

Spray-dried maltodextrin- γ -cyclodextrin microcapsules of garlic essential oil: Release kinetics and application in shelf-life extension of *Nem Chua Hue*

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SUPPLEMENTARY MATERIAL

Supplementary File S1

IAA Production Protocol (Brick *et al.*)

Inoculate bacteria in Luria Bertani (LB) broth supplemented with 0.05-0.5% L-tryptophan and incubate at 28-30°C for 24-48 hours with shaking. Centrifuge culture at 10,000 rpm for 10 minutes, mix 2 ml supernatant with 2 ml Salkowski reagent (50 ml 35% perchloric acid + 1 ml 0.5 M FeCl₃), and incubate 30 minutes for pink color development. Measure absorbance at 530 nm against an IAA standard curve (0-100 µg/ml) prepared similarly, expressing results as µg IAA/ml culture supernatant; normalize to optical density at 600 nm or mg protein if specified. Control uses unsupplemented broth.

IAA Spectrophotometric Standard Curve Preparation

Prepare IAA standards (0-100 µg/mL) in 50% acetic acid or medium supernatant, add Salkowski reagent (0.5 M FeCl₃ in 35% H₂SO₄), incubate 30 min, and plot absorbance at 530 nm vs. concentration for the calibration curve (typical R² > 0.98). Tryptophan supplementation (e.g., 5 mM or 0.2-5 mg/mL) enhances bacterial IAA yield, showing a dose-dependent absorbance increase.

Measurement Protocol

Centrifuge bacterial culture grown with tryptophan (e.g., 72 h at 30°C, pH 7), mix 1 mL supernatant with 2 mL Salkowski reagent, measure OD₅₃₀ after 25-30 min dark incubation, and

quantify IAA via the standard curve. Maximum IAA often occurs at 5 mg/mL tryptophan after 48-72 h.

GA Quantification Protocol (Borrow *et al.*)

Grow isolates in LB broth under drought (PEG 6000) or control conditions for 5-7 days at 28-30°C. Filter culture, acidify supernatant to pH 2.5 with 1 N HCl, and extract three times with equal volumes of ethyl acetate. Evaporate organic phase, dissolve residue in methanol, and measure absorbance at 680 nm against GA3 standards (0-50 µg/ml) for quantification as µg GA/ml. Normalize to culture volume or biomass; confirm via TLC (silica gel, ethyl acetate:hexane solvent) with Rf matching standards.

GA ELISA Standard Curve

Dilute GA3 stock to 10,000, 5,000, 2,500, 1,250, 625, 312.5, 156 pg/mL in sample matrix; add 50 µL/well in triplicate, follow ELISA steps (antibody 1 h, avidin 30 min, TMB 20 min, 450 nm). Fit 4-parameter logistic curve (OD vs. log[GA], $R^2 > 0.95$, dynamic range verified by back-calculation CV <15%). Intra/inter-assay precision CV <10%, recovery 92-108%.

ACC Deaminase Protocol (Penrose and Glick)

Grow bacteria in tryptic soy broth to log phase, harvest by centrifugation, wash with 0.1 M Tris-HCl (pH 7.6), and induce in DF minimal salts medium + 3-5 mM ACC (sole N-source) for 24 hours at 30°C, 200 rpm. Resuspend cells in 0.1 M Tris-HCl (pH 8.5), add 5% toluene for permeabilization (30 s vortex), centrifuge, and mix 100 µl lysate with 950 µl assay mix (100 mM Tris-HCl pH 8.5, 0.5 mM pyridoxal phosphate, 50 µl ACC). Incubate 30 min at 30°C, stop with 1 ml 0.2 M HCl, add 560 µl alkaline-hypochlorite (50 mM NaOH + 2% NaOCl + 0.8% phenol), heat 10 min at 60°C, cool, and read absorbance at 540 nm. Quantify α -ketobutyrate via standard curve (0-1 mM), normalize to mg protein (Bradford assay) as µmol α -KB/mg protein/min.

Ammonia Production Protocol

Grow isolates in peptone water or minimal medium with ammonium source for 48 hours at 30°C. Centrifuge 1 ml culture, add 0.1 ml Nessler's reagent to 1 ml supernatant; yellow-brown color indicates positive ammonia production qualitatively. For quantification, measure absorbance at 420-450 nm against NH₄Cl standards (0-100 µg/ml), expressing as µg NH₃/ml; normalize per mg protein or OD₆₀₀.

HCN Production Protocol (Kremer and Souissi)

Inoculate bacteria on King's B agar amended with 4.4 g/L glycine and overlay with Whatman filter paper saturated in 0.5% picric acid (in 2% Na₂CO₃). Incubate 4-7 days at 28-30°C; orange-to-red color change on paper indicates HCN qualitatively. For quantitative assay, grow in King's B broth + glycine 48 hours, centrifuge, mix supernatant with glycine-picric acid reagent, incubate 30 min, read at 625 nm against KCN standards (0-50 µg/ml), report as µg HCN/ml.

EPS Production Protocol

Grow drought-tolerant isolates (e.g., MD-7, MD-18) in LB broth + 0-30% PEG 6000 for 48-72 hours at 30°C. Centrifuge 10 ml culture (10,000 rpm, 15 min), precipitate EPS from supernatant with 3 volumes cold ethanol overnight at 4°C, recentrifugation, resuspend pellet in water. Quantify total carbohydrates (phenol-sulfuric acid) at 490 nm vs. glucose standards or proteins (Bradford) at 595 nm; report as µg EPS/mg dry weight or per ml culture, normalized to cell protein.

EPS Protein Standard Curve

Prepare BSA standards at 0, 0.2, 0.4, 0.8, 1.2, 1.6, 2.0 mg/mL in PBS; mix 10 µL each with 200 µL BCA working reagent (50:1 reagent A:B), incubate 30 min at 37°C, read absorbance at 562 nm. Plot mean OD vs. concentration using quadratic regression ($y = ax^2 + bx + c$, $R^2 \geq 0.99$). Validate with spiked samples (recovery 95-105%, LOD ~0.05 mg/mL via 3×SD blank).