RESEARCH ARTICLE

Screening of cellulose-degrading fungi that promote growth of vegetable seeds

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Seed germination is often limited by the cellulose barrier in seed coats, and traditional chemical treatments can easily cause pollution. Cellulose-degrading fungi can efficiently break down seed coat cellulose and enhance seed vigor. Currently, there is a scarcity of growth-promoting fungal resources specifically tailored for vegetable seeds. To promote plant growth while improving the utilization of spent fungal substrate (SMS), this study aimed to screen for cellulose-degrading fungi with plant growth-promoting properties from SMS. Through the measurement of carboxymethyl cellulase (CMC) activity and indole-3-acetic acid (IAA) secretion, a strain with high-efficiency cellulose degradation and growth-promoting capabilities was isolated. Morphological observation and 18S rDNA gene sequencing identified this strain as *Penicillium oxalicum*, designated as X3. The CMC enzyme activity of X3 was measured at 298.35 U/mL, and its IAA production capacity reached 17.0 mg/L. Fermentation experiments revealed that the total cellulose degradation rate in the inoculated treatment group (ITG) reached 27.94%, significantly higher than that in the control treatment group (CTG) at 18.01%. The total organic carbon (TOC) content in the ITG rapidly decreased from 54% to approximately 44% within the first 8 days, stabilizing at 42% by the end of fermentation with a degradation rate of 22.22%, markedly higher than that of the CTG ($P < 0.05$). The optimal fermentation period was determined to be 8 days. Growth-promotion experiments on vegetable seeds of eggplant, pepper, and tomato showed that the 60% concentration of the strain fermentation broth resulted in the highest germination index for eggplant, pepper, and tomato seeds at 56.56, 42.06, and 36.19, respectively, which were 1.54, 1.67, and 1.82 times higher than that of the control group (CG). The average plant height of the seedlings treated with 60% concentration of the strain's fermentation broth was 1.26, 1.12, and 1.26 times higher than that of the CG, while the average root length was 1.06, 1.06, and 1.03 times longer than that of the CG. The activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were significantly increased, while malondialdehyde (MDA) content was reduced, demonstrating that the fermentation broth protected plant cells and decreased disease incidence during seed germination. This study laid the foundation for developing safe and effective microbial seed treatment methods while providing new insights into the resource utilization of SMS.

Keywords: *Penicillium oxalate*; cellulose degradation; germination rate; plant height; root length.

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Introduction

The germination of vegetable seeds and the growth of seedlings are critical stages in agricultural production, directly affecting crop yield and quality [1]. The seed coats of many vegetable species contain complex polysaccharides such as cellulose and hemicellulose, which form a natural physical barrier that restricts the absorption of water and oxygen, thereby delaying or inhibiting the germination process [2]. To enhance seed vigor, accelerate germination, and promote root development for robust seedling establishment, traditional methods such as mechanical scarification and chemical treatments like sulfuric acid soaking or gibberellin immersion are commonly used [3]. However, these approaches not only require high technical precision but also pose environmental pollution risks and may cause irreversible damage to the seed embryo [4].

In recent years, microbial degradation of seed coat cellulose has garnered widespread attention due to its environmental friendliness and safety. Cellulose-degrading fungi can secrete a complete cellulase system including endo-1,4-β-glucanase, exo-1,4-β-glucanase, and β-glucosidase, which efficiently break down cellulose components in plant cell walls, thereby promoting seed germination [5]. Yan *et al*. isolated *Trichoderma harzianum* T-24 from tropical rainforest soil, which exhibited a cellulase activity of 58.7 U/mL and increased soybean germination by 62% [6]. Sun *et al*. reported that *Aspergillus niger* C112 isolated from forest humus enhanced soybean germination by 52% [7]. Beyond traditional genera such as *Trichoderma* and *Aspergillus*, certain strains of *Penicillium* and *Fusarium* have also demonstrated remarkable cellulose-degrading capabilities. He *et al*. isolated a *Penicillium chrysogenum* strain from forest soil with a cellulase activity of 38.7 U/mL, improving soybean seed germination by 45% [8]. Dutta *et al*. discovered that a cellulose-degrading *Fusarium solani* strain softened rice seed coats through cellulase secretion, significantly boosting germination rates [9]. Notably, *Trichoderma*, *Aspergillus*, and *Penicillium* not only degrade seed coat barriers but also secrete phytohormones such as indole-3-acetic acid (IAA), thereby promoting seed germination and seedling growth through a dual-action mechanism [10]. He *et al*. isolated a *Penicillium oxalicum* strain (PE12) from compost, exhibiting a cellulase activity of 68.5 U/mL and IAA production of 12.3 μg/mL, which increased soybean germination by 58% [11]. Gao *et al*. screened an IAA-producing *Trichoderma harzianum* strain with strong cellulolytic activity, demonstrating significant growth-promoting effects on cucumber seedlings [12]. Consequently, the screening of cellulose-degrading fungi with plant growth-promoting traits (IAA-producing strains) has attracted considerable research interest. Spent mushroom substrate (SMS), the residual matrix after edible fungus cultivation, is rich in lignocellulose and underutilized fungal mycelia, making it an ideal source for isolating cellulose-degrading fungi [13]. Fungal strains with both cellulose-degrading and plant growth-promoting properties exhibit high colonization rates in SMS [11]. However, studies on the targeted screening of high-efficiency cellulose-degrading fungi from SMS for vegetable seed treatment remain limited, and their mechanistic effects on different crop seeds require further systematic investigation.

This study utilized SMS as the primary material and screened fungal strains with high-efficiency cellulose degradation capability and growth-promoting effects on vegetable seeds and seedlings through Congo red staining, carboxymethyl cellulase (CMC) activity assays, and indole-3-acetic acid (IAA) content measurement. The selected strain was further identified *via* morphological observation and 18S rDNA gene sequencing. SMS fermentation trials, seed germination assays, and measurements of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) activities, and malondialdehyde (MDA) content in seeds were conducted to evaluate the strain's cellulose degradation efficiency and seedling growth-promoting

potential. This research established a foundation for developing safe and effective microbial seed treatments while providing new insights into the resource utilization of SMS.

Materials and methods

Enrichment and screening of strains

3 g of SMS (shiitake mushroom substrate, second flush) obtained from Nanshan Biotechnology Co., Ltd., Zhumadian, Henan, China was mixed with 300 mL of sterile water on a magnetic stirrer for 15 minutes to ensure thorough mixing before centrifuging at 5,000 rpm for 5 minutes to collect the supernatant. The supernatant was streaked into a three-zone Potato Dextrose Agar (PDA) solid culture medium (Hangzhou Baisi Biotechnology Co., Ltd, Hangzhou, Zhejiang, China) and incubated at 28°C for 36 hours. The dominant colonies were transferred onto Congo red medium (Hangzhou Baisi Biotechnology Co., Ltd, Hangzhou, Zhejiang, China) and incubated at 28°C for 48 hours. The strains with cellulose degradation ability were screened by measuring the size of the hydrolyzed transparent zone of Congo red. The strains producing indole-3-acetic acid (IAA) were screened using the method described by Libbert and Manterffel [14].

Determination of CMC activity and IAA content

The CMC activity was determined *via* the 3,5-dinitrosalicylic acid (DNS) method with 1% CMC-Na as substrate [15]. 1 mL of CMC-Na was mixed with 0.50 mL of crude enzyme fermentation broth and incubated at 50°C for 30 min. Reactions were terminated by adding 1 mL DNS reagent and heating at 100°C for 10 min. The absorbance was measured at 540 nm, and the reducing sugar (glucose equivalents) was calculated using a standard curve (0 – 1 mg/mL glucose). One unit (U) was defined as the amount of enzyme releasing 1 µmol glucose equivalents per minute. The enzymatic activity (E) was calculated using the formula below.

$$E\ (U/mL) = 1000 \times C \times V \times D/(t \times v \times 186.16)$$

where C was the concentration of product from the standard curve (mg/mL). V was the total reaction volume (2.5 mL). D was the dilution factor. t was the reaction time (30 min). v was the enzyme volume used (0.5 mL). 180.16 was the molecular weight of glucose (g/mol). The content of IAA was determined using the Salkowski method [16]. 200 µL of test fermentation broth was combined with 400 µL of Salkowski reagent (0.5 M $FeCl_3$ in 35% $HClO_4$) in amber microcentrifuge tubes, while distilled water was used as the blank control. The mixture was incubated at 25°C for 30 min in darkness, and absorbance was measured at 530 nm using a microplate reader. IAA concentration was determined against a standard curve (0 - 100 µg/mL IAA). The IAA was calculated using the formula below.

$$IAA\ (mg/L) = \text{Dilution factor} \times (\text{Measured absorbance} - \text{Intercept})/\text{Slope}$$

Morphological observation and biological identification of strains

The target strains were inoculated onto PDA medium plate and cultured for 5 days. Observations were made on the growth characteristics of the colonies including the colony size, shape, edges, luster, texture, color, and transparency. A slide covered with mycelium was prepared and observed under a microscope. The target strains were sent to Sangon Biotech (Shanghai) Co., Ltd., (Shanghai, China) for 18S rDNA gene sequencing. The resulting sequences were aligned using the BLAST in the National Center for Biotechnology Information (NCBI) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A phylogenetic tree was constructed using the neighbor-joining method in MEGA5.0 software (https://www.megasoftware.net). Based on the results, the species of the strains were determined [17].

Ability of strains to promote decomposition of SMS

The SMS was ground and passed through a 20-mesh sieve. 10 g of the substrate were weighed and placed into 500 mL conical flasks. In the inoculated treatment group (ITG), 15 mL of

identified bacterial strain suspension with a concentration of 1×10^8 cfu/mL was added to the conical flask, while, in the control treatment group (CTG), 15 mL of sterile water was added to the conical flask. Both groups were cultured at 28°C for 14 days. The total organic carbon (TOC) and crude fiber contents of the cultures were measured periodically [18]. TOC content was determined by Potassium Dichromate External Heating (PDEH) method. Briefly, 0.5 g of sample was mixed with 10 mL of 0.4 M $K_2Cr_2O_7$ solution and 20 mL 98% H_2SO_4. After mixing thoroughly, the reaction was placed at 170°C for 5 min until the solution turned orange red. The mixture was cooled down and transferred to a 250 mL conical flask. 3 drops of 0.1% phenanthroline indicator (w/v) was then added before titrating with 0.2 M ammonium ferrous sulfate $(NH_4)_2Fe(SO_4)_2$ standard solution until the color changes from orange red to brownish green. The titrant volume consumed was recorded, and the TOC content was calculated as follows.

$$TOC\ (\%) = 100 \times (V_0 - V_1) \times C \times 3 \times 1.724 / m$$

where V_0 was the blank titrant volume (mL). V_1 was the sample titrant volume (mL). C was the concentration of $(NH_4)_2Fe(SO_4)_2$ (mol/L). 3 was the conversion factor for carbon molar mass (12 g/mol ÷ 4 electron transfer). 1.724 was the organic carbon to organic matter conversion factor (assuming 58% carbon in organic matter). m was the sample mass (g). Crude fiber content was determined by Weende method. Briefly, 1 - 2 g of the sample (m_1) was weighted and placed in a pre-weighed filter paper bag. 200 mL of 1.25% H_2SO_4 solution was added and heated at 100°C for 30 minutes, replenishing with distilled water to maintain a constant volume. The mixture was filtered to collect the residue and rinsed thoroughly with hot water until neutral pH (~7) was obtained. 200 mL of 1.25% NaOH solution was then added and heated at 100°C for 30 minutes while maintaining a constant volume. The sample was filtered again, washed with hot water to neutrality, then degreased by rinsing with ethanol and diethyl ether. The residue with filter paper was transferred to a drying oven and dry at 105°C to constant weight (m_2) followed by incinerating in a muffle furnace at 550°C for 4 hours before cooling down in a desiccator and weighing the ash (m_3). The crude fiber percentage was calculated as follows.

$$\text{Crude fiber}\ (\%) = (m_2 - m_3) / m_1 \times 100$$

Study on the growth-promoting ability of strains

The identified strain with a concentration of 1×10^8 cfu/mL was inoculated into 200 mL of PDA liquid medium and cultured at 28°C, 160 r/min for 15 days. The culture was filtered through filter paper followed by sterilization using a 0.22 μm microporous membrane to obtain sterile filtrate. The sterile filtrate was used to prepare fermentation broths at different concentrations of 0, 20, 40, 60, 80, and 100%. For each type of seed, 6 experiment groups (EG) were set up, while the control group (CG) was prepared using PDA medium without the addition of bacterial solution. Plump and uniformly sized eggplant, pepper, and tomato seeds (Xingyun Seed Industry Co., Ltd., Qingxian, Hebei, China) were soaked in a water bath at 55°C, magnetically stirred for 15 minutes, and then soaked in a water bath at 30°C for 1 hour. Seeds were evenly distributed in the culture dish lined with three layers of filter paper with 50 seeds per dish and 5 mL of sterile water. Each culture dish was evenly sprayed with 10 mL of fermented broth at different concentrations with three replicates per treatment. The dishes were then incubated in a constant-temperature dark environment at 28°C. From the second day after seed treatment, 10 mL of fermentation broth was replenished daily at the same time to maintain the culture conditions, and germination counts were recorded. Seeds that failed to germinate within 7 days were considered non-viable. After 15 days, 20 seedlings of each plant type were randomly selected to measure the plant height and root length. Germination criteria were defined as radicle protrusion exceeding half of the seed's longitudinal diameter. Seeds with abnormal radicle development such as curved or spirally coiled radicles were excluded from the count. The germination rate was calculated using the following formula.

Germination rate (%) = (Number of germinated seeds / Total number of seeds) × 100

Germination index (GI) = ∑(Gt / Dt)

where Gt was the number of germinated seeds on day t. Dt was the corresponding germination day.

Determination of antioxidant enzyme activity and malondialdehyde (MDA) content

On the 4th day of treatment, 0.5 g sample seeds from each treatment was homogenized in ice-cold 0.1 M phosphate buffer (pH 6.0) using a pre-chilled mortar and pestle. After centrifuging at 12,000 rpm at 4°C for 15 minutes, the crude enzyme extract was prepared for the determination of MDA content and enzyme activity, while the control group used heat-denatured crude enzyme extract. MDA content was measured using thiobarbituric acid (TBA) method. 2.5 mL of 10% trichloroacetic acid (TCA) was added to 0.5 mL of crude enzyme extract, vortexed thoroughly, and incubated at 4°C for 10 minutes. After centrifugation at 12,000 rpm for 10 minutes, 2 mL of supernatant was collected, mixed with 2 mL of 0.67% TBA solution, and incubated in a 95°C water bath for 30 minutes. The mixture was centrifuged at 5,000 rpm at 4°C for 5 minutes to remove precipitates. The absorbance at 532 nm was measured using a 752N spectrophotometer (Shanghai Yidian Analytical Instrument Co., Shanghai, China). MDA concentration was determined against a standard curve (0 - 10 μmol/mL MDA) and was calculated as below.

$$MDA(\mu mol/g) = \frac{C \times V}{W \times \varepsilon}$$

where *C* was the concentration of product from the standard curve (μmol/mL). *V* was the total reaction volume (3 mL). *W* was the sample fresh weight (0.5 g). *ε* was TBA-MDA molar extinction coefficient at 532 nm (155/mM·cm). Peroxidase (POD) activity was measured using the guaiacol method. The reaction mixture was prepared by adding 28 μL guaiacol (20 mmol/L final) and 19 μL 30% H_2O_2 (20 mmol/L final) to 50 mL phosphate buffer (pH 6.0). 3 mL of the reaction mixture was added into a cuvette, preheated at 25°C for 5 minutes. After adding 0.1 mL of crude enzyme extract, the reaction was mixed immediately and started timing. The absorbance (A) at 470 nm was measured using a 752N spectrophotometer. Data was recorded every 30 seconds for 3 minutes. One unit of POD activity (U) was defined as the amount of enzyme that oxidized 1 μmol guaiacol per minute. The POD was calculated using the formula below.

$$POD\ (U/g\cdot\min) = \frac{\Delta A_{470} \times V \times D}{W \times \varepsilon \times t}$$

where ΔA_{470} was the change in absorbance per minute at 470 nm (the slope of the linear phase). *V* was total reaction volume (3.1 mL). *D* was dilution factor of the enzyme solution. *W* was sample fresh weight (0.5 g). *ε* was molar extinction coefficient of tetraguaiacol at 470 nm (26.6/mM·cm). *t* was reaction time (min). Catalase (CAT) activity was measured using the ultraviolet absorption method. In a quartz cuvette, 800 μL of 50 mM phosphate buffer (pH 7.0), 100 μL of 30 mM H_2O_2 solution, and 100 μL of crude enzyme extract were sequentially added, mixed immediately, and measured the absorbance at 240 nm using the 752N spectrophotometer. The absorbance of A_{240} was recorded every 30 seconds for 3 minutes. One unit (U) of CAT activity was defined as the amount of enzyme required to decompose 1 μmol of hydrogen peroxide (H_2O_2) per minute and was calculated as follows.

$$CAT\ (U/g\cdot\min) = \frac{\Delta A_{240} \times V \times D}{W \times \varepsilon \times t}$$

where ΔA_{240} was the change in absorbance per minute at 240 nm (slope of the linear range). *V* was the total reaction volume (1 mL). *D* was the dilution factor of the enzyme solution. *ε* was the molar extinction coefficient of H_2O_2 at 240 nm (43.6/M·cm). *W* was the sample fresh weight (0.5

Table 1. Result of Congo red plate transparent ring assay.

Strain	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10
D (cm)	3.30	3.60	3.50	3.60	3.50	3.70	3.10	3.20	3.40	3.20
d (cm)	1.50	1.60	1.50	1.60	1.70	1.80	1.70	1.50	1.50	1.90
D/d	2.20	2.25	2.33	2.25	2.06	2.06	1.82	2.13	2.27	1.68

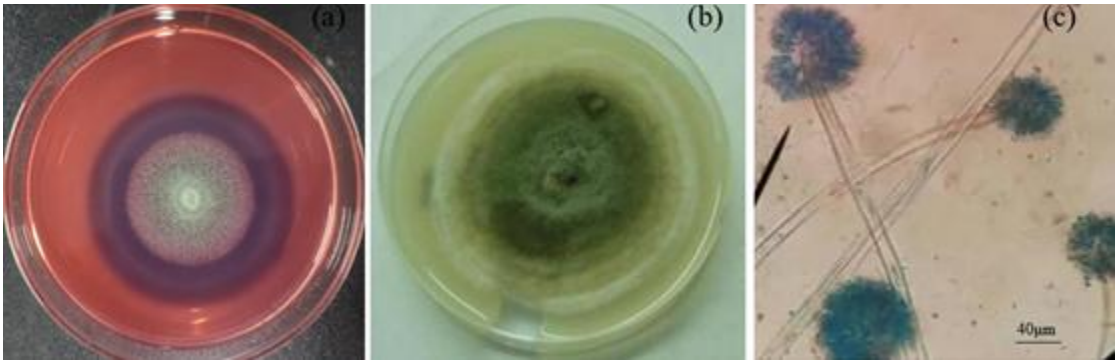

Figure 1. Transparent circle (a), observations of colony (b), and microscopy (c) of X3 strain.

g). *t* was the reaction time (min). Superoxide dismutase (SOD) activity was measured using the nitroblue tetrazolium (NBT) photoreduction method. The reaction mixture was 3 mL, which contained 1.5 mL of phosphate buffer (50 mM, pH 7.8), 0.3 mL each of MET (13 mM), NBT (0.75 mM), riboflavin (0.1 mM), and EDTA-Na_2 (0.1 mM), and 0.3 mL of crude enzyme extract. After 15 min illumination at 5,000 lux, absorbance at 560 nm was measured using the 752N spectrophotometer to quantify SOD-mediated NBT reduction inhibition. One unit (U) of SOD activity was defined as the amount of enzyme required to inhibit 50% of superoxide anion radical ($\cdot O_2^-$) mediated reduction reaction per minute under standardized assay conditions.

$$SOD(U/g) = \frac{(A_{control} - A_{sample}) \times V_{total}}{0.5 \times A_{control} \times W \times V_{enzyme}}$$

where *A* was the absorbance at 560 nm. V_{total} was the total reaction volume (3 mL). *W* was the sample fresh weight (0.5 g). 0.5 was the 50% inhibition threshold (1 unit definition). V_{enzyme} was the enzyme solution volume (0.3 mL).

Data analysis

The experimental data were statistically analyzed using SPSS 23.0 software (IBM, Armonk, New York, USA). The average plant height and root length of the seeds were expressed as mean ± standard error (SE). One-way analysis of variance (ANOVA) was performed on plant height and root length with a significance level of $P < 0.05$ indicating statistically significant differences.

Results

Screening of cellulose-degrading strains

The size of the hydrolysis zone (transparent circle) around each colony was measured. Based on the ratio of the diameter of the transparent circle (D) to the diameter of the colony (d), 10 strains with strong cellulose-degrading ability were identified and labeled as X1 – X10 (Table 1). The results showed that X3 exhibited the highest enzymatic activity (Figure 1a).

Morphological and molecular identification of the cellulose-degrading strain

The colonies of strain X3 exhibited a color gradient ranging from light green to dark green.

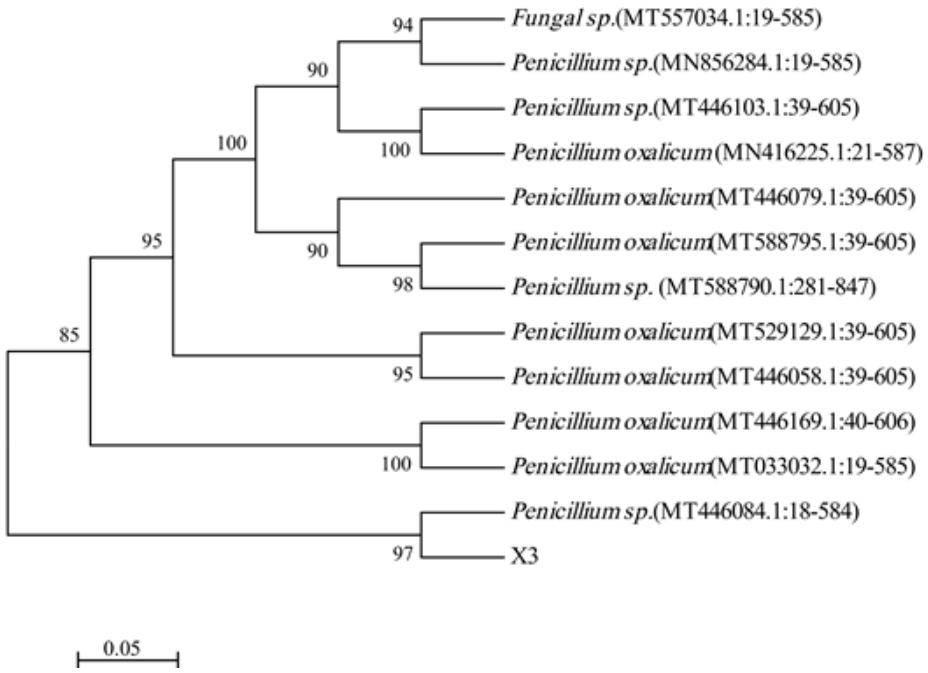

Figure 2. Phylogenetic tree based on the 18S rDNA sequences of strain X3.

The center of the colony transitioned from dark green to pale yellow with a thick, velvety texture, while the periphery displayed a light yellow hue. The colonies were regular in shape either circular or oval with smooth, non-serrated margins. Mature colonies had a rough, granular surface (Figure 1b). Microscopic observation revealed that the hyphae of strain X3 were dark green, well-developed, branched, radial, and flower-like with large spherical vesicles on the hyphae. Conidia appeared slender, smooth, and nearly colorless (Figure 1c). The 18S rDNA sequence demonstrated that strain X3 showed the closest phylogenetic relationship to *Penicillium oxalicum* with a sequence similarity of 97% (Figure 2). Based on both morphological characteristics and molecular identification results, strain X3 was identified as *Penicillium oxalicum*.

Determination of CMC enzyme activity and IAA content in the strains

Among strains X1 to X10, strain X3 exhibited the largest transparent hydrolysis zone and the highest CMC enzyme activity of 298.35 U/mL, significantly outperforming other strains (Figure 3a). In addition, qualitative screening for IAA production identified five positive strains of X1, X3, X4, X5, and X7. Subsequent quantitative analysis revealed that strain X3 possessed the highest IAA production capacity, reaching 17.0 mg/L (Figure 3b).

Compost-promoting ability of strain X3

Cellulose, a long-chain macromolecular polysaccharide composed of glucose units linked by β-1,4 glycosidic bonds, is the most abundant component in SMS and serves as a crucial indicator during the degradation process. The results showed that the cellulose content in the material exhibited a declining trend across different treatments during the fermentation process. During the initial 8 days of fermentation, the cellulose degradation rate in the ITG significantly increased to 21.32% compared to that of 8.26% in the CTG, which was attributed to the initial addition of *Penicillium oxalicum*. From days 8 to 14 of fermentation, the activity of *Penicillium oxalicum* in the ITG decreased due to elevated temperatures, resulting in a slower cellulose degradation rate. However, this rate remained higher than that observed in the CTG. At the conclusion of fermentation, the total

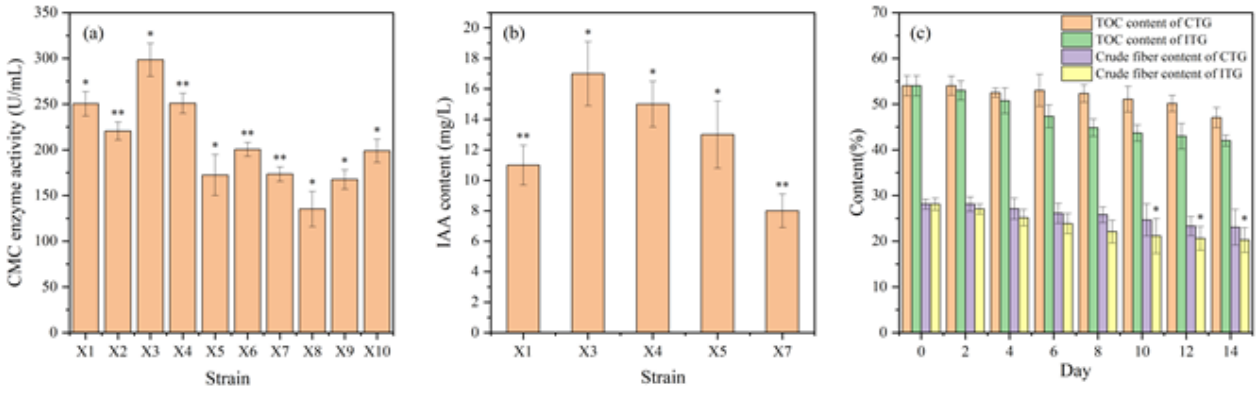

Figure 3. CMC enzyme activities, IAA content of different strains, and the results of SMS fermentation degradation of X3 strain.

Table 2. Germination rate and index of vegetable seeds.

Variety		Fermentation liquid concentration						
		0%	20%	40%	60%	80%	100%	CG
Germination rates (%)	Eggplant	90±0.14^{c}	80±0.04^{d}	96±0.09^{ab}	100±0.15^{a}	100±0.15^{a}	96±0.05^{ab}	86±0.04^{cd}
	Pepper	90±0.12^{ab}	86±0.04^{c}	96±0.21^{b}	100±0.13^{a}	100±0.19^{a}	90±0.15^{ab}	86±0.04^{c}
	Tomato	80±0.13^{bc}	76±0.24^{c}	86±0.18^{a}	86±0.31^{a}	88±0.25^{a}	82±0.12^{b}	80±0.28^{c}
Germination index	Eggplant	39.86±0.12^{b}	33.78±0.35^{d}	50.42±0.17^{c}	56.56±0.36^{a}	55.73±0.28^{a}	52.11±0.26^{c}	36.73±0.34^{d}
	Pepper	26.78±0.16^{d}	21.38±0.22^{de}	33.21±0.35^{c}	42.06±0.28^{a}	39.56±0.16^{b}	37.41±0.22^{bc}	25.13±0.32^{e}
	Tomato	21.86±0.22^{c}	18.23±0.26^{c}	27.81±0.32^{b}	36.19±0.45^{a}	35.24±0.42^{a}	33.26±0.36^{b}	19.88±0.24^{c}

Notes: CG was the control group. The lowercase letters indicated the statistical significance ($P < 0.05$).

cellulose degradation rate in the ITG reached 27.94%, significantly higher than the 18.01% observed in the CTG ($P < 0.05$) (Figure 3c). These results demonstrated that the addition of *Penicillium oxalicum* effectively enhanced cellulose degradation with an optimal fermentation period of approximately 8 days. The degradation rate of the SMS could also be reflected by changes in TOC content. The TOC content of the residue in the CTG showed a gradual decrease from 54% to approximately 52% during the first 8 days, eventually reaching 47.04% by the end of fermentation, representing a degradation rate of 12.89%. In contrast, the ITG exhibited a rapid TOC decline from 54% to about 44% within the initial 8 days period, ultimately stabilizing at 42% with a 22.22% degradation rate at fermentation endpoint, which was a statistically significant improvement compared to CTG ($P < 0.05$). Given SMS was rich in lignocellulose composition, TOC content generally remained relatively stable during fermentation. The introduction of *Penicillium oxalicum* substantially accelerated the lignocellulose degradation, resulting in faster TOC reduction, which was consistent with previous studies [19, 20].

Effect on the germination of three vegetable seeds

The seed germination rates of eggplant, pepper, and tomato of EG significantly exceeded those of the CG across various fermentation broth concentrations ($P < 0.05$). Notably, germination inhibition was observed solely at 20% concentration for both eggplant and tomato seeds. At fermentation broth concentrations of 60% and 80%, eggplant and pepper seeds exhibited peak germination rates, whereas tomato seeds achieved maximal germination specifically at 80% concentration. All three species achieved their highest germination index (GI) values at 60% concentration (Table 2). As GI quantitatively reflects both germination percentage and synchronization [21, 22], these results demonstrated that the 60% cellulose-

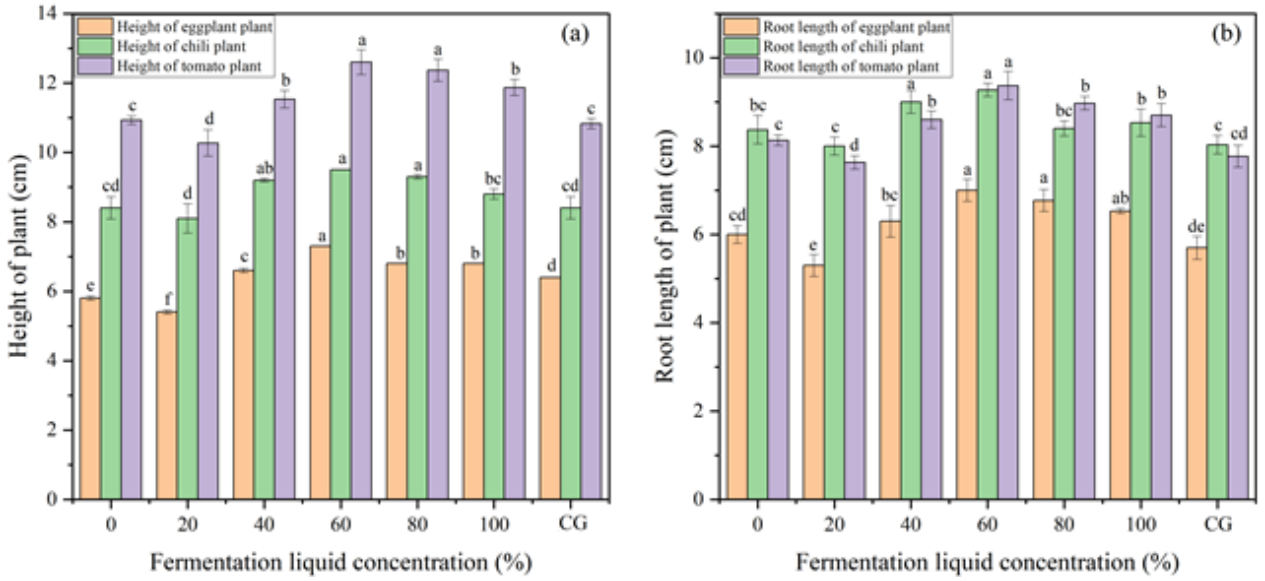

Figure 4. Average height and root length of three kinds of vegetable seedlings. Different lowercase letters indicated significant difference ($P < 0.05$). CG was the control group with PDA medium without the addition of bacterial suspension.

degrading microbial fermentation broth provided optimal growth-promoting effects across all tested seed varieties. The results also showed that *Penicillium oxalicum* had strong phosphorus-solubilizing ability. Some phosphorus-solubilizing bacteria could produce IAA and organic acids to promote seed germination and plant growth [23, 24]. The secretions of strain X3 included CMC enzyme and IAA. During seed germination, the CMC enzyme degraded the cellulose in the seed coat to help seeds break through the hard shell and germinate. As a plant growth regulator, IAA promoted seed germination and vegetative growth.

Effect on the morphological characteristics of three vegetable seedlings

The results of statistical analysis indicated that the average plant height and root length of eggplant, pepper, and tomato seedlings under different fermentation broth concentrations exhibited a unimodal response pattern, initially increasing then decreasing with rising concentration. The growth-promoting effects peaked at 60% fermentation broth concentration with maximum average seedling heights reaching 7.3 cm for eggplant, 9.5 cm for pepper, and 12.6 cm for tomato at this optimal concentration, which were 1.14, 1.13, and 1.17 times higher than those of the CG (Figure 4a). The differences between the 60% concentration group and other groups were also statistically significant ($P < 0.05$). However, there was no significant difference in the plant height of pepper and tomato seedlings when the fermentation broth concentration was 60% and 80% ($P > 0.05$). At a fermentation broth concentration of 60%, the average root lengths of eggplant, pepper, and tomato seedlings reached their maximum values of 7.0 cm, 9.3 cm, and 9.4 cm, respectively, which were 1.23, 1.16, and 1.21 times longer than those of the CG (Figure 4b). Compared with other concentration groups, the differences in the 60% group were statistically significant ($P < 0.05$). For eggplant seedlings, fermentation broth concentrations of 60% and 80% had no significant effect difference on root length ($P > 0.05$). For pepper seedlings, there was no significant difference in root length between the 40% and 60% fermentation broth concentrations ($P > 0.05$).

Effect of fermentation broth on antioxidant enzyme activity

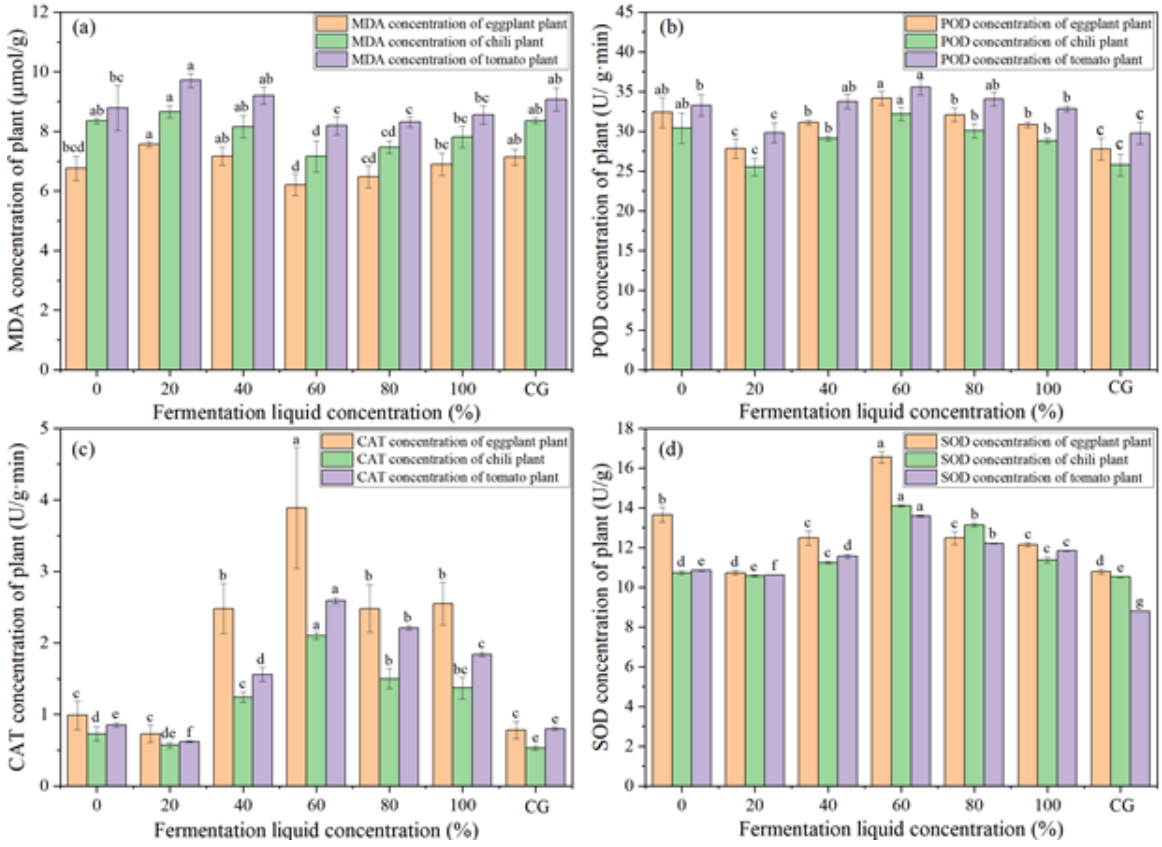

Figure 5. Antioxidant enzyme activity of three kinds of vegetable seedlings. Different lowercase letters indicated significant difference ($P < 0.05$). CG was the control group.

The MDA content of the three vegetable seeds reached the lowest levels at a fermentation broth concentration of 60%, measuring 6.21 μmol/g, 7.16 μmol/g, and 8.19 μmol/g, respectively, which were 0.87, 0.86, and 0.90 times of those in the CG. No significant differences were observed in MDA content between the other treatments and the CG ($P > 0.05$) (Figure 5a). After treatment with different concentrations of the cellulose-degrading strain fermentation broth, the POD activity of the three vegetable seeds was generally higher. At the 60% fermentation broth concentration, POD activities reached the peak, measuring 34.17 U/g·min, 32.17 U/g·min, and 35.6 U/g·min for the seeds of eggplant, pepper, and tomato, respectively, which were 1.23, 1.25, and 1.20 times those of the CG (Figure 5b). The differences between the 60% concentration group and other groups were statistically significant ($P < 0.05$), but no significant difference was observed compared to the pure water group (0% fermentation broth concentration) ($P > 0.05$). The CAT activity of the three vegetable seeds varied with different fermentation broth concentrations. At the 60% fermentation broth concentration, CAT activities reached the highest levels, measuring 3.89 U/g·min, 2.10 U/g·min, and 2.59 U/g·min for the seeds of eggplant, pepper, and tomato, respectively, which were 4.99, 3.96, and 3.24 times higher than those of the CG (Figure 5c). The differences between the 60% concentration group and other groups were statistically significant ($P < 0.05$). At the 20% fermentation broth concentration, CAT activities were inhibited, reaching the lowest levels of 0.73 U/g·min, 0.57 U/g·min, and 0.62 U/g·min, respectively, there was no significant difference compared to the CG ($P > 0.05$). All concentrations of the cellulose-degrading strain fermentation broth promoted SOD activity. At the 60%

fermentation broth concentration, SOD activities reached the highest levels, measuring 16.55 U/g, 14.10 U/g, and 13.59 U/g for the seeds of eggplant, pepper, and tomato, respectively (Figure 5d). The differences between the 60% concentration group and other groups were statistically significant ($P < 0.05$). When the fermentation broth concentration was 60%, the activity of POD, CAT, and SOD in the three vegetable seeds significantly increased, while the MDA content significantly decreased. The results indicated that the 60% concentration of fermentation broth effectively alleviated oxidative stress and promoted plant growth. Thus, the 60% concentration was the optimal choice for improving antioxidant enzyme activity and reducing membrane lipid peroxidation in vegetable seeds.

Discussion

This study successfully screened and isolated a cellulose-degrading strain from SMS, *Penicillium oxalicum*, which exhibited growth-promoting functions. The strain demonstrated the ability to produce CMC enzyme and IAA at levels of 298.35 U/mL and 17.0 mg/L, respectively. The results of the SMS decomposition showed that strain X3 effectively promoted the degradation of cellulose in the SMS with a significant increase in the degradation rate compared to the CTG ($P < 0.05$), which provided a new microbial option for the degradation and utilization of cellulose. The seed growth-promotion results showed that the germination rate, germination index, seedling height, root length, and antioxidant enzyme activity of the three types of seeds varied with the concentrations of the fermentation broth. The optimal growth conditions for the three vegetable seeds were observed at a 60% fermentation broth concentration. However, the specific effects on different vegetable seedlings varied. The follow-up research will further explore how to organically combine microorganisms with different functions to create synergistic effects, providing an excellent microbial community for cellulose degradation, seed germination, and crop growth. The decomposition of SMS is a continuous process, and evaluating degradation capability solely based on a strain's instantaneous enzyme activity has certain limitations [25]. To validate the actual degradation capacity of the strain, this study conducted liquid fermentation experiments and showed that, after 14 days of treatment with strain X3, the decomposition rate of fungal residues reached 22.22%. Previous studies reported the screening of cellulose-degrading strains from SMS such as *Aspergillus niger* and *Pseudomonas koreensis*, which achieved decomposition rates of 21.86% and 17.54%, respectively, under identical conditions [26, 27]. In comparison, strain X3 demonstrated certain advantages. Given that seed coat is primarily composed of cellulose, accelerating cellulose degradation is beneficial for seed germination and plant growth. Numerous studies have reported on microbial enzyme systems for cellulose degradation with fungi attracting greater research attention than bacteria due to their superior enzymatic activity. Li *et al*. screened a cellulose-degrading strain, FLX-7, from a solid waste landfill, which achieved a CMC enzyme activity of 11.94 U/mL after optimizing fermentation conditions [28]. Zhong *et al*. isolated a cellulose-degrading strain, F3, from biogas residue compost with a CMC enzyme activity of 2.73 U/mL [29]. Comparative analysis revealed that strain X3 exhibited superior CMC enzyme production capabilities. Another study found that a 10^3 fold dilution of *Bacillus mucilaginosus* inoculant could increase the germination rate of vegetable seeds and promote root growth in vegetable seedlings [30], while Yang *et al*. also demonstrated that low concentrations of single EM microbial fertilizer significantly promoted the growth of two types of tobacco seedlings, which might be related to the organic acids, plant hormones, and other metabolites produced by the microorganisms [31]. Recent studies have reported that certain fungi possess plant growth-promoting traits such as IAA production and phosphate solubilization [32]. The fungal strain CJAN1179 was found to produce substantial of IAA, particularly in the

presence of tryptophan [33]. The strain CGF-1 demonstrated varying IAA production levels across different extracts, which enhanced stress tolerance and nutrient uptake in wheat plants grown in heavy metal-contaminated soils [34]. In this study, strain X3 secreted 17.0 mg/L of IAA, which might be attributed to differences in fungal species and soil environmental conditions. IAA at certain concentrations not only directly promoted plant growth but also enhanced cell volume and mass by altering the intracellular environment [31]. However, the effects of *Penicillium oxalicum* fermentation broth on seed germination varied with concentrations. High concentrations of fermentation broth could damage root tip epidermis, cause cell deformation and increase intercellular spaces [35]. Research with *Trichoderma harzianum* fermentation broth and another substance showed that high concentrations altered the morphology of epidermal cells, and chemical stress significantly affected seed germination and seedling growth [36]. Numerous studies have shown that POD, SOD, and CAT are closely related to plant stress resistance. Oxygen metabolism during biological activities generates reactive oxygen species (ROS). Under stress conditions, the balance of ROS metabolism is disrupted, leading to the accumulation of ROS, which damages cells and negatively affects plant growth, ultimately reducing yield and quality [37, 38]. The accumulation of ROS under stress can be mitigated by the antioxidant enzyme system, where SOD, POD, and CAT work synergistically to effectively remove excess ROS and prevent membrane lipid peroxidation and other damage. The level of MDA is an important indicator of the extent of membrane lipid peroxidation [39, 40]. In this study, the cellulose-degrading strain significantly promoted the germination of vegetable seeds. The activities of SOD, POD, and CAT in seeds treated with different concentrations of fermentation broth were significantly enhanced with the strongest promotion observed at the 60% fermentation broth concentration. The MDA content was the lowest at this concentration, indicating minimal damage to cell membranes and a protective effect on plant cells, which helped defend against pathogen invasion and reduce disease incidence during seed germination. The results confirmed the stable growth-promoting and stress-resistant effects of the fermentation broth, suggesting its potential as a biological resource to enhance stress resistance during seed germination. However, the mechanisms of action and potential phytotoxicity of the fermentation broth require further investigation. Future research could focus on testing the thermal, pH, and UV stability of the fermentation broth at the optimal concentration for seed germination. Additionally, exploring the growth-promoting potential and mechanisms of *Penicillium oxalicum* could accelerate cellulose degradation, seed germination, and plant growth in straw-returned fields.

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