Effectiveness of the toxin-degrading strain screening strategy: Deficiency and improvement in screening the gossypol-degrading microorganism *Aspergillus niger*

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Methodology article

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Abstract

Background

The gossypol, as an antinutritional factor or natural plant toxin, has antifertility effects on humans and animals, exists in cottonseed oil and meal, and is a typical food or feed contaminant. It is the classic screening method to using acetate gossypol as the sole carbon sources to prepare selective medium and screening gossypol-degrading microorganisms. By the classic screening strategy, many researchers have discovered that some microorganisms, and *Aspergillus niger* is the most reported microorganism. A strain of *A. niger* which can grow in an agar solid medium with gossypol as the sole carbon source is isolated from cattle rumen liquid was obtained by classic screening strategy. The effectiveness of the classic screening strategy was verified, by duplicating and verifying the degradation of gossypol by the *A. niger*.

Results

the *A. niger* can reduce the free gossypol (FG) content through biosorption but has no effect on the total gossypol (TG) content. And it can secrete agarase, utilise agar as carbon source.

Conclusions

The *A. niger* can secrete agarase, but cannot effectively degrade gossypol, In this case, it will mislead researchers and lead them to make wrong judgments. The usual methods of previous screening strategies are not rigorous enough, the classic screening method has defect in screening toxin-degrading strain, so agar control group should be added.

Background

Toxic substances in soil [1], water [2], food and feed [3] can be degraded by microorganisms. Compared with physical and chemical methods, the biodegradation technology has the advantages of safety, efficiency, moderate, environmental friendliness, absence of secondary pollution and low cost and is the most promising treatment method that has been paid attention by researchers. The classic screening method is that [4]: (1) Samples are usually collected from a variety of specific areas where degrading microorganisms may exist. (2) A certain toxic substance is used as the sole carbon (or nitrogen) and energy sources, and microorganisms are inoculated in an agar solid medium. (3)The degradation effect is judged using the diameter of the hydrolysis circle or the growth state of the microorganism, and the strains that can utilise or degrade the toxin are screened and isolated. Through further study, targeted degrading enzymes are obtained, and this strategy has achieved many successes [5–7].

The gossypol, as an antinutritional factor (ANF) or natural plant toxin, has antifertility effects on humans and animals [8]. The gossypol exists in cottonseed oil and meal (CM), and is a typical food or feed

contaminant [9]. Through the classic screening method, many researchers have discovered that some microorganisms, such as *Aspergillus niger* (AN) [10], *Candida tropicalis* [11], *Aspergillus flavus* [12] and *Bacillus subtilis* [13], can degrade gossypol, and AN is the most reported microorganism. On the basis of previous screening strategies, AN that can grow in an agar solid medium with gossypol as the sole carbon source is isolated from cattle rumen liquid. The transcriptome of AN is obtained, and relevant genes or degrading enzymes involved in the degradation of gossypol in AN are attempted to be discovered. However, results have not met our expectations. Therefore, the rigor of the traditional screening strategy for toxin-degrading strains is questioned.

This study has confirmed the deficiencies of previous screening strategies for gossypol-degrading microorganisms and expects to design a scientific and rigorous screening method for toxin-degrading microorganisms on the basis of existing methods.

Methods

Transcriptome sequencing (RNA-seq) and the trait identification of AN

AN cultured in potato dextrose agar (PDA) was used as the control group, and AN cultured in PDA with 2% acetate gossypol (AG) was used as the experimental group. The Illumina Hiseq^{TM} 2500 high-throughput sequencing technology was used to sequence the transcriptome of AN under normal and 2% AG conditions. Differentially expressed genes (DEG) were screened, and 2% AG response-related genes were analysed using the gene ontology (GO) and the KEGG (Kyoto Encyclopedia of Genes and Genomes) databases for annotation in AN (measured by Shanghai Biotechnology Co., LTD).

The AN and spores were observed using scanning (SEM) and transmission (TEM) electron microscopy [14].

Growth in different media

Solid media were prepared as shown in Table 1, and an equal amount of the AN spore solution was inoculated and cultured at 37 °C for 24 h. The growth of AN was observed and recorded. And after growing AN for 30 h, the media were stained with Lugol's iodine solution [15] in the dark for 1 min, washed thrice with clean water and observed under a normal light source.

Table 1. Preparation of solid media for AN

No.	Medium components
1	Sugar-free Czapek's medium (CzA) + 0.5% AG
2	Sugar-free CzA
3	CzA
4	Agar (20 g agar + 1000 mL dH_2O)

Preparation of the crude enzyme and the cell wall pellet suspension

AN was inoculated in potato dextrose broth, and the crude enzyme and the cell wall suspension were prepared as follows.

(1) After 24 h, the AN fermentation broth was collected (before spore production), subjected to repeated freeze–thaw and crushed using ultrasonic wave (in ice water) until no intact cell structure was found using a microscope.

(2) The fermentation broth was centrifuged at 4 °C and 8000 r/min for 10 min. The supernatant and the fungus precipitate were collected. After washing the fungus precipitate thrice with aseptic water, the precipitate was resuspended to the original volume, and the cell wall pellet suspension was obtained.

(3) The supernatant was filtered using the millipore filter (0.45 μm) [16], and the crude enzyme was obtained and stored at −20 °C for later use.

Removal experiment of AG by AN components

The experiment was designed and carried out in accordance with Table 2. Experimental groups were restored to normal temperature, and AG was added to make the concentration reach 0.5% and shaken well. Samples 1, 2, 3 and 4 were used as experimental groups, and sample 5 was used as the blank control group. Samples were collected before and after standing at 30 °C in the dark for 24 h and stored at −20 °C for testing. Before testing samples should been thawed, shaken and freeze dried. The free (FG) and the total (TG) gossypol were detected using the international standard ISO6866-1985, and the FG and TG removal rates were calculated according to the formula (1).

$$Rr = \frac{C1 - C2}{C1} \times 100\% \quad (1)$$

Where, Rr: Removal rate, C_1: Initial content of FG (or TG) and C_2: Rest of FG (or TG)

Table 2. Experimental design of the gossypol removal

No.	Treatment methods
1	Crude enzyme
2	Crude enzyme inactivated using boiling water bath for 10 min
3	Cell wall pellet suspension
4	Cell wall pellet suspension inactivated using boiling water bath for 10 min
5	Aseptic water

Statistical analysis

All statistical analyses were performed using the SPSS version 19 (IBM, Armonk, NY) Statistical differences were determined using the Duncan's multiple range test, and significance was defined as $P < 0.05$.

Results

GO enrichment analysis of differentially expressed genes

The Unigene GO analysis (Fig. 1) showed that 25 112 results were annotated and divided into three functional groups, namely, biological process (33.52%), molecular function (28.65%) and cell component (37.83%). The 10 most significant differences were DNA binding, transcription DNA-templated, zinc ion binding, G2/M transition of mitotic cell cycle, adhesion of symbiont to host, tryptophan biosynthetic process, asexual spore wall assembly, differentially expressed genes are closely related to cell stress resistance, proliferation, tissue repair, adhesion and cell structure [17–19]. Results did not clearly reflect or speculate the existence of the gossypol-degrading gene in AN.

Growth status in different media

AN could grow on all media. At the same culture time, the degrees of growth and maturity followed the order: CzA > sugar-free CzA > sugar-free CzA + 0.5% AG > agar, and spores were produced except in agar (Fig. 2A). The AN in the agar medium was the weakest (Fig. 2A4) followed by sugar-free CzA added with 0.5% AG (Fig. 2A1). Results showed that screening AN by using agar medium added with AG as the sole carbon source was not rigorous.

Lugol's iodine staining

Four media were stained with Lugol's iodine after growing AN for 30 h. Results are shown in Fig. 2B. After staining, corresponding bright spots were observed on the growth position of AN, and the surface of media were slightly dented. The result showed AN could secrete agarase to hydrolyse agar and utilise agar as carbon and energy sources.

Morphological changes in AN

Under the gossypol stress, AN had increased hyphae and decreased number of spores compared with AN grown on PDA alone (Fig. 3A). The spores grown on AG were deformed; had blisters on the surface; thin, uneven and damaged cell wall and small ratio of organelles to cell volume (Fig. 3B). Results showed that gossypol had a negative effect on the AN growth and could cause a bubblelike structure to form on the spores' surface and structural injury in the cell wall. However, AN had a certain tolerance to gossypol injury.

Removal efficiency of fungal components on AG

AG was added to each group, and the mixture was placed at room temperature for 1 h (Fig. 4). Flocculation and sedimentation were observed in all experimental groups. The influence of the inactivated cell wall pellet (Fig. 4B4) was most notable followed by that of the un-inactivated cell wall pellet (Fig. 4B3).

Different lowercase letters indicate significant differences amongst treatment groups ($P < 0.05$)

Compared with the control group, the crude enzyme and the cell wall of AN had significant removal effects on the FG in the sample ($P < 0.05$, Fig. 5), and the inactivated cell wall group had the highest removal rate (42.94%). The FG removal rate of each component after inactivation was higher than that before inactivation. The FG removal rates of cell wall groups were higher than that of the crude enzyme groups, but no significant difference was observed amongst experimental groups ($P > 0.05$).

Different lowercase letters indicate significant differences amongst treatment groups ($P < 0.05$)

The components of AN had different removal effects on TG in the sample (Fig. 6). The removal rate of each group was low (all < 15%), and the inactivated crude enzyme group had the highest TG removal rate (13.38%). The FG removal rate of each component was higher after inactivation, but no significant difference was observed amongst the same component groups ($P > 0.05$). The crude enzyme group had significant differences amongst cell wall groups ($P < 0.05$), but no significant difference was observed between the control and the experimental groups ($P > 0.05$).

Discussion

In this experiment, AN can grow in four kinds of media, and screening the degrading strains simply by using an agar medium added with toxin as the sole carbon source is not rigorous. The Lugol's iodine solution can stain polysaccharides in agar into a dark brown colour and cannot stain the degraded oligosaccharides of agar [15]. This solution has been used to visualise the agarase activity to screen the agarase production by microorganisms on a culture plate [20]. Similar to the AN in this study, some strains [15, 21] can utilise agar to grow normally on the medium without any sugar, interfere with the expected experimental results and affect the experimenter to make a true judgment on the results.

Enzymes are metabolites secreted by living organisms and can be divided into primary (constitutive enzymes) and secondary (inducible enzymes) metabolites. This study first discovers that AN can secrete

a constitutive enzyme that hydrolyses agar and utilise agar as sole carbon source. Therefore, we suspect that the gossypol-degrading enzyme of AN may not be a primary metabolite and that the gossypol cannot be directly utilised. This enzyme may be an inducible enzyme and can be secreted only under the condition of the gossypol as an inducer. In this study, the gossypol has a negative effect on the normal growth of AN. However, AN has a certain tolerance to the gossypol injury and produces active spores. Thus, AN may produce some special inducing enzymes under the induction of the gossypol. Given that the premise of the basic physiological metabolism of the cell is guaranteed, when exogenous stimuli interfere with the normal metabolism of cells or cause cell damage, certain proteins (such as cytokines and inducible enzymes) are secreted abundantly by the cells, which are under the exogenous stimulus stress against exogenous substances and protect cells [22, 23]. Therefore, when screening for a toxin-degrading strain by using a medium with the toxin as the sole carbon (or nitrogen) and energy sources, the function of inducing enzymes may be overlooked by usual screening methods of the predecessors.

For the removal effect of FG (or TG), no significant difference is observed before and after inactivation in crude enzyme groups (or cell wall groups). The TG removal rate of each group is lower than the FG removal rate (Fig. 5 and Fig. 6). For AN, the FG removal function and metabolic enzymes may not be closely related. Heating promotes proteolysis and increases the content of free amino acids [24, 25]. The crude enzyme solution is composed of proteins, and the cell wall suspension also contains some little cell wall proteins. After heat inactivation, the content of free amino acids in the solution increases. The Maillard reaction occurs and is strengthened between the carbonyl group from the gossypol and free amino acids under ISO detection conditions. As a result, the TG content decreases, and the TG removal rates of the crude enzyme and the cell wall groups increase when inactivated. Significant differences between the crude enzyme and the cell wall groups are observed due to different protein contents. The gossypol is unstable at room temperature, and a small amount of gossypol is degraded [26]. No significant difference in the TG removal rate is observed between the control and the crude enzyme or the cell wall group ($P > 0.05$). Thus, we believe that the decrease in TG content is not due to AN.

Hyphae secrete enzymes that convert the organic material from media into small molecules that can be taken up by the fungus to serve as nutrients [27]. Hyphae in filamentous fungi also serve as store house for nitrogen and phosphorus in the form of DNA, giving hyphal tips the capability of persistent extension and foraging in new areas [28]. The inadequate nutrition in sugar-free medium prompts AN to increase absorbable nutrients indirectly by increasing the number of hyphae. The toxic effect of the gossypol prompts AN to extend away from the gossypol damage by increasing the number and the length of hyphae. The agar medium lacks minerals and is not enough to support the growth of AN. AN relies on stored nutrients in spore growth and grows weakly. The main components of the AN cell wall are chitin and glucan and used as biosorbent for heavy metals [29]. The removal effect of AN on FG may be due to the biosorption of fungi. The gossypol can destroy the structure of AN and increase the gene expression of fungus cell stress resistance, proliferation, tissue repair, adhesion and cell structure. The reduction in gossypol is more likely to be consumed by damaging AN or protein.

When screening toxin-degrading microorganisms, we usually regard the toxin as the sole carbon source, in the preparation of agar solid medium tend to ignore the agar could be a potential carbon source, moreover, in culturing, is likely to ignore the carbon dioxide (CO_2) [30] in the air even light [31] may be a carbon or energy for some microorganisms. Thus, factors, such as agar, CO_2, illumination and inducible enzymes should be fully considered.

The degradation effect of microorganisms on toxin is an important basis to evaluate whether strains have further research value. Toxins can be absorbed by microbial adsorption, and free toxins are remarkably reduced. but no degradation, will free again under specific conditions, when evaluating the toxin degradation effect of a strain, the reduction binding toxins should be considered fully, basing on the change in total toxin to determine whether the target strain adsorbs or degrades toxins.

Besides, substances or toxins can be directly, exactly and efficiently analysed quantitatively and qualitatively by using a mass analyzer and other equipment [32, 33]. The toxin can be more intuitive judged whether or not to be degraded or form a new substance. Yet the toxin degradation products by microorganisms usually have many characteristics, such as low content and mixed with a variety of complex substances, the composition is complicated and difficult to extract and purify. In addition, the mass analyser has high requirements on operating ambient detection conditions and is not suitable for use at the initial phases of screening degrading strains.

During the processes, such as agricultural production, the exploitation and the utilisation of resources, sewage treatment and food storage and processing. Various degrees of toxin pollution, including organochlorine pesticides, polycyclic aromatic hydrocarbons (PAHs), dyes, mycotoxins and others, are available, and most of them are refractory organic pollutants, bioaccumulative, persistent and toxic with carcinogenic and mutagenic activities [34–36]. The successful screening and the domestication of microorganisms with degradation effect on a certain pollutant are important for environmental protection and human health. When screening toxin-degrading strains by using a toxin as the sole external carbon source and the solid medium as gel, the selection strategy should be optimised, and control groups with agar, CO_2 and light should be established at the same time. The screening scope can be extended to toxin-resistant organisms, and the content can be reduced by the inducible enzyme or consumed by the injury of resistant organism. The screening of pollutant-degrading strains is a tedious task and contingent in nature. Therefore, screening strategies should be fully considered when conducting strain screening for improved accuracy.

Conclusions

In this study, AN is found for the first time to secrete agarase and utilise agar as carbon source and energy source. AN can reduce the FG content by biosorption but cannot degrade the gossypol and reduce the content of the TG. The classical screening method has limitation in screening gossypol-degrading microorganism.

Abbreviations

AN: *Aspergillus niger*, ANF: Antinutritional factor; AG: Acetate gossypol; CM: Cottonseed oil and meal; CzA: Czapek's medium; DEG: Differentially expressed genes; FG: Free gossypol; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; RNA-seq: Transcriptome sequencing; SEM: Scanning electron microscopy; TG: Total gossypol; TEM: Transmission electron microscopy; PAHs: Polycyclic aromatic hydrocarbons; PDA: Potato dextrose agar

Declarations

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

CZ, GQS, HW, DJL, YX, YHZ, MJC, LJC designed and analyzed the experiments. C Z and GQS performed the experiments. CZ and LJC interpreted the data and wrote the manuscript, and were major contributors in writing the manuscript. All authors read and approved the final manuscript.

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References

1. Ghazali F M, Rahman R N Z A, Salleh A B, Basri M. Biodegradation of hydrocarbons in soil by microbial consortium. Int Biodeter Biodegr. 2004; 54:61-67. doi: 10.1016/j.ibiod.2004.02.002

2. Hazrat Ali. Biodegradation of Synthetic Dyes-A Review. Water Air Soil Poll. 2010; 213:251-273. doi:10.1007/s11270-010-0382-4
3. Ji C, Zhao L H. Research and prospects of aflatoxin biological d Chinese Journal of Animal Nutrition 2010; 22: 241-245. doi: 10.3969/j.issn.1006-267x.2010.02.001
4. Lewis W H, Tahon G, Geesink P, Sousa D Z, Ettema T J G. Innovations to culturing the uncultured microbial majority. Nat Rev Microbiol. 2020; doi: 10.1038/s41579-020-00458-8
5. Cheng K J, Jones G A, Simpson F J, Bryant M P. Isolation and identification of rumen bacteria capable of anaerobic rutin degradation. Can J Microbiol. 1969; 15:1365-1371. doi: 10.1139/m69-247
6. Dai P F, Cai T M, Wang L, Zhang W M, Lu L V, Shao Y, Zhao X M, Qian D U, Yang J, Li L F, Shi J J. Isolation, identification, degradation characteristics and pathway of a nicosulfuron-degrading bacteria. Environ Sci Technol. 2015; 28:12-21. doi: 10.3969/j.issn.1674-4829.2015.04.003
7. Liu B, Ju M, Liu J, Wu W, Li X. Isolation, identification, and crude oil degradation characteristics of a high-temperature, hydrocarbon-degrading strain. Mar Pollut Bull. 2016; 106:301-307. doi: 1016/j.marpolbul.2015.09.053
8. Randel R D, Chase C C, Wyse S J. Effects of gossypol and cottonseed products on reproduction of mammals. J Anim Sci. 1992; 70:1628-1638. doi: 10.1080/00071669208417483
9. EFSA Panel on Contaminants in the Food Chain (CONTAM), Knutsen H K, Barregård L, Bignami M, Brüschweiler B, Ceccatelli S, et al. Presence of free gossypol in whole cottonseed. EFSA J. 2017; 15:e04850. doi: 10.2903/j.efsa.2017.4850
10. Yang X, Sun J Y, Guo J L, Weng X Y. Identification and proteomic analysis of a novel gossypol-degrading fungal strain. J Sci Food Agr. 2012; 92:943-951. doi: 10.1002/jsfa.4675
11. Mageshwaran V, Sharma V , Chinnkar M, Parvez N, Krishnan V. Biodegradation of gossypol by mixed fungal cultures in minimal medium 1. Appl Biochem and Micro+. 2018; 54:301-308. doi: 10.1134/S0003683818030092
12. Yang X, W X, Guo J, Sun J. Screening and Identification of Gossypol-degraded Strains Isolated from a soil microcosm. Cotton Sci. 2010; 22:539-546. doi: 10.1080/00949651003724790
13. Zhang Y, Zhang Z, Dai L, Liu Y, Cheng M, Chen L. Isolation and characterization of a novel gossypol-degrading bacteria *Bacillus subtilis* strain Rumen Bacillus Subtilis. Asian Austral J Anim. 2018; 31:63-70. doi: 10.5713/ajas.17.0018
14. Tizro P, Choi C, Khanlou N. Sample Preparation for Transmission Electron Microscopy. Methods Mol Biol. 2019; 1897:417-424. doi: 10.1007/978-1-4939-8935-5_33
15. Fu X T, Kim S M. Agarase: Review of major sources, categories, purification method. enzyme characteristics and applications. Mar Drugs. 2010; 8: 200-218. doi: 10.3390/md8010200
16. Sultana N, Saha R. Studies on potential application of crude keratinase enzyme from *Stenotrophomonassp*. for dehairing in leather processing industry. J Environ Biol. 2018; 39: 324-330. doi: 10.22438/jeb/39/3/MRN-572

17. Sharma D, Singh A, Pathak M, Kaur L, Kumar V, Roy B G, Ojha H. DNA binding and antiradical potential of ethyl pyruvate: Key to the DNA radioprotection. Chem-biol Interact. 2020; 332:1-9. doi: 10.1016/j.cbi.2020.109313
18. Judit Zámborszky. Cell Cycle Transitions, G_2/M. Encyclopedia of Systems Biology. 2013; 329-333. doi: 10.1007/978-1-4419-9863-7_38
19. Swirnoff A H, Milbrandt J. DNA-binding specificity of NGFI-A and related zinc finger transcription factors. Mol and Cell Biol. 1995; 15:2275-2287. doi: 10.1093/brain/awh544
20. Kawai S K . Purification and characterization of an extracellular β-agarase from *Bacillus* MK03. J Biosci and Bioeng. 2003; 95:456-463. doi: 10.1016/S1389-1723(03)80063-4
21. Chi W J, Park D Y, Seo Y B, Chang Y K, Lee S Y, Hong S K. Cloning, expression and biochemical characterization of a novel GH16 β-agarase AgaG1 from *Alteromonas* GNUM-1. Appl Microbiol Biot. 2014; 98:4545-4555. doi: 10.1007/s00253-014-5510-4
22. Gu C, Hou C, Zhang S. miR-425-5p improves inflammation and septic liver damage through negatively regulating the RIP1-mediated necroptosis. Agents and Actions. 2020; 69:1-10. doi: 10.1007/s00011-020-01321-5
23. McClain C J, Barve S, Deaciuc I, Kugelmas M, Hill D. Cytokines in Alcoholic Liver Disease. Sem Liver Dis. 1999; 19: 205-219. doi:10.1055/s-2007-1007110
24. Chang Y N, Zhao G M, Liu Y X, Li M Y, Huang X Q, Sun L X. Changes of free amino acids in chicken and its broth during cooking. Sci Technol Food Ind. 2014; 35:333-337+342. doi: 10.13386/j.issn1002-0306.2014.09.064
25. Jiang M, Dong M S, Rui X, Li W, Chen X H. Effects of high pressure homogenization and heat treatment on protein solubility in soymilk. Food Sci. 2013; 34:125-130. doi: 10.7506/spkx1002-6630-201321026
26. Nomeir A A, Abou-Donia M B. Gossypol: High-performance liquid chromatographic analysis and stability in various solvents. J Am Oil Chem Soc. 1982; 59:546-549. doi: 10.1007/BF02636320
27. Vinck A , Terlou M , Pestman W R , Martens E P, Ram A F, van den Hondel C A M J J , Wösten H A B. Hyphal differentiation in the exploring mycelium of Aspergillus niger. Mol Microbiol. 2005; 58:693-699. doi: 10.1111/j.1365-2958.2005.04869.x
28. Ramesh Maheshwari. Nuclear behavior in fungal hyphae. Fems Microbiol Lett. 2005; 249:7-14. doi: 10.1016/j.femsle.2005.06.031
29. Filipovic-Kovacevic Ž, Sipos L, Briški F. Biosorption of Chromium, copper, nickel and zinc ions onto fungal pellets of *Aspergillus niger* 405 from aqueous solutions. Food Technol Biotech. 2000; 38:211-216. doi: 10.1016/S0141-0229(00)00214-3
30. Peng Y L, Zhao H Z, Yang Q Z, Song J. Progress in microbial and enzyme immobilization of carbon d Chemistry and Bioengineering. 2010; 27:10-13. doi: 10.3969/j.issn.1672-5425.2010.07.003
31. Shi Y Z, Zhang Q G, Wang Y, Jing Y Y. Experimental study on continuous culture of photosynthetic bacteria for hydrogen production from biomass. Transactions of the CSAE. 2008; 24:218-221. doi

3321/j.issn:1002-6819.2008.06.046

32. Yan J, Feng S, Wu T, Liu R H, Tsao R. Chemical constituents and antioxidant activity of flaxseed during germination. Journal of Chinese Mass Spectrometry Society. 2018; 39:112-119. doi: 10.7538/zpxb.2017.0003
33. Zhou J L, Zhou Z, Chen B L, Ma L K. Identification of an unknown derivative of sildenafil by UHPLC/LITQ Orbitrap MS. Journal of Chinese Mass Spectrometry Society. 2016; 37:422-430. doi: 10.7538/zpxb.2016.37.05.0422
34. Jones K C, Voogt P D. Persistent organic pollutants (POPs): state of the science. Environ Pollut. 1999; 100:209-221. doi: 10.1016/s0269-7491(99)00098-6
35. Kelly B C, Konomou M G, Blair J D, Morin. Food Web-Specific biomagnification of persistent organic pollutants. Science. 2007; 317:236-239. doi: 10.1126/science.1138275
36. Huen K, Yousefi P, Bradman A, Yan L, Harley K G, Kogut K, Eskenazi B, Holland N. Effects of age, sex, and persistent organic pollutants on DNA methylation in children. Environ Mol Mutagen. 2014; 55:209-222. doi: 1002/em.21845

Figures

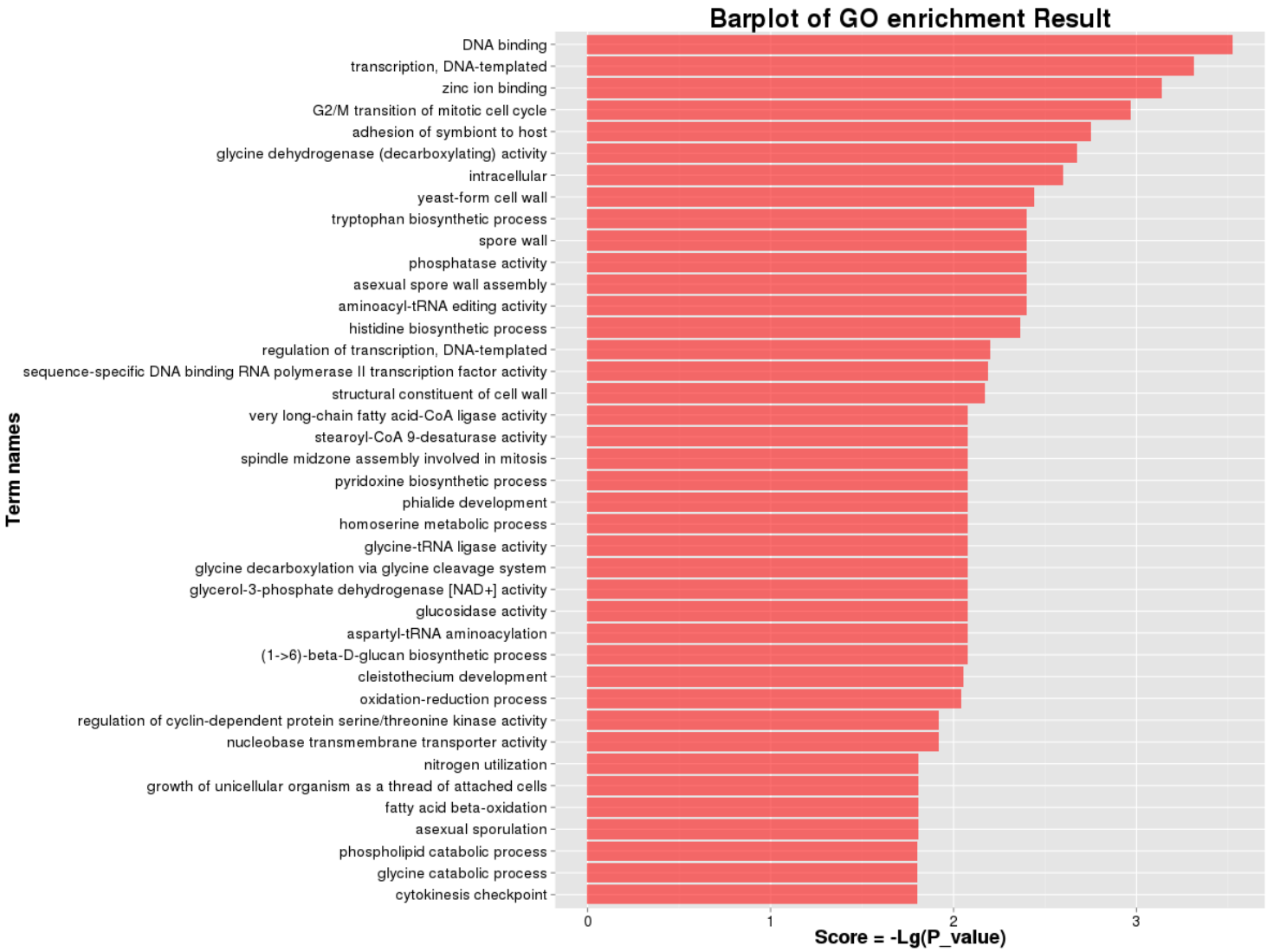

Figure 1

GO functional annotation of differentially expressed genes under the gossypol stress

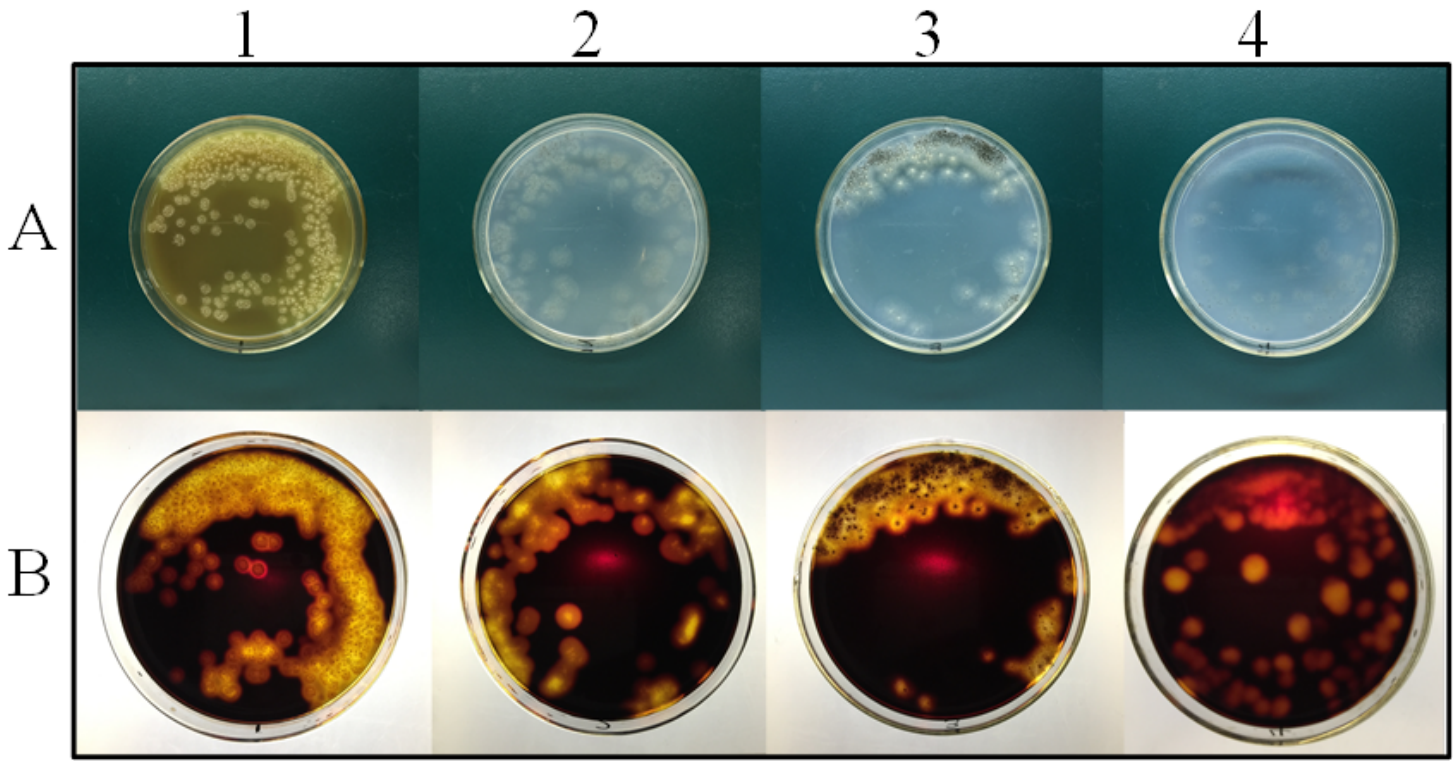

Figure 2

Growth status of AN in media and results of Lugol's iodine staining A: Before and B: after staining with Lugol's iodine 1: Sugar-free CzA + 0.5% AG, 2: Sugar-free CzA, 3: CzA, 4: Agar medium

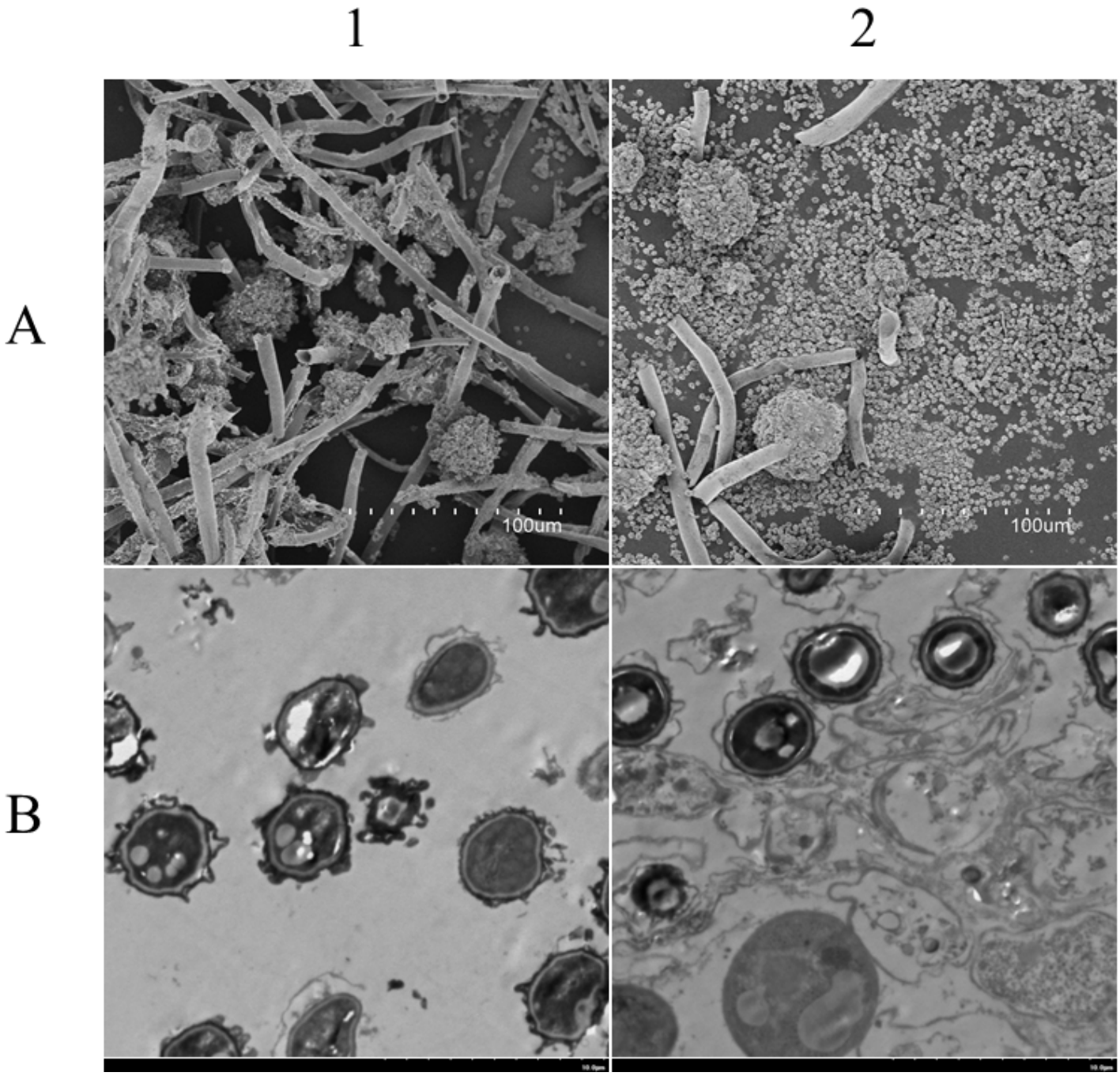

Figure 3

Micrographs of AN and spores A: SEM image of AN, B: TEM image of spores 1: Growth of AN on PDA added with 2% AG, 2: Growth of AN on PDA

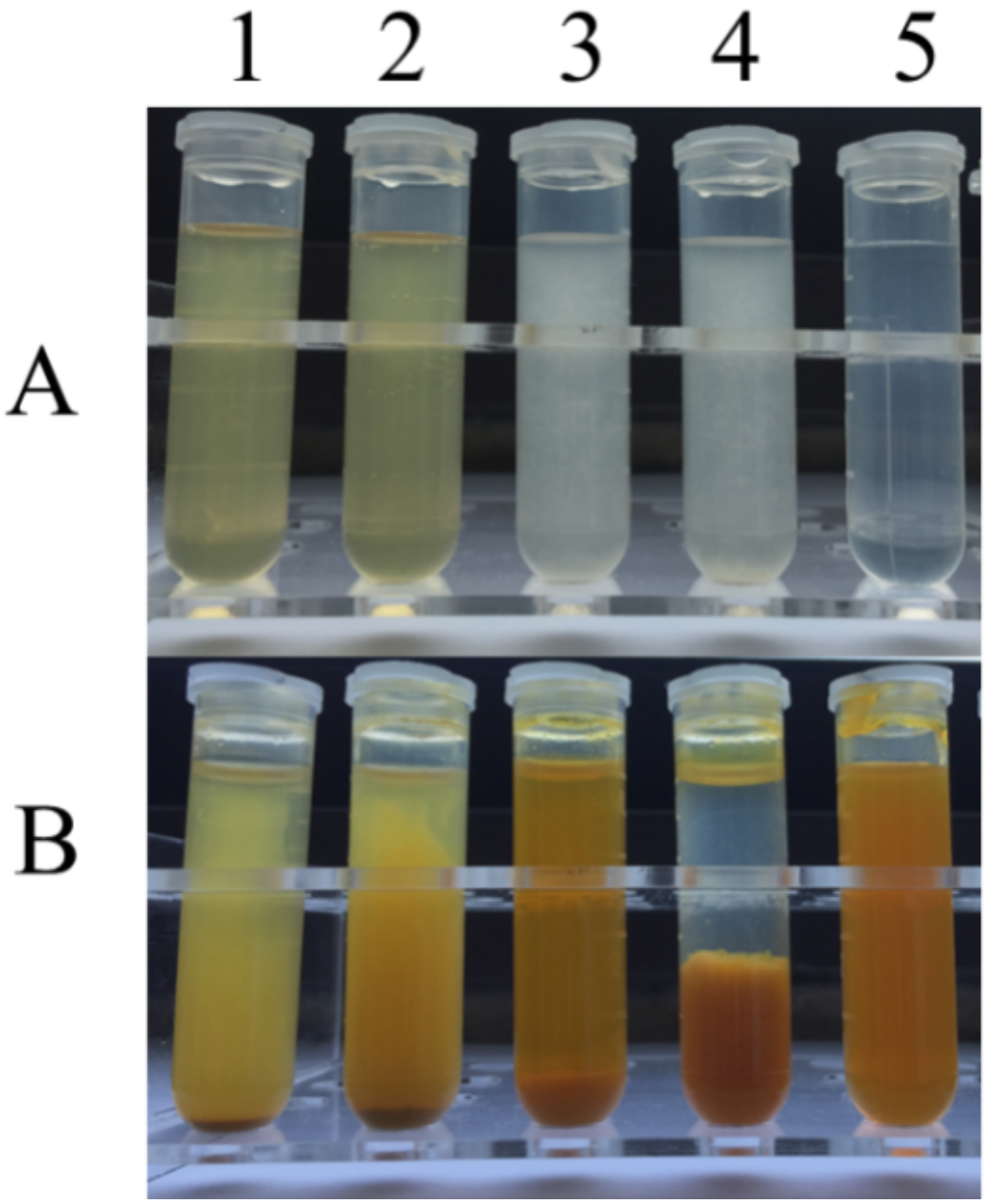

Figure 4

Flocculation and sedimentation of fungal components in AG A: Before and B: After adding AG 1: Crude enzyme, 2: Inactivated crude enzyme, 3: Cell wall pellet suspension, 4: Inactivated cell wall pellet suspension, 5: Blank control (aseptic water)

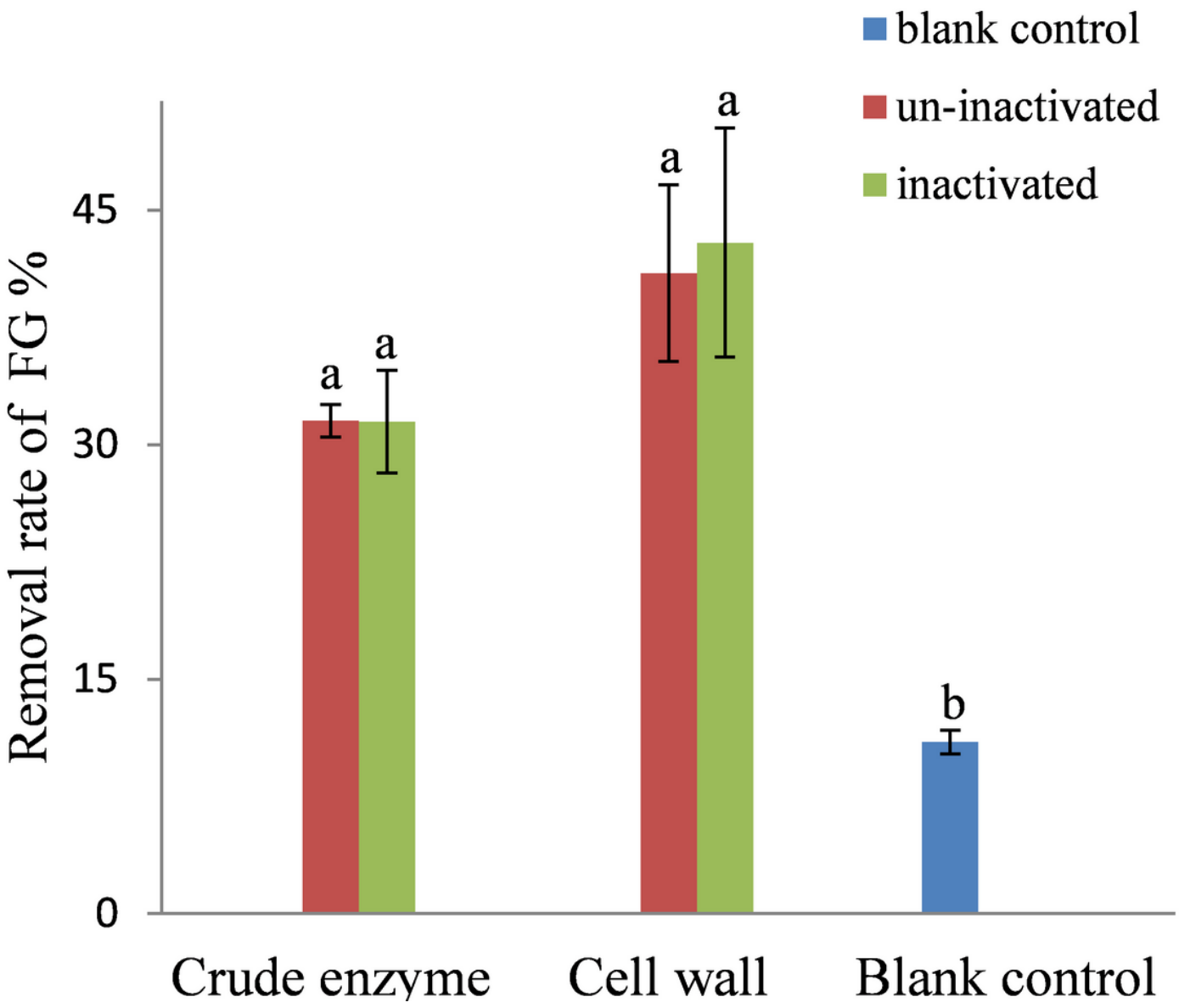

Figure 5

Removal efficiency on FG by fungal components

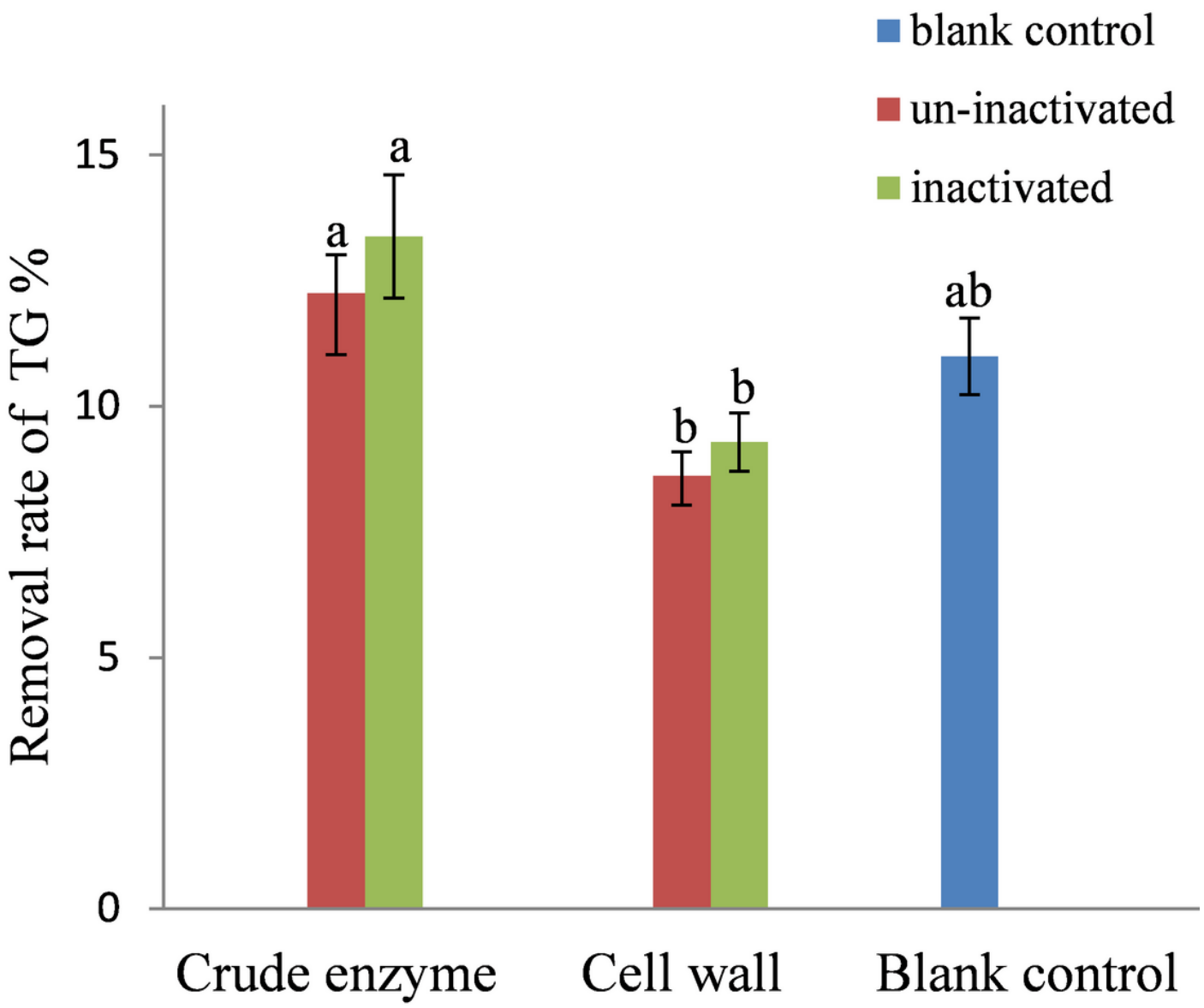

Figure 6

Removal efficiency on TG by fungal components