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BIOCONTROL POTENTIAL AND PROTEIN EXTRACTION PROCESSES FROM *PRIESTIA MEGATERIUM*: A REVIEW

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Abstract: *Bacillus megaterium* has been extensively studied as a biofungicide, biofertilizer, enhancer in plant growth, plant growth-promoting rhizobacteria (PGPR), and broad-spectrum biocontrol agent in the agriculture industry. They are environmentally valuable, avirulent to humans and animals, have minor generation time, simple nutritional requirements, and robust stress resistance, and are mostly in agriculture. Due to their biocontrol ability against pathogens, they frequently appear in rhizospheres and phylloplanes owing to their capability to endure and flourish in different environments and have revealed the possibility to hinder the growth of plant diseases, happening on both the roots and aerial parts of the plant. This review highlights the immense potential of *B. megaterium* for a range of biotechnological applications by offering a thorough overview of the biocontrol abilities and techniques for protein production, and the wide range of commercially significant products obtained from this bacterium.

Keywords: *Priestia megaterium*, biocontrol ability, protein extraction, cell disruption, estimation, purification, commercial uses.

I. INTRODUCTION

Previously known as Bacillus megaterium (Gupta et al. 2020), *Priestia megaterium* acts as a model bacterium for genetic studies and recombinant protein manufacturing (Vary 1992; Vary 1994). It has a large size of up to 2.5×10 µm owing to its name "megaterium" which means "big beast" as it has a considerably great size compared to that of *Escherichia coli* (Vary et al. 2007). They are Gram-positive in nature, rod-shaped, aerobic, and form endospores and their genome have a low G+C (~38%) content extensively circulated in environments such as soil, seawater, sediments, rice paddies, dried food, honey, and milk (Lee et al., 2016).

Several Bacillus species along with *Subtilis* and *Cereus* clade consist of a total of 17 new individual clades based on conserved signature indels and it was proposed that these clades should be documented as new genera, with the term Priestia gen. nov. for the Megaterium clade comprising of the former *Bacillus* species *B. megaterium*, *B. abyssalis*, *B. aryabhattai*, *B. endophyticus*, *B. filamentosus*, *B. flexus*, and *B. koreensis* owing to the two CSIs in the oligoribonuclease NrnB which were exclusively common by all clade members (Gupta et al. 2020). *P. megaterium* can be found in various products including honey (López and Alippi 2009), wine (Cosmos et al. 2017), raw meat (Yucel et al. 2009), fish (Al Bulushi et al. 2010), and also in habitats like sea water (Xu et al. 2014), the oral cavity of humans (Al-Thubiani et al. 2018) sediments, rice paddies, dried food milk as well as in endophyte (Salgaonkar et al., 2013) and most typically plants and soil (Dobrzanski et al. 2018). Therefore, their metabolism is modified to diverse carbon sources including xylose, which is a byproduct of hemicellulose, glycerol (Korneli

et al. 2013), disaccharides such as cellobiose, maltose or sucrose (Youngster et al. 2017), and a series of cheap mixed saccharide sources such as sugarcane molasses (Kanjanachumpol et al. 2013).

They are utilized in several fields because of their superiority in various areas, such as they are environmentally beneficial, avirulent to humans and animals, minor generation time, simple nutritional requirements, and robust stress resistance (Nascimento et al., 2019) and mostly in agriculture. *B. megaterium* has been widely studied as a biofungicide, biofertilizer, enhancer in plant growth, plant growth-promoting rhizobacteria (PGPR), and broad-spectrum biocontrol agent in the agriculture industry (Chakraborty et al., 2014), which can possibly improve the soil microbial network and cause a drop in the quantity of soil-borne plant pathogens. Moreover, isolated active antibacterial constituents could be applied to the soil to enhance the development of plants and cause a reduction in the number of plant pathogens (Ryan et al., 2013).

Like its adaptive behaviour, *B. megaterium* has a multipurpose than commercial usage as they are identified to yield vitamin B12, oxetanocin which is an anti-viral agent, and penicillin amidase as well as its use in AIDS diagnostics and as a host to direct foreign proteins without being degraded (Vary, 1994; Morita et al., 1999). They are also well known in the field of agriculture for the promotion of plant development and biocontrol ability against pathogens and they frequently appear in rhizospheres and phylloplanes owing to their capability to endure and flourish in different environments and have revealed the possibility to hinder the growth of plant diseases, happening on both the roots and aerial parts of the plant (Islam and Nandi, 1985; Liu and Sinclair, 1992; Bertagnolli et al., 1996; Pengnoo et al., 2000; Chiou and Wu, 2001; Jock et al., 2002; Wiwattanapatapee et al., 2004; Jung and Kim, 2005). They are non-pathogenic to humans (Vary, 1992, 1994; Vary et al., 2007). and can be tremendously resistant to heat, chemicals, and UV radiation, and therefore support the endurance of the bacterium in nature (Roberts and Hitchins, 1969). By the production of spores, it improves the possibility of the biocontrol ability of the bacterium (Rhodes, 1993; Fravel and Larkin, 1999; Emmert and Handelsman, 1999).

Bacillus spp., together with *B. megaterium*, form a popular source of biocontrol agents because of antagonistic or antibacterial activity to counter phytopathogens (Quigley, 2010; Fira et al., 2018) due to the making of inhibitory metabolites as a mechanism. For example, *B. subtilis* is used in the isolation of surfactin and it established the inhibitory properties to counter plant pathogenic fungi such as *Botrytis cinerea, Sclerotinia sclerotiorum, Colletotrichum gloeosporioides* (Płaza et al., 2013). When 2,5-Diketopiperazines were secreted from *Bacillus* sp. N strain, it displayed a surge in antimicrobial activity to combat plant pathogenic fungi (Nishanth et al., 2012).

An essential characteristic that *B. megaterium* has is the extraordinary aptitude for the large-scale synthesis of vitamins and proteins. The *B. megaterium* strain was isolated by cultivating environmental samples in (Nutrient Agar) NA media and then picking colonies that exhibit the required traits. Furthermore, the use of LB (Luria-Bertani) media for the isolation of strains of IBBPo17 of *B. megaterium* will be covered. Cell lysis techniques are employed to disrupt the robust cell wall and membrane of B. megaterium that act as a barrier and release the cellular contents. Enzymatically the cell can be disrupted by enzymes like lysozyme that specifically cleaves the peptidoglycan layer in the bacterial cell wall. By incubating *B. megaterium* cells with lysozyme, the cell wall is weakened, allowing for efficient cell disruption (Renganathan et al., 2011).

Mechanically the cell can be disrupted by bead beating which employs high-speed agitation with small beads (glass, ceramic, or plastic) in a bead beater. The forceful collisions between the beads and cells lead to cell wall breakage and the release of intracellular components. Certain detergents can also disrupt the cell membrane by solubilizing phospholipids. Soluble protein fractions can be isolated by centrifugation. The lysed cell suspension is subjected to high-speed centrifugation which separates the heavier cell debris (pellet) from the supernatant containing the soluble proteins. The supernatant, enriched with the desired proteins, can then be further processed for purification.

After the proteins of interest are extracted, the review will investigate techniques such as ion exchange chromatography, gel filtration chromatography, and ammonium sulfate precipitation to further purify the proteins. Ion exchange chromatography separates proteins based on their net surface charge at a specific pH. It utilizes a stationary phase containing charged functional groups (anion or cation exchangers) that interact electrostatically with oppositely charged protein groups. Proteins with different isoelectric points (pI) will exhibit varying affinities towards the stationary phase, allowing for their separation. Gel filtration chromatography also known as size exclusion chromatography separates proteins based on their size and shape. The stationary phase consists of porous beads with defined pore sizes. Larger molecules are excluded from these pores and elute first, while smaller molecules penetrate the pores and elute later. Ammonium sulfate precipitation is a simple and cost-effective salting-out technique for protein precipitation which utilizes the principle of decreasing protein solubility with increasing ammonium sulfate concentration.

In downstream applications, protein concentration is an essential benchmark by Coomassie Brilliant Blue G-250 based assay (Konstantinos et al., 2015). The CBB G-250 assay relies on the ability of the dye, Coomassie Brilliant Blue G-250, to bind non-covalently to basic and aromatic amino acid residues in proteins. This binding alters the dye's spectral properties, resulting in a shift in its absorbance spectrum between 562-595 nm. The intensity of the color change at the specific wavelength for protein-bound dye is directly proportional to the protein concentration in the sample. Bacillus megaterium is a useful source of numerous products that are significant to the global economy. The different enzymes that this bacterium produces, including vitamin B12, glucose dehydrogenase, amylases, and penicillin amidase, will be discussed in this review along with their varied uses in different areas (Narendra and Lingayya, 2019). This review highlights the immense potential of *B. megaterium* for a range of biotechnological applications by offering a thorough overview of biocontrol abilities and techniques for protein production, purification, quantification, and the wide range of commercially significant products obtained from this bacterium.

II. BIOCONTROL ACTIVITY

A biological control agent (BCA) is typically a fungus, bacteria, virus, or a combination of the same, in plant pathology, which applies to the use of microbial antagonists to overpower diseases. They are beneficial as they are extremely specific for a pathogen and are harmless for non-target species (Vásconez et al., 2020). *B. megaterium* has also garnered significant attention for its biocontrol activity against several disease-causing bacteria and fungi.

2.1. Against fungi

When water-soluble preparation of *B. megaterium* was used against some of the diseases of the rice variety called Pitsanulok-2, it caused a reduction in the occurrence of sheath blight, lesion/leaf, and dirty panicle. It was also capable in dropping the incidence % of the disease after only a few sprays (Kanjanamaneesathian et al., 2009). Moreover, when volatiles yielded by *B. megaterium* KU143 were used to counter *Aspergillus flavus* and aflatoxin production on stockpiled rice grains, it strikingly decreased the growth area of the mycelia and curbed the *A. flavus* AF57 significantly by repressing the % of its conidial germination. Likewise, when investigating the effects of *B. megaterium* KU143 on *A. flavus* AF57 on unhulled rice grains, it demonstrated that it decreased the fungi. When the effect of the volatile on aflatoxin production was inspected on unhulled rice grains with the aflatoxigenic *A. flavus* KCCM 60330, it decreased the whole aflatoxin manufacture (Manna et al., 2018).

Similarly, when a strain of marine *Bacillus megaterium* was assessed for its activity in combating postharvest deterioration of peanut kernels triggered by *Aspergillus flavus*, the outcomes exhibited that the concentrations of antagonist had a significant outcome on biocontrol effectiveness *in vivo*: when the concentration of the washed bacteria cell suspension was used at 1×10^9 CFU/ml, the rate percentage of rot of peanut kernels was less (31.67%±2.89%). The observations also displayed that unwashed cell culture as well as the washed cell suspension of *B. megaterium* showed similar effectiveness and an enhanced biocontrol was achieved when longer incubation time was applied. At the incubation time of 60-h, the rate of decay deteriorated to 41.67%±2.89% (Kong et al., 2010). In another case, lipopeptides resulting from *Bacillus megaterium* WL-3 strain contained three subfamilies: Surfactin (C13– C15), Iturin A (C14 – C16), and Fengycin A (C15 – C19) upon Electrospray Ionization Mass Spectrometry (ESI-MS). The Iturin A and Fengycin A lipopeptide families were a long-established anti-oomycete against *P. infestans* mycelium growth as well as combating potato late blight in greenhouse experiments and field assays. Besides, Iturin A and Fengycin A were able to stimulate the efficiency of photosynthesis in plants, growth in plants, and production of potatoes. Above all, the amalgamation of Iturin A and Fengycin A (I + F) was greater than distinct lipopeptides in regulating the subjects mentioned above (Wang et al., 2020). Similarly, Iturin A2 is an anti-Rhizoctonia solani peptide was reportedly isolated from *B. megaterium* B196 (Qin, 2013). 12-hydroxyjasmonic acid, an active monomeric compound, was isolated from *B. megaterium* LB01 and it has a suppressing effect against *C. gloeosporioides* (Ding et al., 2020). Furthermore, *B. megaterium* L2 was also a source of erucamide, behenic acid, palmitic acid, phenylacetic acid, and b-sitosterol which displayed positive antibacterial activities on the tested bacteria. Amongst which, phenylacetic acid showed high antibacterial activity against all the tested bacteria (Xie et al., 2021).

The eluted contents of crude extract from *B. megaterium* L2 showed inhibitory activity to counter *A. tumefaciens*, *E. carotovora*, *and R. solanacearum*, which are some common plant pathogens (Ji et al., 2019). *Bacillus megaterium* (strain MKB135)] also showed its capability to regulate septoria tritici blotch (STB) caused

by *Mycosphaerella graminicolais* on adult wheat plants where it constantly slowed down the growth of STB by up to 80%. Furthermore, in vitro seedling studies displayed that both cell wall constituents and culture filtrate of *B. megaterium* had the potential to hinder disease expansion by 62 and 36 % individually (Kildea et al., 2007). The strain TRS-4 can encourage the development of tea plants and induce resistance to brown root rot disease caused by Fomes lamaoensis (Murr.) Sacc. and Trott (Chakraborty et al., 2006).

2.2. Against Bacteria

Upon disk-diffusion technique, the antibacterial action of *P. megaterium* KD7 was determined to counter *E. amylovora* in which the cell-free supernatant (CS) displayed better antimicrobial action in comparison to the bacterial cell disruption (BD), demonstrating that the extracellular secondary metabolites secreted are the reason behind its antibacterial activity. Four extracts with methanol (EM), n-hexane (EH), ethyl acetate (EEA) and n-butanol (EB) from strain KD7 were prepared to check growth inhibition activity against *E. amylovora*. Following incubation, only the bacteria culture broth (BC) and methanol extracts (EM) displayed an antibacterial activity due to the presence of amino acids in methanol extract by TLC analysis (Cui et al., 2023).

2.3. Against nematodes

The bacteria from the rhizosphere that is from *Bacillus* viz. *B. subtilis*, *B. megaterium*, and *B. pumilus* have revealed nematicidal activity against root-knot nematodes *Meloidogyne* incognita as well as enhance the growth parameters of sugar beet (Youssef et al. 2017). Similarly, when *B. megaterium* is added with nemastrol, humisun, dried sweet basil, and oxamyl, it made systemic resistance against Meloidogyne spp. in sugar beet wherein the population of nematode densities in 250 g soil and a number of females (1 g /root) were curbed with single and concomitant applications and a reduction percentage in final nematode population ranged from 48.2 to 95.7% (Mostafa et al., 2018). The other examples of the biocontrol ability of B. megaterium are listed below in Table 1.

Table 1. *B. megaterium* is a biocontrol agent against various fungi, nematodes, and insects.

	Test organism	**Crop/feeds**	**Target organism**	**References**
Fungi	*Bacillus megaterium*	Poultry feeds	*Aspergillus flavus*	Djellel and Larous et al., 2018
	B. megaterium in granulated formulations	Rice	*Rhizoctonia solani* Kuhn	Chumthong et al., 2008; Wiwattanapatapee et al., 2013
	Bacillus megaterium A6	Oilseeds	*Sclerotinia sclerotiorum*	Hu et al., 2013
	Bacillus megaterium strain B153-2-2	Soybean	*Rhizoctonia solani* isolate 2B12 (ISG-2B).	Zheng and Sinclair et al., 2000
	Bacillus megaterium KL39	Red pepper	*Phytophthora capsici*	Jung et al., 2005

	Bacillus megaterium	Tea	*Sclerotium rolfsii*	Chakraborty et al., 2015
	Bacillus megaterium	Greenhouse and nursery crops	*Rhizoctonia, Pythium, Fusarium*, and *Phytophthora*	Zheng et al., 2013
Insect	*Bacillus megaterium* isolate Bmin23	Apple	*Aphis pomi* De Geer	Aksoy and Sullivan et al., 2008
	Bacillus megaterium	-	*Culex quinquefasciatus*	Aksoy and Sullivan et al., 2008
	Bacillus megaterium	-	*Culiseta longiareolata*	Aksoy and Sullivan et al., 2008
Nematodes	*Bacillus megaterium*	Sugar beet	*Heterodora schachtii*	Aksoy and Sullivan et al., 2008
	Bacillus megaterium	Potatoes	*Meloidogyne chitwoodi* Golden, O'Bannon, Santo, & Finley and *Pratylenchus penetrans* Cobb	Aksoy and Sullivan et al., 2008
	Bacillus megaterium	Rice	*Meloidogyne graminicola*	Aksoy and Sullivan et al., 2008
	Bacillus megaterium	Tomato	*Meloidogyne incognita*	Singh and Siddiqui, 2008
	Bioarc (*Bacillus megaterium*)	Sunflower	*Meloidogyne javanica*	Hammad et al., 2007
	Bacillus megaterium	Tomato and Pepper	*Meloidogyne incognita*	Peregrín et al., 2012

III. ISOLATION OF *Bacillus megaterium* STRAINS

A 250 mL Erlenmeyer flask containing 25 ml of nutritional broth was autoclaved for 20 minutes at 121º C and 15 psi. Following sterilization, the medium was allowed to cool to room temperature before being infected with a loopful of *Bacillus megaterium* and incubated for 24 hours at 35º C with 120 rpm of agitation (Zeeshan et al., 2016).

The L2 strain was grown on NA (Nutrient Agar) to generate active bacteria for 48 hours at 30° C. To prepare seed broth, colonies were sub-cultured twice, or three times before being injected into NB (Nutrient Broth) medium and incubated for 20 hours at 30° C and 150 revolutions per minute. (Yudan et al., 2021) [10] For large-scale cultivation, the seed broth was moved into a 400-liter fermentation tank with 80 L of NB medium. The tank was then ventilated at a rate of 1.5 L/min and 150 r/min for 48 hours at 30º C (Ji et al., 2018).

LB (Luria-Bertani) medium was infected with the *Bacillus megaterium* strain IBBPo17. The flask was incubated on a rotary shaker (200 rpm) for 24 hours at 30°C. To harvest the cells of the bacteria centrifuge it, and then wash it two times. The resulting cell pellets were then resuspended (OD660, 0.07) in basal medium (pH 7.2) complemented with either 0.5% carbon (starch, cellulose) or 0.1% nitrogen (yeast extract, protease peptone) resources. Lastly, long-chain n-alkane was incorporated into the cell suspensions, such as 5% n-hexadecane. The flasks have been sealed and placed on a rotary shaker (200 rpm) and incubated for 72 hours at 30°C. Using a previously developed process by Darsa et al., the estimation of carbon dioxide (CO2) generation was also used to track the development of the cells (Mihaela, 2022).

IV. PROTEIN PRODUCTION AND EXTRACTION

Samples of *Bacillus megaterium* from the overnight culture can be frozen in LB medium with 25% glycerol at a temperature of -80°C. Individual portions of LB medium with 6.8 µM erythromycin should be inoculated with aliquots (1% of the culture volume) of the aerobically developed overnight culture. The cells should be allowed to grow at 30°C in an aerobic environment until they reach an OD578 of 1, which indicates a cell density of roughly 109 cells/mL. (Heiko et al., 2005) [12] Then add 33 mM xylose to stimulate xylA promoter-driven gene expression. For every distinct protein expression, the xylA promoter's induction period needs to be adjusted. The ideal expression was attained at OD578 0.3–1.5 cell densities. Regular variations in the xylose content ranged from 0.5% to 1.5%. After 3-5 hours gene expression is induced, and protein synthesis reaches its highest level. Harvest the cells by centrifugation at 4500g for 15 min at 4ºC. Resuspend the pellet in 1–2 volumes of lysis buffer (20 mM ethylenediaminetetraacetate disodium salt (EDTA), 100 mM Na3PO4, and 5 mg/mL lysozyme, and adjust to pH 6.5 with H3PO4). Incubate the tubes at 37ºC for 30 minutes. Examine under a microscope the extent of lysis. Crush the protoplasts by intense shaking. Take samples for protein analysis and OD578nm measurement every 30 to 60 minutes (up to 6 hours after induction). To analyze extracellular proteins, take 2 ml of the cell culture. A sample volume larger than 2 ml is needed for intracellular protein analysis. To extract the cells and cell-free supernatant from each sample, centrifuge it. Store the cell-free supernatant for extracellular protein analysis at 4° C, and freeze the cell pellet at -20 °C for intracellular protein analysis. The lysate is further examined by standard molecular biology techniques such as sodium dodecyl sulfate– polyacrylamide gel electrophoresis (SDS-PAGE) (Sambrook et al., 1989).

2.1 Analysis of Intracellular Proteins

Resuspend the cells in 30 µl of lysis buffer. Using a thermomixer, shake the cells at 1,000 rpm for 30 minutes at 37 °C, for efficient lysis of the cells shake the samples at regular intervals of 10 minutes. Centrifuge the cell lysate at 13,000 rpm for 30 minutes at 4 °C. Add 13 µl of SDS sample buffer into 27 µl of the soluble protein supernatant. Discard the supernatant and add the pellet back into 30 µl of 8 M urea. Centrifuge at 13,000 rpm for 30 minutes at 4 C. Add 13 µl of SDS sample buffer into 27 µl of the supernatant. For 5 minutes, heat each sample at 95 °C. Load 7.5 µl of every sample onto a gel made of SDS-PAGE.

V. PROTEIN ESTIMATION

5.1 2 N HCl: Prepare by mixing the highly concentrated 12 N HCl 6 times with ddH2O.

5.2 CBB-2 N reagent: The CBB reagent is made by dissolving 60 mg CBB in 100 ml 2 N HCl by mixing for 40 minutes and eliminating particulates that are not dissolved either through centrifugation (15,000 g at RT for 10 minutes) or through filtering and protect it from light. Before use, dilute the reagent 2 times with 2 N HCl [CBB 2 N:2 N HCl (1:1) reagent].

5.3 1 mg BSA ml^{-1}: Prepare by dissolving 1 mg BSA in 1 ml ddH2O. For the standard curve, dilute this stock to several standards with concentrations of 1–40 µg BSA ml^{-1}.

5.4 Standard curve construction: In ddH2O, prepare a set of BSA standard solutions that vary in protein concentrations (1–40 µg BSA ml^{-1}). In a microtiter plate, add 200 µl of BSA solution each with 50 µl of the CBB 2 N:2 N HCl (1:1) reagent. Add 200 µl of ddH2O for reagent blank instead of protein lysate. Additionally, add 250 µl of ddH2O in a few wells to establish the microtiter plate's baseline as a blank. Incubate for 5-10 minutes at RT and determine the absorbance of every assay mixture at 610 and 470 nm. Then, establish a standard graph using either the absorbance ratio 610/470 nm (the absorbance of the reagent blank for the zero-protein sample) or a total absorbance at 610 nm (against the reagent blank).

5.5 Calculate the protein concentration in the sample: Add 200 µl of the diluted sample (S) with 50 µl of CBB 2 N:2 N HCl (1:1) reagent, then compare the absorbance at 610 and 470 nm to a reagent blank that has sample buffer or ddH2O instead of the sample. Additionally, add 250 µl of ddH2O in a few wells to establish the microtiter plate's baseline as a blank. Measure the absorbance of the assay solutions at 610 and 470 nm in a spectrophotometer after 5 to 10 minutes of incubation at RT (Konstantinos et al., 2015).

VI. PURIFICATION OF PROTEASE

6.1 Ammonium sulfate precipitation

A 500 ml aliquot of crude enzyme preparation was subjected to ammonium sulfate precipitation with a saturation gradient of 0-85%, employing slow, continuous stirring while maintaining a chilled environment. The resulting protein pellet was resolubilized in 0.05 M Tris-HCl buffer (pH 10.0) and subsequently dialyzed against the same buffer to remove residual salts. The dialyzed protein solution was then concentrated via lyophilization for further purification steps.

6.2 Sephadex G-100 gel filtration

Overnight, Sephadex G-100 chromatographic media was left to swell in sterile distilled water. Non-sedimented gel beads were separated, and the gel slurry was filled into a glass column sized 100 × 2.5 cm, having a sintered filter at the base. Avoid air bubbles while packing. The packed column was brought to pH 10.0 using 0.05 M Tris-HCl buffer. The dialyzed protein mixture was put onto a Sephadex G-100 column and allowed to elute at a flow rate of 3.0 ml every 15 minutes with 0.05 M Tris-HCl buffer at pH 10.0. The activity of proteins and proteases was measured. The portions exhibiting protease activity were mixed, dialyzed, and then concentrated via lyophilization (Renganathan et al., 2011).

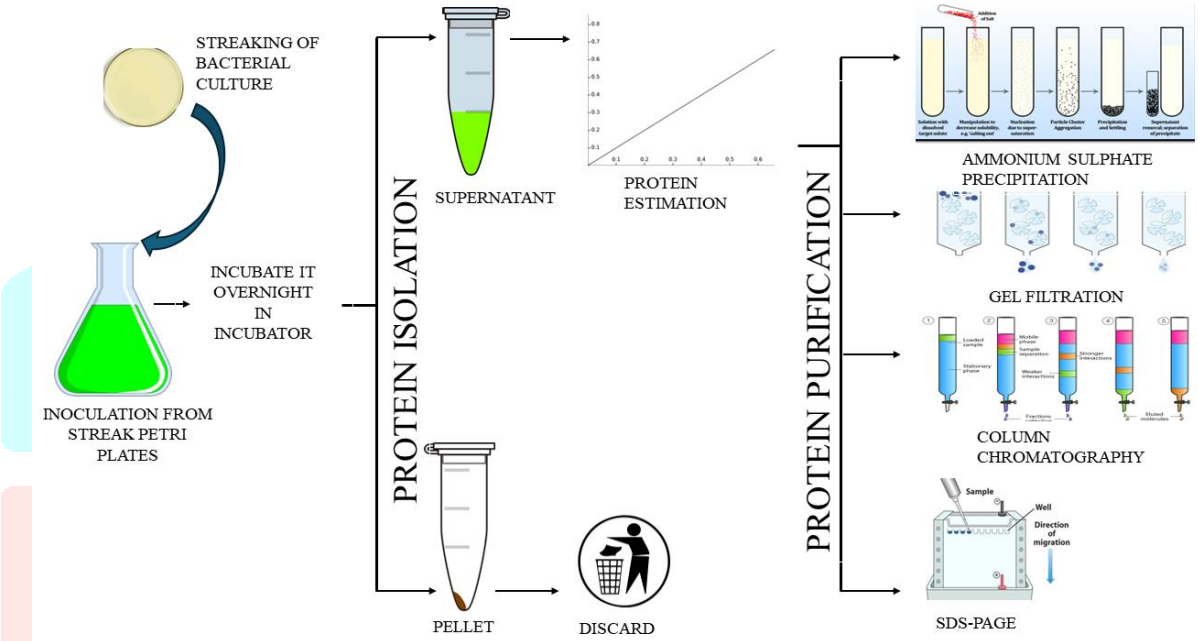
6.3 Q-Sepharose column chromatography

The concentrated protein sample was filled into a Q-Sepharose ion-exchange chromatography column (dimensions: 10 cm x 1.6 cm), and washed with 0.05 M Tris-HCl buffer (pH 10.0) to remove unbound material. In the same buffer, the bound enzyme was eluted out by a linear gradient of sodium chloride (NaCl) from 0 M to 0.5 M. An automatic fraction collector was used to gather 3ml fractions every 12 minutes. Protein concentration in each fraction was monitored by measuring the absorbance at 280 nm. Protease activity was assayed for all collected fractions. Fractions exhibiting protease activity were mixed, dialyzed against the same buffer, concentrated, and finally stored at 4°C for subsequent experiments.

6.4 Polyacrylamide gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was employed to assess the purity and estimate the molecular weight of the purified enzymes. The methodology was described by Laemmli, 1970, utilizing a 5% stacking gel and a 12%resolving gel. Samples were prepared by mixing aliquots of the purified enzymes with glass-distilled water in a 1:5 ratio. The sample buffer contained 10 mM Tris-HCl (pH 8.0), 2.5% SDS, 5% β-mercaptoethanol, and 0.002% bromophenol blue. Before electrophoresis, samples were heated at 100°C for 5 minutes. Alongside the purified protein samples, a concurrent SDS-PAGE run was performed using standard protein markers including phosphorylase b (97.4 kDa), bovine serum albumin (66.0 kDa), ovalbumin (43.0 kDa), carbonic anhydrase (29.0 kDa), soybean trypsin inhibitor (20.1 kDa), and lysozyme (14.3 kDa). Following electrophoretic separation, the gel was stained with silver nitrate using the protocol established by Blum et al., 1987.

Fig.1. Inoculation, protein extraction, protein estimation, protein purification.



VII. COMMERCIALLY IMPORTANT PRODUCTS OF *Bacillus megaterium*

a-amylase, P-amylase, penicillin amidase, neutral protease, P-glucanase, megacins (the phospho-lipase MegA), glucanotransferase, and chitosanases belong to the enzymes released by *B. megaterium* (Vary, 1992). This species may also generate special enzymes like alphostatin, which reduces calf alkaline phosphatase, an epoxide hydrase, an isomerase for maleylpyruvate, and α-glucanase that is employed as an indicator for morphological investigation of yeast cell walls (Narendra and Lingayya, 2019). Table 1 describes different enzymes that are produced by *Bacillus megaterium* and some of the applications of those enzymes.

- Amylase: *Bacillus megaterium* generates two types of amylases: α-amylase and β-amylase. *Bacillus megaterium* amylases serve a purpose in the bread industry to break down branched saccharides into a structure that hydrolyzes glucoamylases (Akinfemiwa et al., 2023).
- Glucose dehydrogenase: Numerous glucose dehydrogenase (GDH) genes from *B. megaterium* species have been recognized in laboratories in Germany and Japan. It frequently serves as a biosensor and produces NADH in industrial operations due to its ease of immobilization (Kittsteiner-Eberle et al., 1989).
- Penicillin amidase: Penicillin acylase, also known as amidase, is utilized to break down the side-chain of penicillins to produce novel synthetic antibiotics (Krishika and Rahul, 2021).
- Vitamin B12: The primary aerobic source of cobalamin, known as vitamin B12, is *B. megaterium*. It is produced by the haem-biosynthesis (Rebekka et al., 2009).

- Oxetanocin and other antimicrobial agents: *B. megatrium* yields a few antibiotics, such as emimycin (Vary, 1992), but the most interesting one is a special analog antibiotic called oxetanocin, that acts upon a wide range of significant pathogenic viruses, involving thymidine kinase that is not generated (Kohlbrenner et al., 1990).
- Bioremediation: *B. megaterium* can act as a detoxifying agent because it is found in strange and occasionally harmful conditions. According to Quinn et al. (1989), *Pseudomonas* and *B. megaterium* have carbon-phospholyase that splits C-P bonds, enabling the two species to break down 14 of the 15 C-P herbicide substances examined.

Table 1: Different types of enzymes produced by *B.megaterium* and its application

ENZYMES	USE	REFERENCES
α amylase	● removing starch after weaving, thus making the fabric softer and more comfortable. ● creating specific textures in fabrics by partially degrading the starch content in specific areas called biopolishing.	Nadia et al., 2012
β amylase	fruit development, ripening, seed germination, and abiotic stress response.	Niu et al., 2018
Glucose dehydrogenase	Biosensors and bio-fuel cells.	Krzysztof et al., 2020
Penicillin amidase	Production of synthetic antibiotics.	Krishika and Rahul, 2021
Vitamin B12	● DNA synthesis. ● Amino acid metabolism. ● Enables the bacteria to utilize ethanolamine as a source of carbon and nitrogen.	Roof and Roth 1989; Wolf and Brey 1986
Oxetanocin	Bacterial metabolite, an antibacterial agent, and an anti-HIV agent.	Aoshu et al., 2021
Bioremediation	Oil degradation in saline conditions.	Xiaoyan et al., 2022

VIII. CONCLUSION

Priestia megaterium is a model bacterium for genetic studies and recombinant protein manufacturing. It has a large size of up to 2.510 m and is gram-positive, rod-shaped, aerobic, forms endospores and their genome has a low G+C content extensively circulated in environments such as soil, seawater, sediments, rice paddies, dried food, honey, and milk. It has been widely studied as a biofungicide, biofertilizer, enhancer in plant growth, plant growth-promoting rhizobacteria (PGPR), and broad-spectrum biocontrol agent in the agriculture industry. It has been identified to yield vitamin B12, oxetanocin, and penicillin amidase, as well as its use in AIDS diagnostics and as a host to direct foreign proteins without being degraded. It is also well known in the field of agriculture for the promotion of plant development and biocontrol ability against pathogens and has revealed the possibility of hindering the growth of plant diseases.

IX. ACKNOWLEDGMENT

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