Sys Rev Pharm 2024; 15(10): 331-334
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453/DOI: 10.31858/0975-8453.15.10.331-334

Research Article

Chemical Elucidation and Kinetics of Antibiotics Production from Soybean (*Glycine max*) Seeds by Soil *Bacillus* Isolate

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Article History: *Submitted: 02.10.2024* *Accepted: 22.10.2024* *Published: 29.10.2024*

ABSTRACT

Bacillus is a gram-positive bacterium, with potential to synthesize several enzymes and secondary metabolites. Soybean (*Glycine max*) is a leguminous edible seed that is highly rich in proteins and other nutrients. This study chemically elucidates the various antibiotics produced by soil *Bacillus* during the fermentation of soybean seeds.

Bacillus species were isolated from a soil sample on sterilized nutrient agar and tested by gram staining and starch hydrolysis. Dried seeds of *Glycine max* were mechanically ground to obtain *Glycine Max* Seed Powder (GMSP). Fermentation of GMSP was carried out in a 250 mL Erlenmeyer flask for 192 hours. The time-courses of antibiotic production like peptide, macrolide and fluoroquinolone in fermentation broths were monitored spectrophotometrically every 24 hours, while High Performance Liquid Chromatography (HPLC) was used to analyze the antibiotics in the broths. Whitish bacterial colonies *Bacillus* formed were positive for gram staining and starch hydrolysis. Time-course analysis showed highest concentrations of peptide, macrolide and fluoroquinolone antibiotics at 117.16 μg/mL, 342.57 μg/mL and 2584.81 μg/mL, respectively in the broths. The HPLC indicated formation of bacillomycin D, surfactin A and 1-deoxynojirimycin as major antibiotics in GMSP broth. This study demonstrates that *Bacillus* species can synthesize antibiotics from soybean seeds, an innovation that extends the economic value of this food crop.

Keywords: Antibiotics, *Bacillus*, Fermentation, *Glycine max* seed, *Bacillus subtilis*

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INTRODUCTION

Bacillus bacteria are gram-positive, rod-shaped organisms that can be either facultative aerobes or anaerobes, commonly found in soil, water and air. According to Contesini FJ, *et al.*, 2018 the *Bacillus* species have the potential to synthesize many enzymes and antibiotics. For instance, *Bacillus subtilis* has been reported to produces a peptide antibiotic called bacitracin, which is clinically used to treat certain bacterial infections (Dame ZT, *et al.*, 2021). Antibiotics are microbial or botanical secondary metabolites capable of killing or inhibiting microbial growth in the treatment of infections (Thakur D and Prasanna R, 2020; Hooper DC, 2021).

Soybean is a leguminous crop belonging to the Fabaceae family and is a major global crop, largely cultivated in the United States of America, Brazil and Argentina (Doyle JJ and Luckow MA, 2003). Soybean is a rich source of plant-based protein and contains high levels of B vitamins, unsaturated fatty acids, calcium, fiber, zinc and iron, making it a good functional food. According to Rizzo G and Baroni L, 2018 the seeds are commonly used in various forms, including soy milk, tofu and edamame. Additionally, soybean meal, a byproduct of oil extraction, is used as a high-protein feed for livestock (Doyle JJ and Luckow MA, 2003).

The high nutrient content in soybean seeds makes them a versatile substrate for *Bacillus* growth and the production of important compounds. The present study was designed to investigate the ability of soil *Bacillus* isolates to produce antibiotics by fermentation of GMSP as a substrate.

MATERIALS AND METHODS

Soil sample 1 g were collected from a non-agricultural site and processed for the isolation of *Bacillus* species on sterilized Nutrient Agar (NA) at 37°C. The bacterial isolate was subjected to gram staining, starch hydrolysis and light microscopy for identification. GMSP was obtained by grinding dried soybean seeds. This powder was then subjected to batch fermentation with the *Bacillus* isolate at 37°C for antibiotics production for 192 hours, with pH monitored daily. The fermentation was conducted in a 250 mL Erlenmeyer flask containing 100 mL of sterilized production medium, 10 mL of inoculum 1.5 x103 viable cells and 10 g of GMSP, with constant mechanical agitation.

Standard protocols were used to determine the concentrations of peptide, macrolide and fluoroquinolone antibiotics in the cell-free fermentation broths spectrophotometrically on a daily basis, using amoxicillin, erythromycin and ciprofloxacin as standard antibiotics, respectively. After 192 hours, cell-free broths were analyzed by Ultraviolet (UV) spectroscopy and High Performance Liquid Chromatography (HPLC) for antibiotic identification and quantitation.

RESULTS AND DISCUSSION

Bacillus isolates were obtained as whitish colonies (*Figure 1*) with rod shape red/pink (*Figure 2*), which tested positive for gram staining and starch hydrolysis (*Table 1*). Lu Z, *et al.*, 2018 documented that *Bacillus* species grow as a gram-positive, rod-shaped and white or slightly yellow colonies. The pH values were found to be 3.88 ± 0.35 in glutamic acid broth and 6.38 ± 0.74 in GMSP broth (*Table 2*).

Time-course analysis shows the highest concentrations of peptide (*Figure 3*), macrolide (*Figure 4*) and fluoroquinolone (*Figure 5*) antibiotics at 117.16 μg/mL after 144 hours, 342.57 μg/mL after 144 hours and 2584.81 μg/mL after 96 hours respectively, in the GMSP broth. The UV spectral values of γ-GA 202.5 nm-231 nm (*Figure 6*) and GMSP 201 nm-249.5 nm (*Figure 7*) broths were comparable to standard amoxicillin 200 nm-244 nm (*Figure 8*), Erythromycin 200 nm-204.5 nm (*Figure 9*) and ciprofloxacin 200 nm-201.5 nm (*Figure 10*).

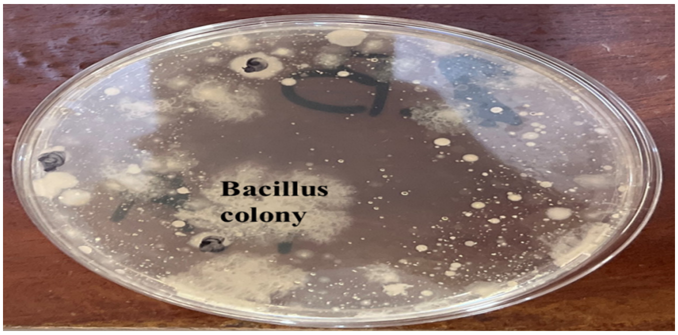

Figure 1: Soil *Bacillus* isolates grown as whitish colonies on nutrient agar

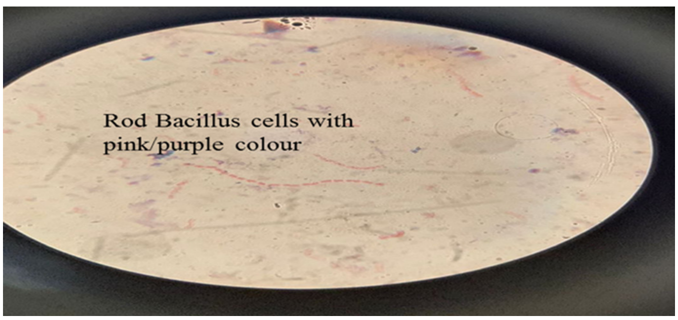

Figure 2: Microscopic rod shape of *Bacillus* isolate

Table 1: Gram and starch reactions of soil *Bacillus* isolate

Slide number	Microscopic view	Gram reaction	Appearance of zone around the bacterial cells in starch reaction
1	Rod shape	Red/pink colour	Clear zone
3	Rod shape	Red/pink colour	Clear zone
4	Rod shape	Red/pink colour	Clear zone
5	Rod shape	Red/pink colour	Clear zone
6	Rod shape	Red/pink colour	Clear zone

Table 2: pH change during fermentation of glutamic acid and *Glycine Max* Seed Powder (GMSP)

Time (Hours)	pH values	
	Glutamic acid	GMSP
24	3.09 ± 0.01	5.28 ± 0.20
48	4.17 ± 0.10	6.34 ± 0.14
72	4.14 ± 0.04	6.55 ± 0.52
96	4.38 ± 0.11	6,27 ± 0.02
120	4.29 ± 0.14	7.10 ± 0.08
144	4.55 ± 0.23	7.32 ± 0.33
168	4.44 ± 0.10	7.53 ± 0.41
192	4.37 ± 0.51	7.22 ± 0.22
M ± SD	3.88 ± 0.35	6.38 ± 0.74

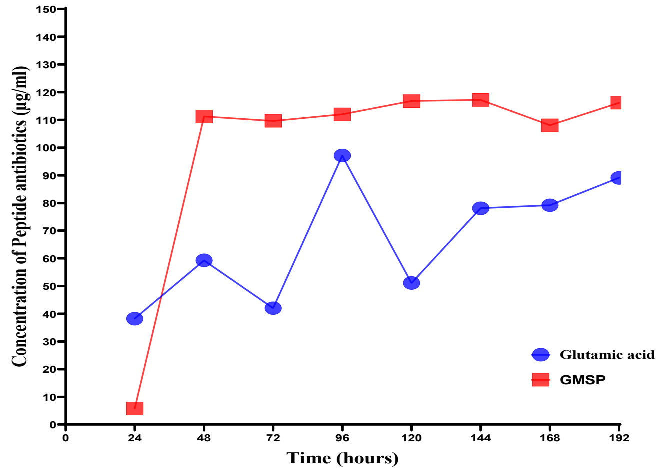

Figure 3: Time-course of peptide antibiotics production during fermentation of glutamic acid and *Glycine max* seed powder with soil *Bacillus* isolate

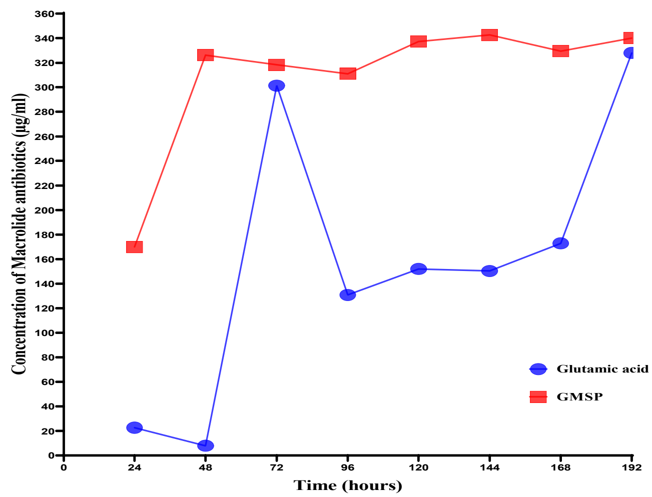

Figure 4: Time-course of macrolide antibiotics production during fermentation of glutamic acid and *Glycine max* seed powder with soil *Bacillus* isolate

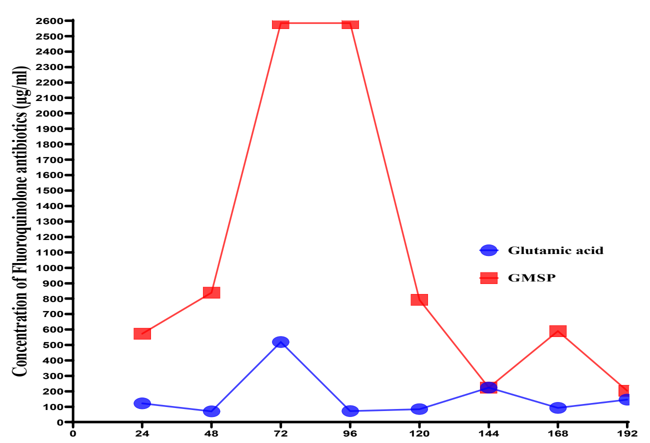

Figure 5: Time-course of fluoroquinolone antibiotics production during fermentation of glutamic acid and *Glycine max* seed powder with soil *Bacillus* isolate

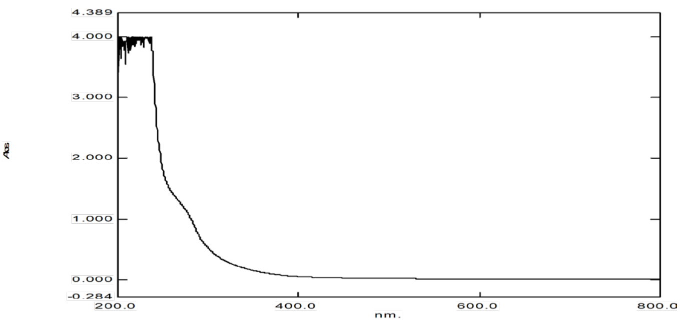

Figure 6: UV spectrum of glutamic acid fermentation broth

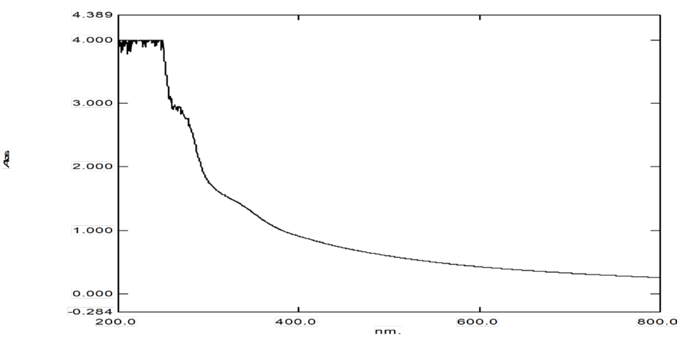

Figure 7: UV spectrum of *Glycine max* seed powder fermentation broth

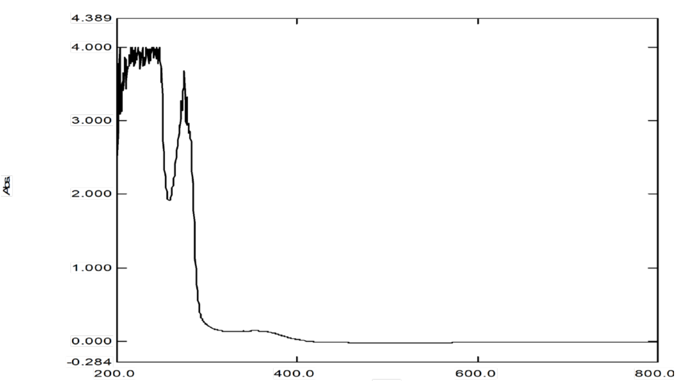

Figure 8: UV spectrum of standard amoxicillin (peptide antibiotics)

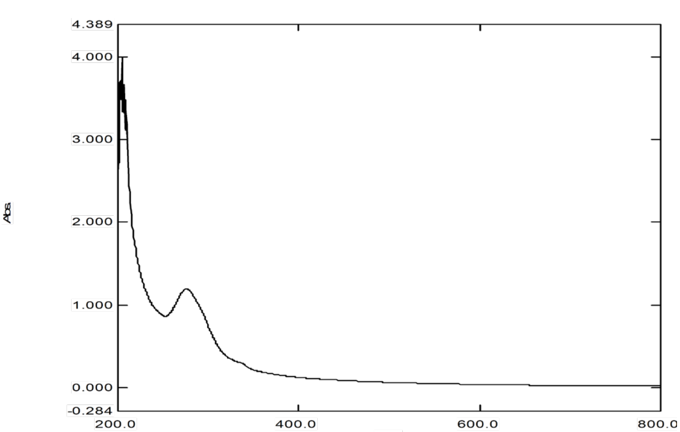

Figure 9: UV spectrum of standard erythromycin (macrolide antibiotics)

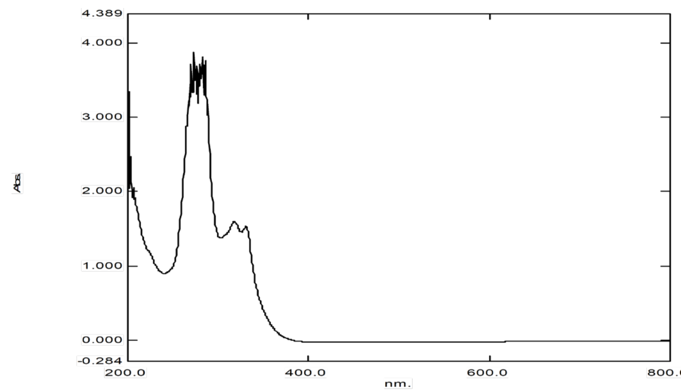

Figure 10: UV spectrum of standard ciprofloxacin (fluoroquinolone antibiotics)

The HPLC data indicated the presence of 3-phosphoglyceric acid 2.59% and poly-gamma-glutamic acid 97.41% and in γ-GA broth (*Figure 11*). In contrast, the GSMP broth contained Phenylacetic acid 3.28%, bacillomycin D 38.34%, Poly-Y-Glutamic Acid (Y-PGA) 6.93%, surfactin A 16%, bacteriocin 2.20%, Iturin 1.98%, alpha-amylase 2.14%, 1-deoxynojirimycin 13.14%, daidzein 2.39%, fengycin 4.89%, cephamycin C 4.84% and geinstein 3.87% (*Figure 12*). 1-Deoxynojirimycin is classified as a macrolide, (Bajpai S and Rao AV, 2014) while bacillomycin, surfactin, bacteriocin, iturin and fengycin are polypeptide antibiotics derived from *Bacillus* species (Chen X, *et al.*, 2022). In a recent study by (Adeleke GE, *et al.*, 2024) it was demonstrated that bacitracin B, bacilysin and bacillomycin D were produced from yellow maize seeds, while spectinomycin and kanamycin were produced from mango fruit juice through fermentation by soil *Bacillus* isolates.

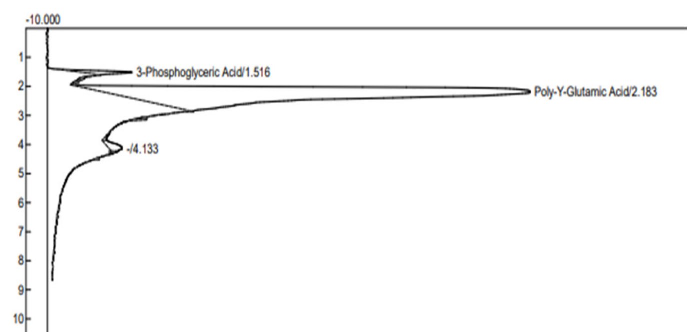

Figure 11: HPLC chromatogram of Glutamic acid fermentation broth

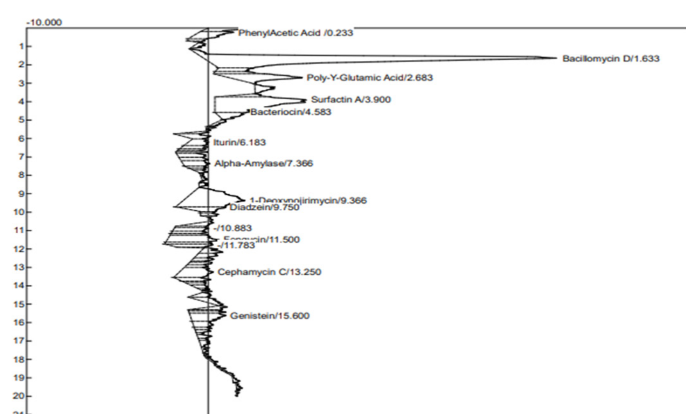

Figure 12: HPLC chromatogram of *Glycine max* seed powder fermentation broth

CONCLUSION

This research has demonstrated that soil *Bacillus* can potentially produce important antibiotics such as, bacillomycin D, surfactin A and 1-deoxynojirimycin through fermentation of GMSP. These antibiotics may be employed in combating infections in both humans and veterinary animals. This research serves as an innovation to increase the economic value chain of soybean, extending its application from food production to the pharmaceutical production of significant antibiotics.

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