Screening of Glucosinolate-Detoxifying Strains and Their Effects on Fermented Rapeseed Meal Postprint

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Abstract

This study aimed to screen strains applicable for rapeseed meal detoxification and investigate their effects on rapeseed meal fermentation. Using glucosinolate, the primary antinutritional factor in rapeseed meal, as the sole carbon source, 54 strains exhibiting good growth on screening medium were isolated and purified from a mixture of fermented silage and soil. Rescreening with glucosinolate degradation rate as the dependent variable initially yielded 5 strains with degradation rates exceeding 30%. Through single-strain fermentation experiments using a rapeseed meal solid-state fermentation medium, one strain A9 with a glucosinolate degradation rate of 23.69% was obtained. Morphological observation and 16S rDNA sequencing and analysis of strain A9 identified it as *Lactobacillus acidophilus*, which was subsequently used in mixed-strain fermentation of rapeseed meal with *Saccharomyces cerevisiae* and *Bacillus subtilis*. The results demonstrated that mixed-strain fermentation reduced the glucosinolate content of rapeseed meal from 37.48 mol/g to 25.96 mol/g, achieving a degradation rate of 30.73%; crude protein content increased from 38.41% to 45.44% ($P<0.05$); protein structure was altered, with decreased protein molecular weight, and peptide content increased from 0.84% to 2.62% ($P<0.05$); the fermented rapeseed meal exhibited a yellowish-brown, fluffy appearance with a rich sour aroma, and total acid content increased from 1.01% to 3.91% ($P<0.05$). These findings indicate that the nutritional value and flavor of rapeseed meal were effectively improved through mixed-strain fermentation.

Full Text

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Abstract

This study aimed to screen bacterial strains for detoxifying rapeseed meal and investigate their fermentation effects. Using glucosinolate (GS), the main anti-nutritional factor in rapeseed meal, as the sole carbon source, 54 strains with robust growth were isolated and purified from a mixture of fermented silage and soil. Five strains exhibiting GS degradation rates exceeding 30% were selected through secondary screening. Single-strain solid-state fermentation of rapeseed meal identified strain A9 with the highest GS degradation rate of 23.69%. Morphological observation and 16S rDNA sequencing identified strain A9 as *Lactobacillus farciminis*. Subsequent mixed fermentation of rapeseed meal with *L. farciminis*, *Bacillus subtilis*, and *Saccharomyces cerevisiae* reduced GS content from 37.48 mol/g to 25.96 mol/g, achieving a degradation rate of 30.73%. Crude protein content increased significantly from 38.41% to 45.44% ($P<0.05$), while protein structure changed with reduced molecular weight. Polypeptide content rose from 0.84% to 2.62% ($P<0.05$). The fermented rapeseed meal exhibited a yellow-brown, fluffy appearance with a rich sour aroma, and total acid content increased from 1.01% to 3.91% ($P<0.05$). These results demonstrate that mixed fermentation effectively improves the nutritional value and flavor of rapeseed meal.

Keywords: glucosinolate; detoxification; fermentation; rapeseed meal; nutritional value; flavor

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With the rapid development of China's animal husbandry industry, conventional feed ingredients, particularly protein sources, are becoming increasingly scarce. The traditional corn-soybean meal dietary structure is evolving, and diversified diets incorporating alternative protein sources such as rapeseed meal will become the new norm [1]. Rapeseed meal, a byproduct of rapeseed oil extraction, contains 35%-45% crude protein and represents a common protein feed resource [2]. However, its utilization is limited by anti-nutritional factors including glucosinolates, tannins, and phytic acid, along with high crude fiber content [3]. Researchers have conducted extensive studies on detoxification and degradation of these anti-nutritional factors to enhance the feed value of rapeseed meal.

Early detoxification methods primarily involved physical and chemical approaches [4]. While physical heating and solvent extraction effectively reduced glucosinolate content, these methods suffered from high operational costs, significant nutrient loss, and chemical residue issues [5]. In recent years, biological fermentation has gained attention due to its low cost, minimal nutrient loss, and ability to simultaneously improve nutritional value during detoxification [6-8]. Rozan et al. [9] reported that fermentation with *Rhizopus oligosporus* for 40 hours degraded 30% of lignin and 47% of glucosinolates. Mixed fermentation can produce multiple enzyme systems, achieving synergistic effects [10-11]. However, most strains used for rapeseed meal fermentation, such as *Aspergillus niger*, *Aspergillus oryzae*, and various bacilli, are not approved feed additives by the Ministry of Agriculture, raising safety concerns. Additionally, most research has focused primarily on glucosinolate degradation rather than comprehensive evaluation of nutritional and flavor parameters [12]. Therefore, this study aimed to screen highly efficient glucosinolate-degrading microorganisms from fermented silage-soil mixtures that comply with Ministry of Agriculture regulations, and to conduct mixed solid-state fermentation of rapeseed meal with *S. cerevisiae* to improve flavor and *B. subtilis* to enhance nutritional structure, thereby simultaneously achieving detoxification and improving nutritional value and palatability.

Materials and Methods

1.1 Experimental Materials

High-glucosinolate rapeseed cake (108 mol/g) was obtained from Dali, Yunnan Province for glucosinolate extraction. Fermentation-grade rapeseed meal (37 mol/g) was provided by Beijing Jinshengxiang Technology Development Co., Ltd. as the fermentation substrate. *Bacillus subtilis* and *Saccharomyces cerevisiae* were obtained from the State Key Laboratory of Microbial Technology at Shandong University. Glucosinolates were crudely extracted in our laboratory.

1.2 Crude Extraction of Glucosinolates

Glucosinolate extraction followed the method described in reference [13], using 70% ethanol solution at 70°C for 6 hours, followed by centrifugation, filtration, and rotary evaporation of the supernatant to obtain the crude glucosinolate product.

1.3 Culture Media

MRS + Glucosinolate Solid Screening Medium: Peptone 10 g/L, yeast extract 5 g/L, beef extract 10 g/L, glucosinolate 5 g/L, K HPO 2 g/L, sodium acetate 5 g/L, ammonium citrate 2 g/L, MgSO 0.1 g/L, MnSO 0.05 g/L, Tween-80 1 g/L, agar 20 g/L, sterilized at 121°C for 20 min.

MRS + Glucosinolate Liquid Rescreening Medium: Same composition

as above without agar.

MRS Medium: Peptone 10 g/L, yeast extract 5 g/L, beef extract 10 g/L, glucose 20 g/L, K HPO 2 g/L, sodium acetate 5 g/L, ammonium citrate 2 g/L, MgSO 0.1 g/L, MnSO 0.05 g/L, Tween-80 1 g/L, sterilized at 121°C for 20 min.

YPD Agar Medium: Peptone 20 g/L, yeast extract 10 g/L, glucose 20 g/L, sterilized at 121°C for 20 min.

LB Medium: Peptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L, sterilized at 121°C for 20 min.

Rapeseed Meal Fermentation Medium: 50 g rapeseed meal, 50 mL sterile water, sterilized at 105°C for 15 min.

1.4 Primary Screening of Glucosinolate-Degrading Bacteria

Twenty grams of fermented silage and 10 g of soil were inoculated into a 250 mL flask containing 120 mL sterile water, shaken at 37°C and 200 rpm for 20 min, then left to stand for 30 min. Twenty milliliters of the supernatant was inoculated into rapeseed meal fermentation medium and incubated statically at 37°C for 48 h. After mixing thoroughly with a sterile glass rod, 5 g was diluted with sterile water, and 1 mL of the supernatant was serially diluted (10^{1} to 10) and plated on MRS + glucosinolate solid screening medium. Following 48 h static incubation at 37°C, colonies that were numerous, fast-growing, and large were selected for streaking, isolation, purification, and preservation for rescreening.

Purified single colonies were inoculated into MRS medium and incubated statically at 37°C for 24 h, then transferred at 5% (v/v) inoculation rate to MRS + glucosinolate liquid rescreening medium. After 48 h static incubation at 37°C, cultures were centrifuged at 3,000 rpm for 15 min, and the supernatant was analyzed for glucosinolate content. The five strains with highest degradation rates were preserved.

1.5 Secondary Screening of Glucosinolate-Degrading Bacteria

The five selected strains were cultured and inoculated into rapeseed meal fermentation medium at 10% (w/w) inoculation rate with a 1:1 substrate-to-water ratio, followed by static fermentation at 37°C for 48 h with three replicates. After fermentation, portions were dried at 60°C and ground for glucosinolate analysis, while others were stored at -20°C. The strain showing the best degradation performance was selected for mixed fermentation with *B. subtilis* and *S. cerevisiae*.

1.6 Strain Identification

Preliminary identification was performed based on colony characteristics and morphology according to the *Standard Atlas of Food Hygiene Microbiology*. The strain was subjected to 16S rDNA amplification using primers 1492R (5 -GGTTACCTTGTTACCACTT-3) and 27F (5 -AGAGTTTGATCCTGGCTCA-3). Purified PCR products were sequenced by Qingdao Tsingke Biotechnology Co., Ltd., and the sequences were compared against the GenBank nucleotide database using BLAST analysis to identify homologous sequences.

1.7 Determination of Strain Growth Curve

The target strain was streaked on plates, and single colonies were inoculated into 10 mL liquid MRS medium, incubated at 37°C for 24 h to prepare seed culture. This was inoculated at 1% (v/v) into 150 mL liquid MRS medium and incubated statically at 37°C for 48 h. Samples were taken every 3 h to measure optical density (OD) at 600 nm using a UV-visible spectrophotometer, and the growth curve was plotted.

1.8 Mixed Fermentation with Glucosinolate-Degrading Bacteria, *B. subtilis*, and *S. cerevisiae*

The selected strain was co-cultured with *B. subtilis* and *S. cerevisiae* at a 4:3:3 volume ratio, with total inoculation at 10% (w/w) and substrate-to-water ratio of 1:1. Fermentation was conducted at 34°C for 48 h with three replicates. Fermented samples were analyzed for glucosinolate, crude protein, crude fiber, crude fat, polypeptide, and total acid contents.

1.9 Measurement Indicators and Methods

Glucosinolate content before and after fermentation was determined using the palladium chloride colorimetric method [14-15]. Crude protein was measured using a Dumas automatic nitrogen analyzer. Crude fiber was determined by the filter bag method, and crude fat by Soxhlet extraction. Polypeptide content was analyzed according to GB/T 22492-2008 for soybean peptide powder. Total acid content was measured by titration (expressed as lactic acid) [16-17].

Protein molecular weight distribution was analyzed by SDS-PAGE: 1.0000 g of rapeseed meal powder (passed through 60-mesh sieve) was extracted with 0.1 mol/L Tris-HCl buffer (pH 8.0), centrifuged, and 20 L of the supernatant was loaded onto a two-layer SDS-PAGE gel (5% stacking gel, 15% separating gel). Electrophoresis was performed at constant current (20 mA, 80 V) for 2 h, followed by Coomassie brilliant blue staining.

1.10 Statistical Analysis

Data were divided into two categories. Strain screening data were analyzed by direct observation, while fermentation data were analyzed using SPSS 19.0 general linear model with Duncan's multiple comparison. Results were expressed as mean ± standard error, with $P<0.05$ indicating significant difference. Graphs were prepared using Excel 2007.

Results

2.1 Primary and Secondary Screening Results

Using glucosinolate as the sole carbon source, 54 microbial strains with large, fast-growing colonies capable of utilizing glucosinolate were isolated on MRS + glucosinolate solid medium. Rescreening in liquid medium measured degradation rates, as shown in . Nine strains achieved degradation rates exceeding 30%, with the maximum reaching 39.25%. The five most effective strains were selected for further fermentation: A1 (39.25%), A9 (34.58%), D6 (34.58%), E6 (34.58%), and F3 (35.51%).

2.2 Single-Strain Solid-State Fermentation Results

Single-strain solid-state fermentation of rapeseed meal was conducted with the five selected strains in triplicate. Glucosinolate degradation rates were calculated based on content changes before and after fermentation. As shown in , strain A9 exhibited the highest degradation rate at 23.69%.

2.3 Strain Identification Results

Strain A9, obtained through primary and secondary screening, formed circular, transparent colonies with smooth edges after 24 h cultivation. Microscopic observation revealed rod-shaped cells. PCR amplification yielded a 948 bp sequence. BLAST analysis against GenBank showed the strain belonged to *Lactobacillus*, with 99% similarity to *Lactobacillus farciminis* KJ04 (accession number KX139184.1), confirming strain A9 as *L. farciminis*.

2.4 Strain Growth Curve

As shown in [Figure 1: see original paper], *L. farciminis* exhibited a brief lag phase, entered logarithmic growth at 3-21 h, reached maximum concentration at 30 h, then entered decline phase. Therefore, 21 h cultured broth was selected as seed culture for subsequent experiments.

2.5 Effect of Mixed Fermentation on Glucosinolate Content

Mixed fermentation of rapeseed meal with *L. farciminis*, *B. subtilis*, and *S. cerevisiae* (4:3:3 ratio, 10% inoculation, 1:1 substrate-to-water) at 34°C for 48 h reduced glucosinolate content from 37.48 mol/g to 25.67 mol/g, achieving a

30.73% degradation rate that complies with Ministry of Agriculture standards for low-glucosinolate rapeseed meal.

2.6 Effect of Different Fermentation Methods on Glucosinolate Degradation

Different fermentation methods yielded varying degradation efficiencies (). The liquid screening medium achieved 39.25% degradation, while single-strain and mixed fermentation resulted in 23.69% and 30.73%, respectively—both lower than the screening medium. Mixed fermentation outperformed single-strain fermentation.

2.7 Changes in Nutritional Components of Fermented Rapeseed Meal

Microbial fermentation improved rapeseed meal nutritional value while detoxifying. Both single-strain and mixed fermentation increased crude protein, polypeptide, and total acid contents, while altering crude fiber and crude fat levels. As shown in , crude protein increased significantly ($P<0.05$) from 38.41% to 44.94% (single-strain) and 45.59% (mixed), with no significant difference between fermentation groups ($P>0.05$). Polypeptide and total acid contents increased significantly ($P<0.05$), with mixed fermentation showing superior results ($P<0.05$).

2.8 Changes in Protein Molecular Composition

SDS-PAGE analysis ([Figure 2: see original paper]) revealed that rapeseed meal proteins primarily ranged from 17-34 kDa. After fermentation, high-molecular-weight proteins (17-34 kDa) in region A decreased, while low-molecular-weight proteins in region B increased substantially, particularly those below 10 kDa. Both single-strain and mixed fermentation produced similar effects.

2.9 Changes in Flavor Profile

After 48 h fermentation with *L. farciminis*, rapeseed meal developed a yellow-brown, fluffy appearance with a rich sour aroma—an important palatability indicator [18]. Total acid content increased significantly ($P<0.05$) from 1.01% to 3.10% (single-strain) and 3.91% (mixed fermentation).

Discussion

3.1 Effects of Fermentation Methods on Glucosinolate Degradation

Microbial fermentation effectively degrades glucosinolates by direct utilization as a carbon source and through enzymatic hydrolysis into volatile isothiocyanates [19]. In MRS + glucosinolate medium, glucosinolate is the sole carbon source, enabling direct microbial utilization and superior degradation compared to rapeseed meal medium where preferential carbon source utilization occurs.

Mixed fermentation outperformed single-strain fermentation, likely because the 120+ glucosinolate types [20] require diverse enzyme systems for complete degradation. Mixed cultures produce richer enzyme systems that degrade multiple glucosinolate types synergistically [21]. Wang et al. [22] reported 91.36% degradation using a 9%:9%:6% ratio of *Lactobacillus plantarum*, *B. subtilis*, and *Aspergillus oryzae* at 33°C for 96 h. Ju et al. [23] similarly found mixed fermentation superior to single-strain fermentation, consistent with our results.

3.2 Improvement of Protein Quality in Fermented Rapeseed Meal

Microbial fermentation significantly improves rapeseed meal protein quality. Despite high protein content, rapeseed meal proteins have large molecular weights and low digestibility, especially in press-extracted meals [24]. The predominant 12S globulin consists of six subunit pairs (20-30 kDa polypeptide chains). *L. farciminis* and *B. subtilis* produce -amylase, neutral protease, and other extracellular proteases [25] that degrade large proteins into absorbable small peptides and convert plant proteins into superior microbial proteins and functional peptides [26]. Protein and amino acid digestibility are key quality indicators; smaller molecular weights and higher polypeptide content enhance digestibility. Electrophoresis confirmed increased small-molecular-weight proteins and significantly elevated polypeptide content. Polypeptides offer superior nutritional value, are more readily absorbed than amino acids, and promote mineral absorption [27] while providing immunological, neurological, and antioxidant functions [28]. These improvements align with findings by Fu et al. [26] and Wang et al. [29].

3.3 Mechanism of Nutritional Improvement Through Microbial Fermentation

Fermentation created a porous, fluffy structure, likely due to enzymatic hydrolysis of surface fiber structures and gas exchange during *B. subtilis* and *S. cerevisiae* metabolism, increasing enzyme-substrate contact area [30] for enhanced nutrient utilization. The nutritional and flavor improvements resulted from synergistic fermentation. Increased crude protein and polypeptide contents occurred because rapid *B. subtilis* growth during early fermentation secreted abundant proteases that converted rapeseed proteins into microbial proteins and peptides while consuming carbon sources, causing "protein concentration effects" through dry matter loss [31]. Significantly increased total acid content and sour aroma improved palatability. Organic acids produced during *L. farciminis* and *S. cerevisiae* metabolism can alleviate stress-induced feed intake reduction, stimulate olfactory senses, and promote consumption [32]. Lactic acid acts as an acidifier that improves palatability, reduces gastrointestinal pH, and inhibits pathogen proliferation while promoting digestive enzyme activation and gut microflora balance [33-34]. The diverse enzyme systems produced during fermentation predigest rapeseed meal, reduce anti-nutritional factors, im-

prove protein quantity and quality, increase polypeptide content, and enhance palatability for better animal utilization.

Conclusions

1. This study screened a natural microbial strain capable of efficiently degrading glucosinolates. Identified as *Lactobacillus farciminis*, strain A9 achieved a 39.25% degradation rate in screening medium.

2. Both single-strain fermentation with *L. farciminis* and mixed fermentation with *B. subtilis* and *S. cerevisiae* effectively reduced rapeseed meal glucosinolate content (23.69% and 30.73% degradation, respectively) while significantly increasing crude protein, polypeptide, and total acid contents, thereby substantially improving the nutritional value and flavor of rapeseed meal.

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Note: Figure translations are in progress. See original paper for figures.