139–147. Available from: https://doi.org/10.1111/1751-7915.14176