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A Rapid and Reproducible Protocol for Protein Extraction from Actinomyces

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We use this protocol and it's working

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Abstract

Actinomyces are filamentous, Gram-positive bacteria with medical and biotechnological significance, particularly as producers of enzymes and bioactive metabolites. Protein extraction from these organisms is difficult because of their slow growth, aggregation behavior, and complex cell wall composition. Rapid and reliable extraction protocols are essential for downstream biochemical and proteomic studies. Cultures were grown in starch casein nitrate (SCN) broth for 8–10 days at 30 ± 2 °C under agitation (100 rpm). Biomass was harvested by centrifugation and repeatedly washed with phosphate-buffered saline (PBS, pH 7.0) to eliminate residual media components. Two critical steps were incorporated to improve protein recovery: (i) treatment of the washed pellet with chilled acetone, which precipitates proteins and removes interfering compounds, and (ii) solubilization of the acetone-treated pellet with 1% sodium dodecyl sulfate (SDS), which disrupts the robust actinomyketal cell wall and facilitates protein release. Extracts were stored at −20 °C for short-term use and lyophilized for long-term preservation. The combined acetone precipitation and SDS solubilization strategy yielded protein extracts of sufficient purity and concentration for electrophoresis, enzyme assays, and proteomic applications. This rapid method provides a reproducible and scalable approach for protein extraction from Actinomyces, overcoming key challenges associated with filamentous bacterial growth and cell wall resilience.

Image Attribution

Figure 1. Instrumentation used for the optimal culture growth and cell separation.

Figure 3. Protein characterization using 10% SDS-PAGE gel, silver nitrate staining.

Guidelines

Note: the supernatant was transferred to a fresh tube, decontaminated and discarded using WHO biosafety hazardous guidelines.

Materials

Conical flask, Non-absorbent cotton, SCN broth, 50mL sterile centrifuge tubes, Acetone (Ice-cold), 1% SDS solution.

i. Starch Casein Nitrate broth (Media composition per liter, pH: 7.0±0.2)

Starch: 10.0 g
Casein: 0.30 g
Soya peptone: 10.0 g
Potassium nitrate (KNO_3): 2.0 g
Magnesium sulfate ($MgSO_4$): 0.050 g
Di-potassium hydrogen phosphate (K_2HPO_4): 2.0 g
Sodium chloride (NaCl): 2.0 g
Calcium carbonate ($CaCO_3$): 0.20 g
Ferrous sulfate ($FeSO_4$): 0.010 g
Ampicillin: 10 mg
Nystatin: 25 mg
Double distilled water: 1000 mL

ii. 0.05M Phosphate buffered saline (PBS) pH 7.0±0.2.

Sodium dihydrogen phosphate (NaH_2PO_4): 2.231 g
Disodium hydrogen phosphate (Na_2HPO_4): 11.555 g
Double distilled water: 1000 mL

Note. Adjust the pH using 6 M HCl or 6 M NaOH to obtain pH 7.0.

iii. 1% SDS (Sodium Dodecyl sulfate)

Sodium Dodecyl sulfate – 1.0 g
Double distilled water – 100 mL

Procedure

1 Retrieve the Actinomyces culture pellet from the SCN (Suggested optimal growth measured at 600 nm OD of 0.6-0.8) broth incubated for 8-10 days at 30±2°C, 100 rpm. Culture pellet was separated using cooling centrifuge at 7000 rpm for 10 mins at 4 °C.

2 Separate the culture pellet (Tube A) and spent media (Tube B) (Note: the supernatant was transferred to a fresh tube, decontaminated and discarded using WHO biosafety hazardous guidelines).

3 

Figure 1. Instrumentation used for the optimal culture growth and cell separation.

4 The culture pellet washed 2-3 times using 0.05M PBS, pH 7.0 (Note: Free from chelating element), re-suspended in PBS buffer and recentrifuged at 7000 rpm for 10 mins at 4 °C.

5

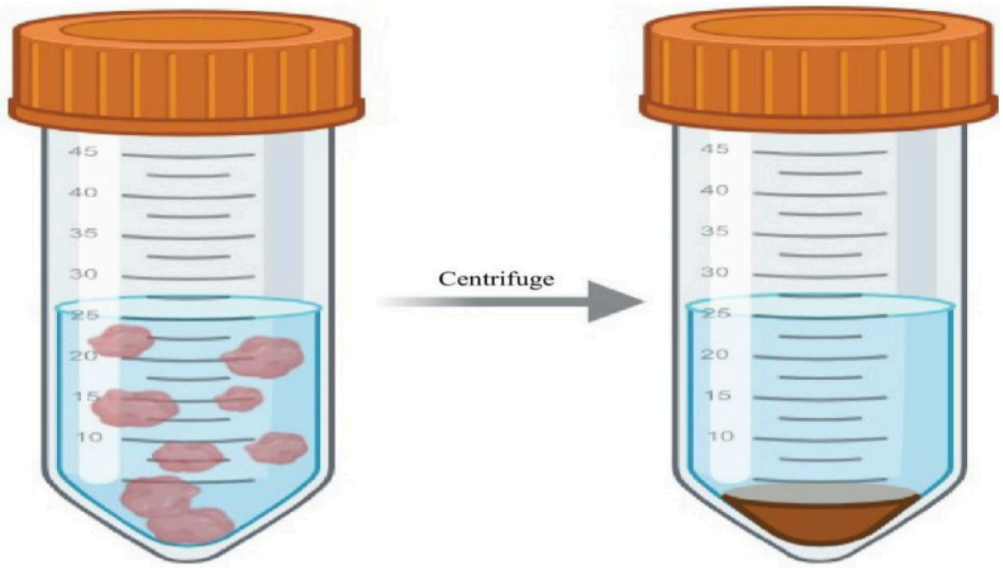

Figure 2. Diagrammatic representation of cell separation.

Procedure

6 Discard the supernatant and add 10 mL of chilled Acetone solvent to culture pellet kept for 15-30 min. Residual Acetone solvent was removed using steam of nitrogen.

7 Add 1 mL of 1% SDS was incubated for 2 min, yielded protein extracts of sufficient purity and concentration for electrophoresis, enzyme assays, and proteomic applications, kept under -20 °C for short term shortage and lyophilized for further use.

8 The Protein was quantified using Bradford assay with BSA standard graph showed 224 mg per gram dry weight of cells.

Result

9 Linear regression: Absorbance = 0.0022 × [Protein] (µg/mL) + 0.001 (R^2 ≈ 0.999)

10 Absorbance at 595 nm = 0.495 (in triplicates)
From regression: [Protein] = (0.495 – 0.001) ÷ 0.0022 ≈ 224 µg/mL
Extract volume measured: 1 mL
Protein amount = 224 µg ≈ 0.224 mg
Cell dry weight used for extraction: 1 mg (0.001 g)
Therefore: 0.224 mg/g = 224 mg/g

Protein Characterization using SDS-PAGE

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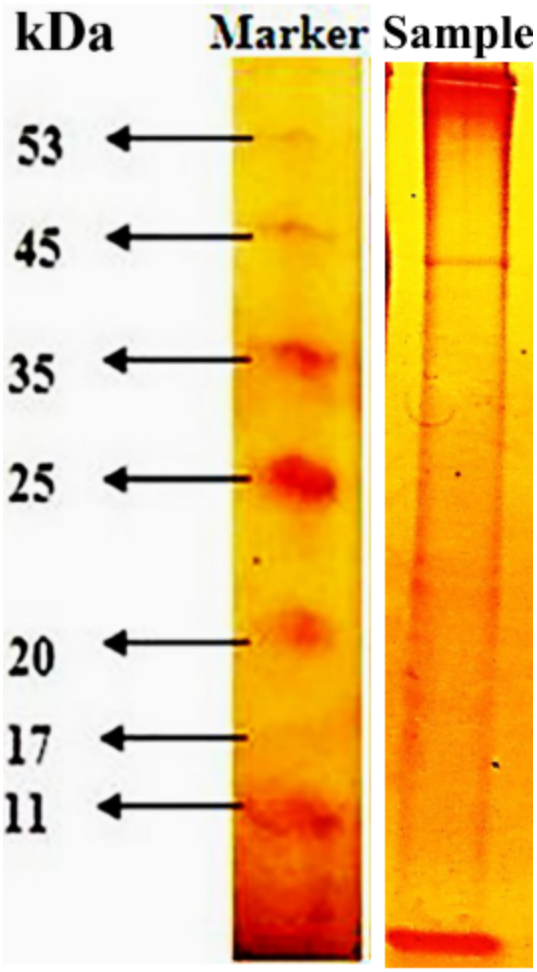

Figure 3. Protein characterization using 10% SDS-PAGE gel, silver nitrate staining.

12 Molecular weight marker: Bands are clearly visible at ~53, 45, 35, 25, 20, 17, and 11 kDa, which serve as reference standards.

13 Sample lane: A dominant intense band is observed at the top of the gel (~45 kDa), suggesting the presence of a major protein of approximately 45 kDa. A faint band is also visible slightly lower (~17-25 kDa), which may represent a degradation product or a less abundant protein. Minimal or no other strong bands are seen, indicating that the sample is relatively homogeneous.

Protocol references

Babu, P.M., Panda, N., Nayak, R.K., Sethi, D., Biswal, S., Mishra, M.K., Datta, S., Karubakee, S., Suchitha, N.S., Prusty, M., Nayak, A., Dash, R., Pattanayak, S.K., 2025. Isolation, characterization and screening of phosphate (P) solubilizing actinomycetes and exploring its potency in finger millet (Eleusine coracana L.). BMC Plant Biol 25, 362. https://doi.org/10.1186/s12870-025-06385-1

Bhaduri, S., Demchick, P.H., 1983. Simple and rapid method for disruption of bacteria for protein studies. Applied and Environmental Microbiology 46, 941–943. https://doi.org/10.1128/aem.46.4.941-943.1983

Burdock, T., Brooks, M., Ghaly, A., Dave, D., 2011. Effect of assay conditions on the measurement of dehydrogenase activity of Streptomyces venezuelae using triphenyl tetrazolium chloride. Advances in Bioscience and Biotechnology 02, 214. https://doi.org/10.4236/abb.2011.24032

Jiang, Y., Li, Q., Chen, X., Jiang, C., Jiang, Y., Li, Q., Chen, X., Jiang, C., 2016. Isolation and Cultivation Methods of Actinobacteria, in: Actinobacteria - Basics and Biotechnological Applications. IntechOpen. https://doi.org/10.5772/61457

Karthik, Y., 2022. Bioactive peptides from Mangrove Soil Actinomycetes and characterization for antimicrobial and anticancer activity. Mangalore University.

Zhang, L., Zhang, H., Huang, Y., Peng, J., Xie, J., Wang, W., 2021. Isolation and Evaluation of Rhizosphere Actinomycetes With Potential Application for Biocontrolling Fusarium Wilt of Banana Caused by Fusarium oxysporum f. sp. cubense Tropical Race 4. Front. Microbiol. 12. https://doi.org/10.3389/fmicb.2021.763038

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