Supplementary information on "Colourless agar for enhancing colour contrast between microbial colonies and agar"

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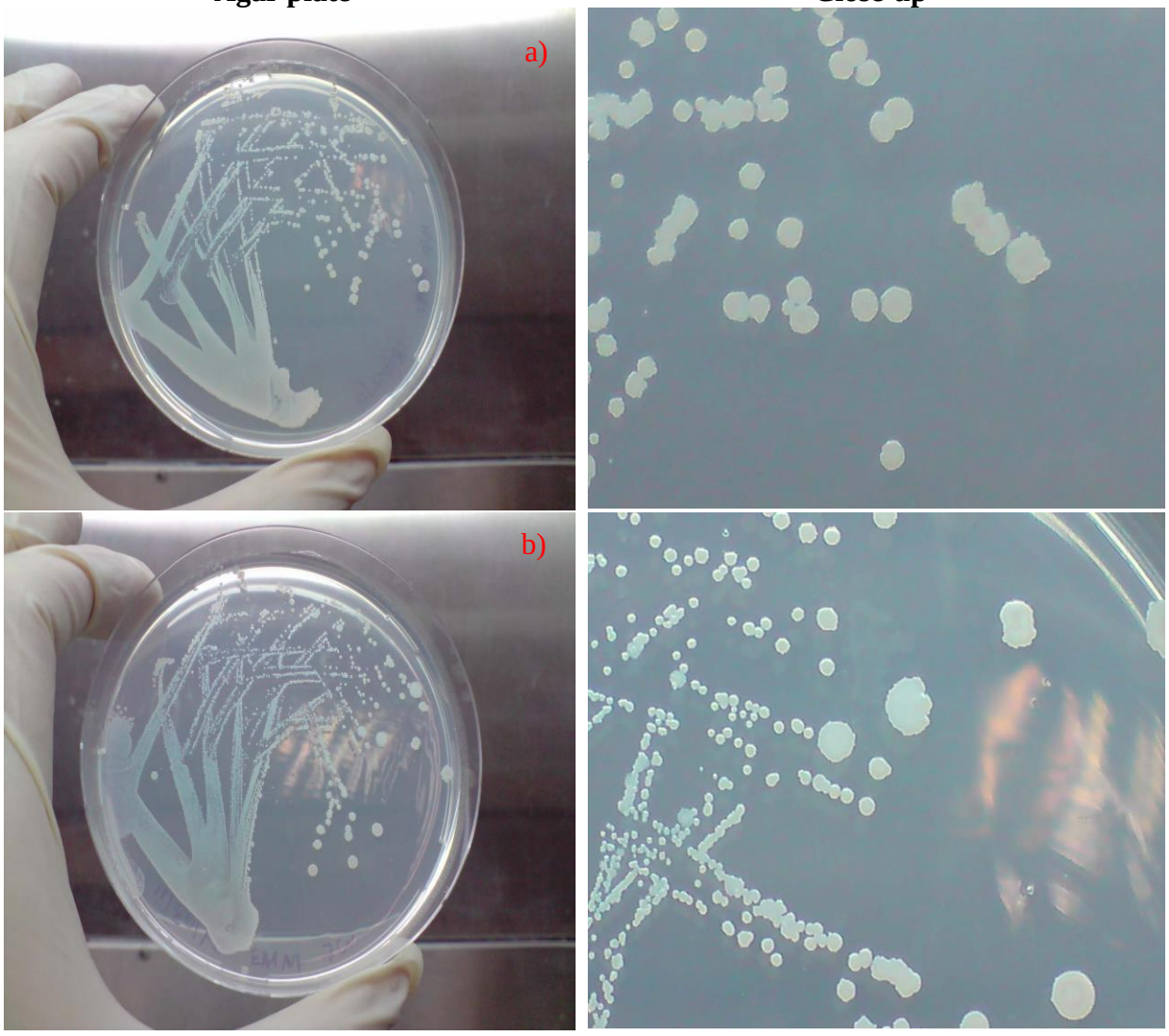
Agar plate **Close-up**



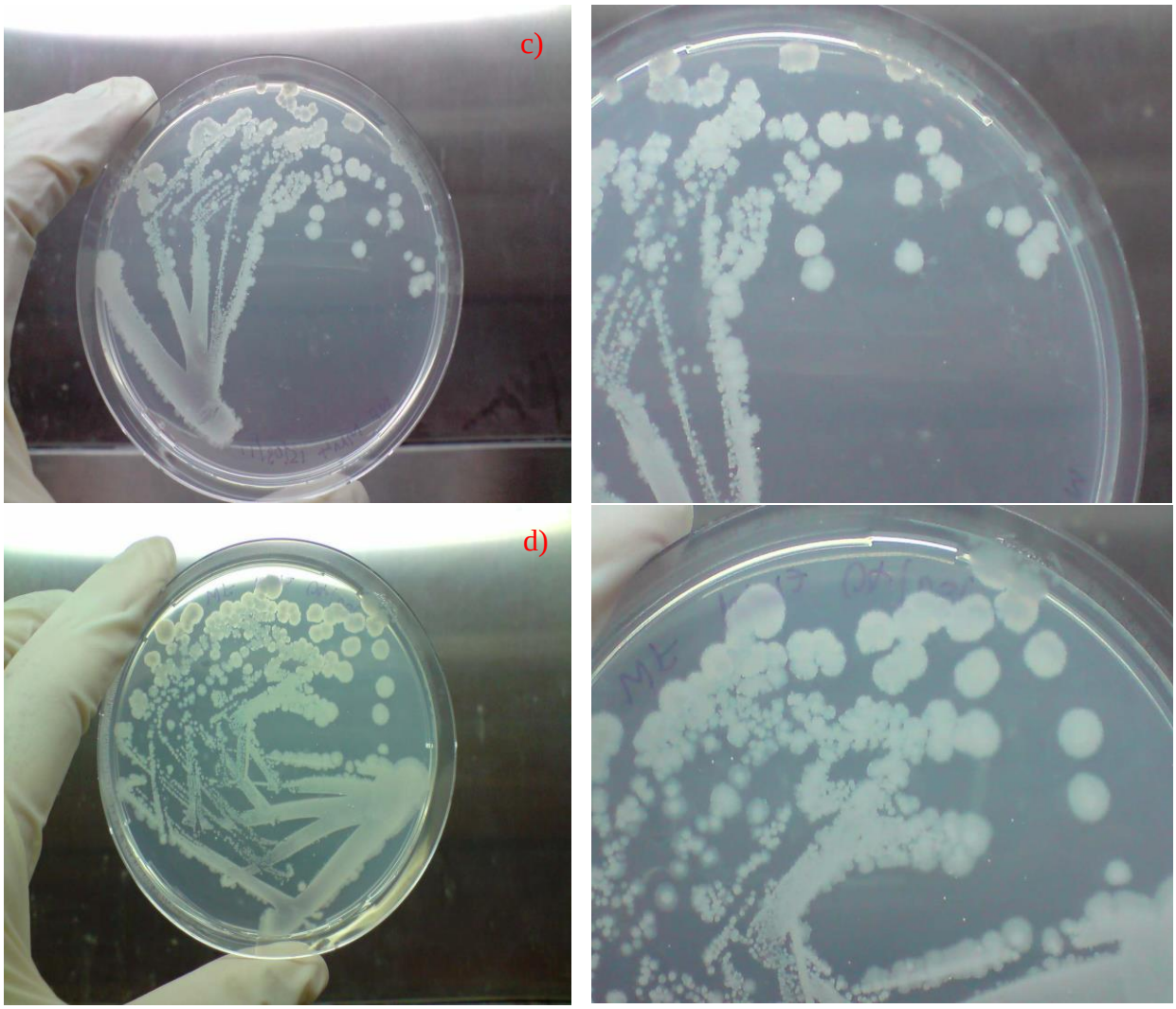
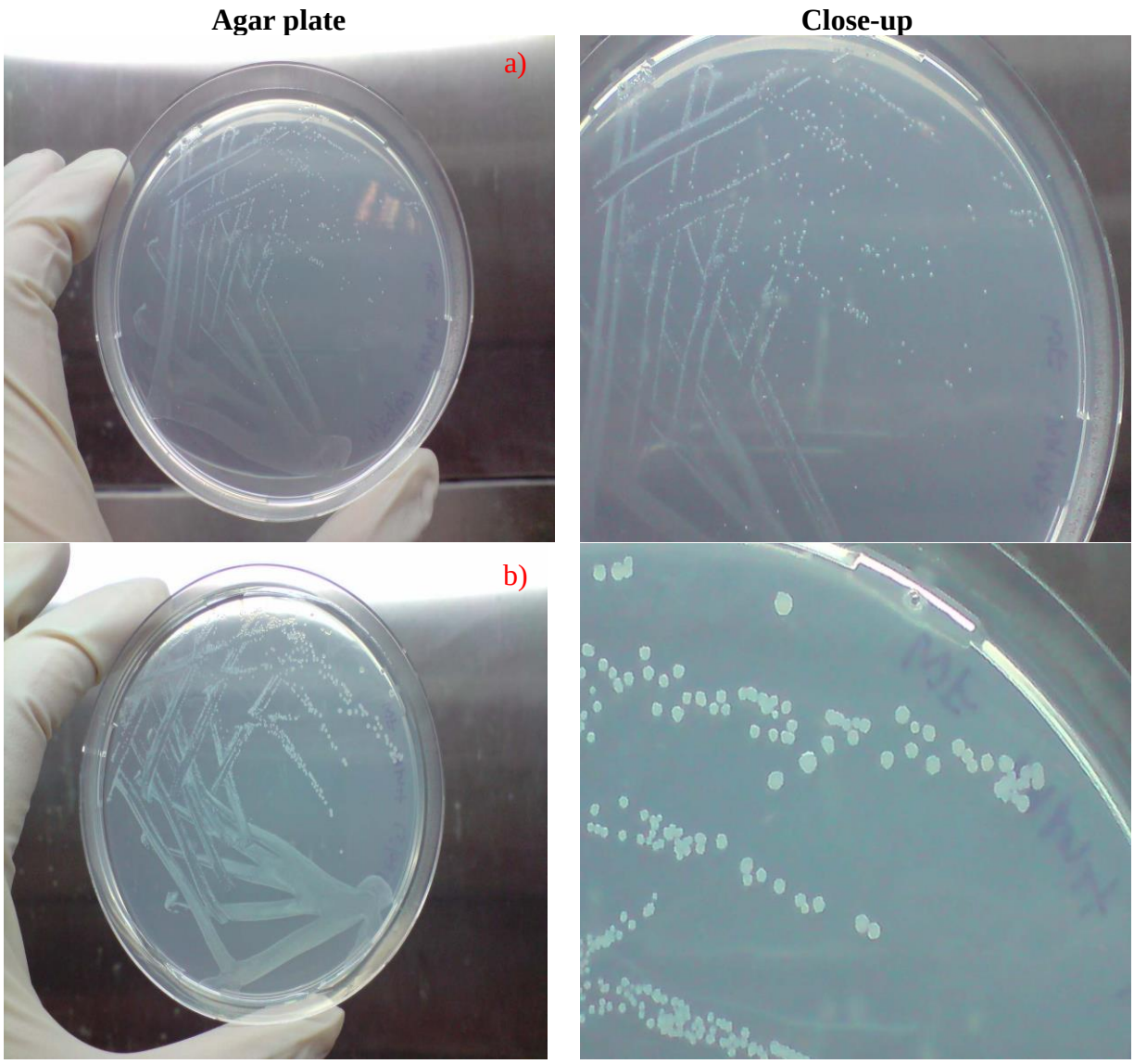


Figure S1: Growth of *Bacillus subtilis* NRS-762 on formulated colourless agar with different yeast extract concentration at 30 ^{o}C. a) No yeast extract supplementation, 6 days of incubation, b) 0.1 g/L yeast extract supplementation, 5 days of incubation, c) 0.5 g/L of yeast extract supplementation, 1 day of incubation, d) 1.0 g/L of yeast extract supplementation, 1 day of incubation.

Bacillus subtilis NRS-762 could grow on the formulated colourless agar medium without yeast extract supplementation at 30 ^{o}C since traces of vitamins and growth factors were present in the LB Lennox broth from which the inoculum for the streak plate culture was obtained (Figure S1a). More importantly, growth rate of the bacterium was positively correlated with concentration of yeast extract supplementation. Specifically, as yeast extract supplementation increased, growth rate and colony size increased correspondingly (Figure S1a, b, c, and d). For example, small, round white colonies were recovered on formulated colourless agar with 0.1 g/L of yeast extract, while large, round, wrinkled colonies were obtained on the same medium with 0.5 or 1.0 g/L of yeast extract supplementation. However, in all cases, growth on formulated colourless agar medium at

different yeast extract concentration generated colonies without the typical beige colouration found during growth on LB Lennox agar.



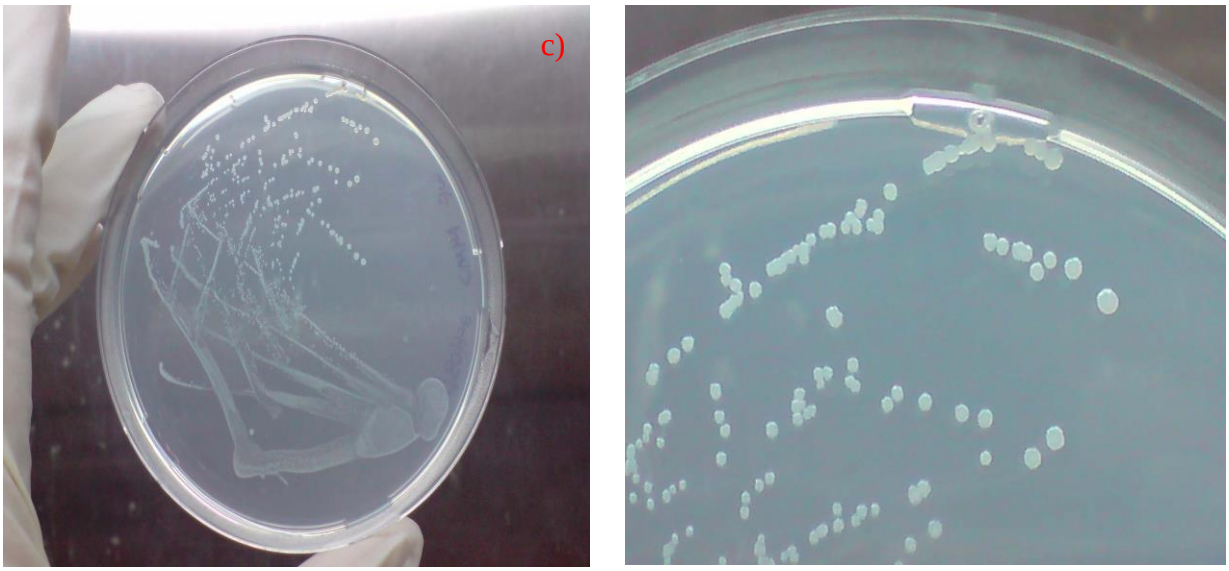
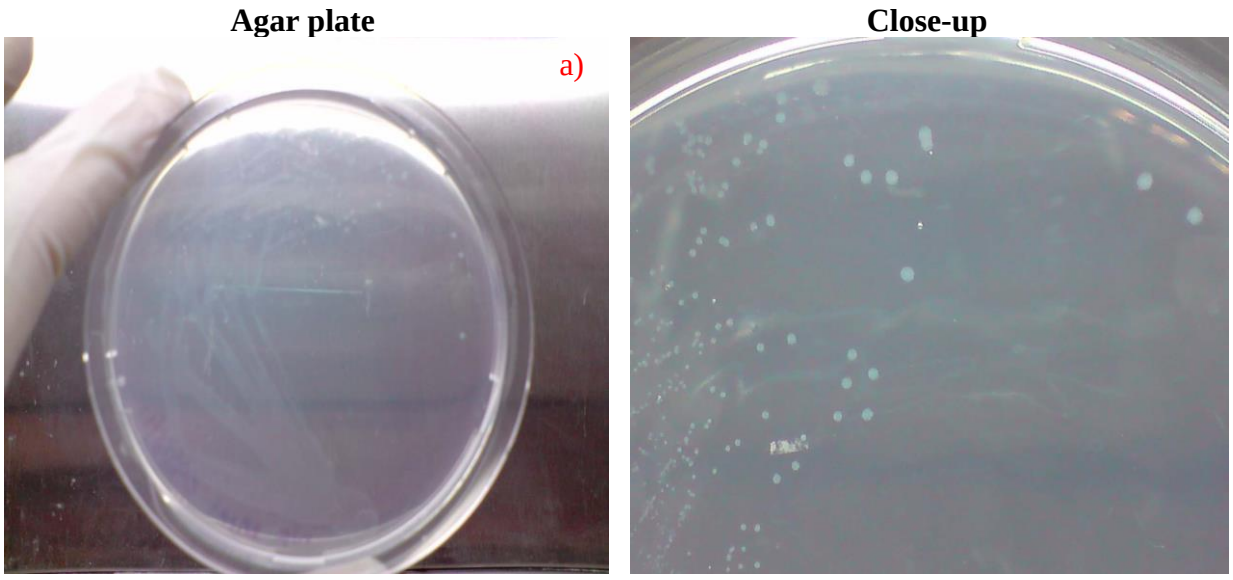


Figure S2: Growth of *Escherichia coli* DH5α on formulated colourless agar at 37 ºC with different concentrations of yeast extract supplementation. a) 0.1 g/L yeast extract, 1 day of incubation, b) 0.5 g/L yeast extract, 7 days of incubation, c) 1.0 g/L yeast extract, 2 days of incubation.

Escherichia coli DH5α could not grow in formulated colourless agar without yeast extract supplementation. Growth of *E. coli* DH5α on formulated colourless agar at 37 ºC showed similar trends to those of *B. subtilis* NRS-762 on the same medium. Specifically, growth rate increased with yeast extract supplementation in a concentration-dependent manner (Figure S2a, b, c). Colony size also increased with yeast extract supplementation. Small, white, round colonies were obtained during growth of the bacterium on formulated colourless agar, which differed from the beige colonies obtained during growth on LB Lennox medium.



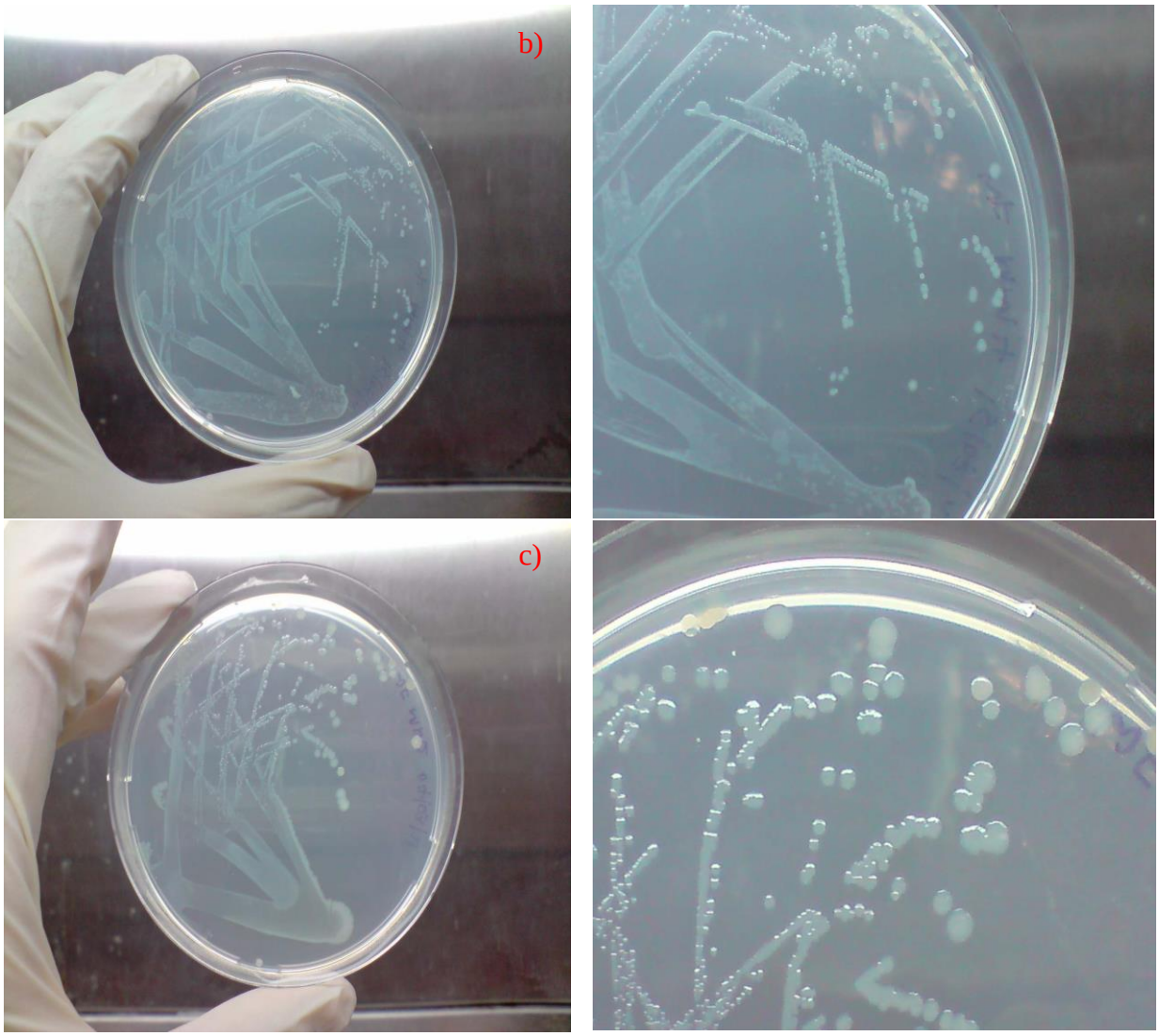


Figure S3: Growth of *Pseudomonas protegens* Pf-5 on formulated colourless agar at 30 ºC with different concentrations of yeast extract supplementation. a) 0.1 g/L yeast extract, 5 days of incubation, b) 0.5 g/L yeast extract, 1 day of incubation, c) 1.0 g/L yeast extract, 2 days of incubation.

Growth rates and colony size of *Pseudomonas protegens* Pf-5 increased with yeast extract supplementation in formulated colourless agar medium in a concentration-dependent manner (Figure S3). Specifically, small, round, greenish mucoid colonies were obtained during cultivation of *P. protegens* Pf-5 on formulated colourless agar medium with different levels of yeast extract supplementation. The extent of the mucoid morphotype was more evident at yeast extract supplementation of 1 g/L.

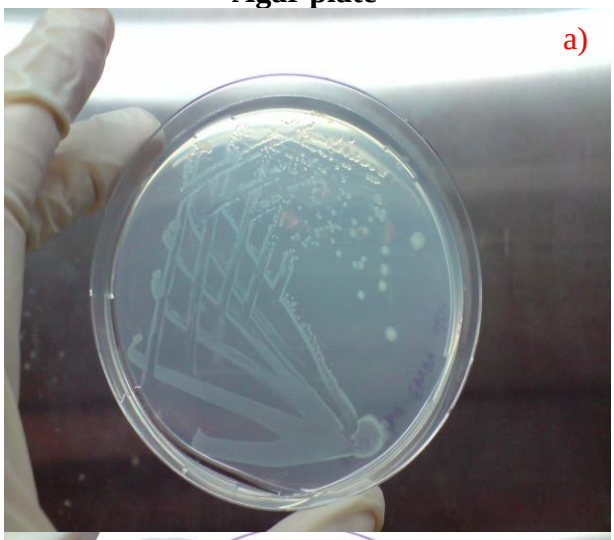
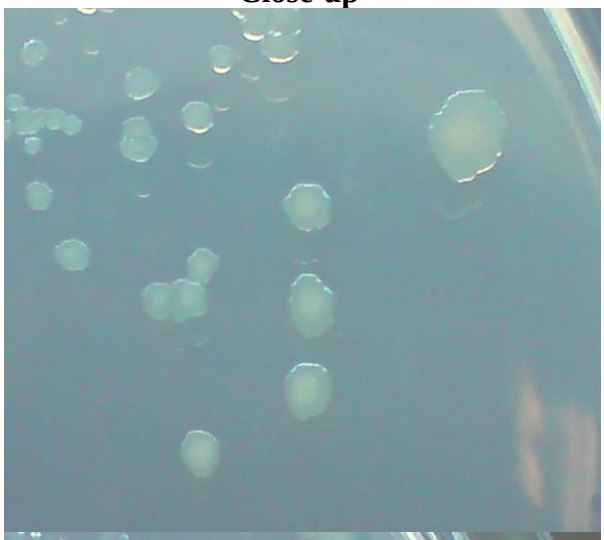
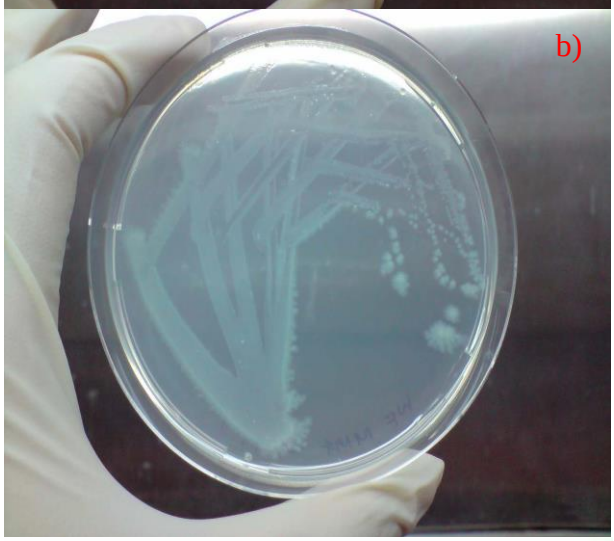
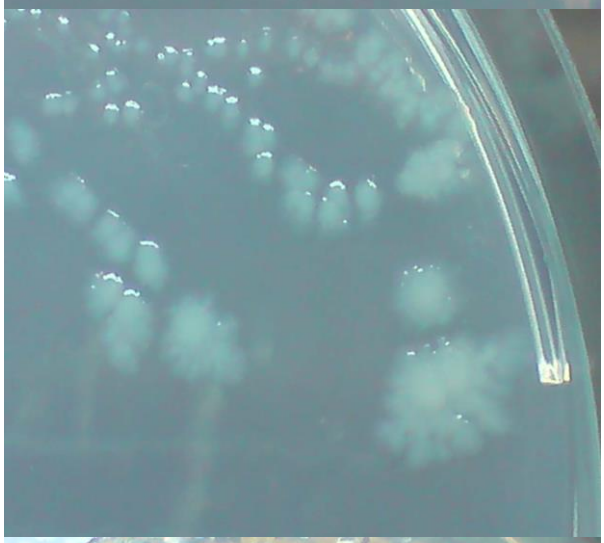
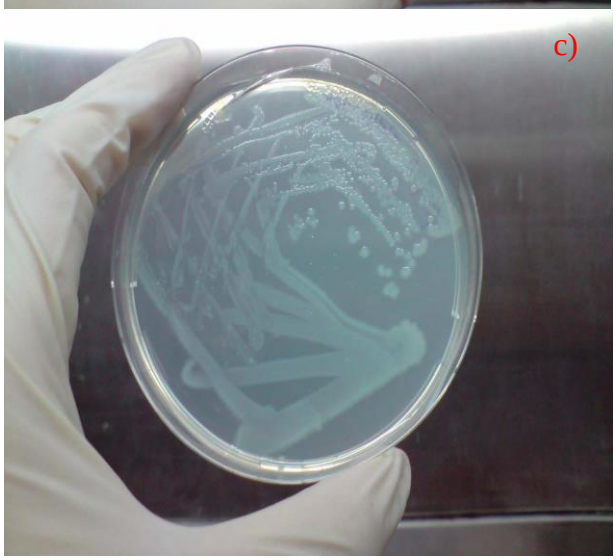
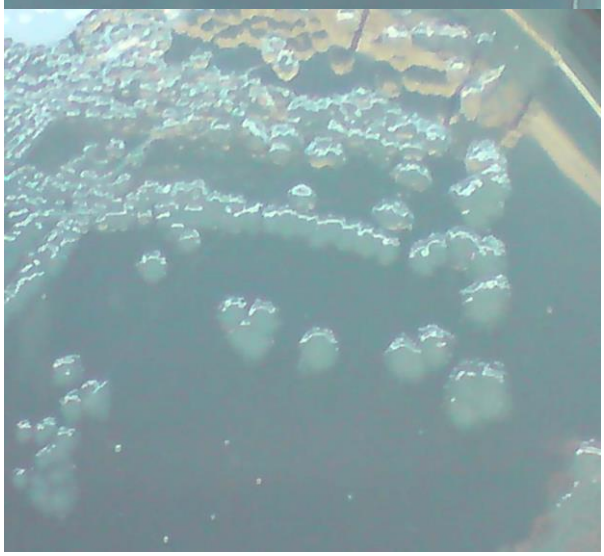
Agar plate	Close-up
a) 	
b) 	
c) 	

Figure S4: Growth of *Pseudomonas aeruginosa* PRD-10 (ATCC 15442) on formulated colourless agar with differing concentrations of yeast extract at 37 ^{o}C. a) 0.1 g/L yeast extract, 5 days of incubation, b) 0.5 g/L yeast extract, 7 days of incubation, c) 1.0 g/L yeast extract, 2 days of incubation.

Pseudomonas aeruginosa PRD-10 was cultivated in LB Lennox medium at 37 ^{o}C and 230 rpm rotational shaking prior to streak plate culture on solid formulated colourless agar medium. Cultivation of *P. aeruginosa* PRD-10 (ATCC 15442) on formulated colourless agar at different yeast extract concentrations at 37 ^{o}C revealed that the bacterium could grow well on the medium even at yeast extract supplementation of 0.1 g/L (Figure S4). Interestingly, colony morphology correlated with yeast extract supplementation. Specifically, round, greenish colony differentiated between the colony centre and periphery was observed on formulated colourless agar with 0.1 g/L of yeast extract. However, as yeast extract supplementation increased, colony morphology became more irregular with signs of initiation of swarming motility, which is collective cellular migration to a different habitat in time of environmental fluctuations or nutritional stress. For example, greenish irregular colony was observed for *P. aeruginosa* PRD-10 cultivated on formulated colourless agar with 0.5 g/L of yeast extract. At 1.0 g/L of yeast extract supplementation, *P. aeruginosa* PRD-10 exhibited a different phenotype of greenish, round mucoid colony. More importantly, slimy material appeared to be secreted by the bacterium during growth on formulated colourless agar with 1.0 g/L yeast extract. Unlike growth on LB Lennox agar, no green diffusible substance was secreted by *P. aeruginosa* PRD-10 into the colourless agar medium.

Conflicts of interest

The author declares no conflicts of interest.

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