Growth of *Pseudomonas protegens* Pf-5 in M9 minimal salts medium

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Abstract

Growth in minimal salts medium meant that a microorganism has the full repertoire of biosynthetic machinery for surviving in habitats lacking in vitamins and growth factors. This significantly expands the types of habitats suitable for colonization by the microbe. Such evolutionary selected and endowed survival advantage provided a critical growth advantage and colonization potential to particular microbes. *Pseudomonas protegens* Pf-5 (ATCC BAA-477) potential for growth in minimal salts medium was evaluated by aerobic growth experiments at 25 and 30 ^{o}C in M9 medium. Experiment results revealed that the bacterium could grow in M9 medium without nutrient and yeast extract supplementation, but with a long lag phase of 18 hours and a small optical density of 0.7 and 1.0 at 25 and 30 ^{o}C, respectively after 45 hours of incubation. Variation in pH, on the other hand, revealed similar pH profiles of net secretion of acidic metabolites during growth in M9 medium, that stabilized prior to attainment of maximal optical density in the cultures at the two temperatures. Supplementation of M9 medium with 1 g/L yeast extract at 25 ^{o}C resulted in a shorter lag phase of 3 hours in *P. protegens* Pf-5 and a maximal optical density of 3.1, which indicated the importance of vitamins and growth factors in yeast extract in supplying necessary building blocks for biomass formation. Similarly, net secretion of acidic metabolites was observed in pH profile prior to exponential phase growth in *P. protegens* Pf-5, which was followed by a gradual rise in culture broth's pH. Collectively, *P. protegens* Pf-5 could grow in minimal salts medium without supplementation of vitamins and growth factors at 25 and 30 ^{o}C; thereby, pointing to a significant competitive advantage in survival and colonization of new habitats in the face of nutritional and environmental stressors.

Keywords: *Pseudomonas protegens*, minimal medium, biosynthesis, vitamins, growth factors, optical density, lag phase, overflow metabolism, acidic metabolites,

Subject areas: biotechnology, ecology, microbiology, biochemistry, cell biology,

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Highlights

1) *Pseudomonas protegens* Pf-5 (ATCC BAA-477) was able to grow in M9 minimal salts medium without nutrient supplementation at 25 and 30 °C. Specifically, following a long lag phase of 18 hours, optical density increased to 0.7 and 1.0 at 25 and 30 °C, respectively.

2) Variation in pH during growth at 25 and 30 °C pointed to net secretion of acidic metabolites into the medium followed by a stabilization of pH around 6.5, independent of growth temperature.

3) Supplementation of M9 minimal salts medium with 1 g/L yeast extract at 25 °C reduced the lag phase to 3 hours with enhanced biomass formation recorded at optical density of 3.1 after 24.5 hours of incubation.

Introduction

Ability to grow in a minimal salts medium without infusion of vitamins and growth factors meant that the microorganism possesses all the biosynthetic machinery for generating the building blocks necessary for biomass formation and cell division. Hence, the microbe would not be limited by the availability of vitamins and growth factors in the environment in its choice of potential habitats. This meant that a greater variety of habitats are available for colonization, which places the microbe at a selective advantage relative to other microorganisms unable to grow in environments without a source of vitamins and growth factors. More importantly, the ability to grow in a minimal salts medium also meant that the microbe is able to compete better with other microorganisms for the same habitat as it may be more efficient in utilizing nutrients from the environment for biomass formation and increasing cell numbers.

In this study, the ability of a common soil bacterium, *Pseudomomas protegens* Pf-5 (ATCC BAA-477), to grow in M9 minimal salts medium was investigated using aerobic growth experiments in shake flasks at 25 and 30 °C.

Materials and Methods

Materials

M9 medium was constituted using its components and its composition is as follows, [g/L]: D-Glucose, 4.0; NH_4Cl, 1.0; NaH_2PO_4, 3.0; Na_2HPO_4, 6.78; NaCl, 0.5. Composition of M9 minimal medium supplemented with 1 g/L yeast extract was [g/L]: D-Glucose, 4.0; NH_4Cl, 1.0; NaH_2PO_4, 3.0; Na_2HPO_4, 6.78; NaCl, 0.5; yeast extract, 1.0.

Growth of P. protegens Pf-5 in liquid medium

Stock cultures of *Pseudomonas protegens* Pf-5 (ATCC BAA-477) were prepared in 40% glycerol and stored at -70 °C until use. Stock glycerol cultures of *P. protegens* Pf-5 was used in inoculating 100 mL of M9 medium contained in 250 mL glass shake flasks. Incubation of the seed cultures

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was at 30 ºC and 230 rpm rotational shaking in a temperature controlled incubator. After 36 hours of incubation, 1 mL of seed culture was used in inoculating 100 mL of M9 medium or M9 medium supplemented with 1 g/L yeast extract in 250 mL glass shake flask as experiment cultures. The incubation conditions were 25 ºC and 250 rpm on a non temperature controlled orbital shaker, and 30 ºC and 230 rpm rotational shaking in a temperature controlled incubator (Yih Der LM-570RD, Taiwan). Three biological replicates were performed for each experiment.

Measurement of optical density and pH

At appropriate time points, aliquots of experiment cultures were withdrawn for measurement of optical density (at 600 nm) via a Shimadzu Biospec Mini UV-Visible spectrophotometer with a quartz cuvette of 10 mm pathlength (volume: 3.5 mL). Dilution with deionized water was performed when the optical density exceeded 1. pH was measured using an Orion 9156 BNWP pH probe fitted to a Mettler Toledo Delta 320 pH meter.

Results and Discussion

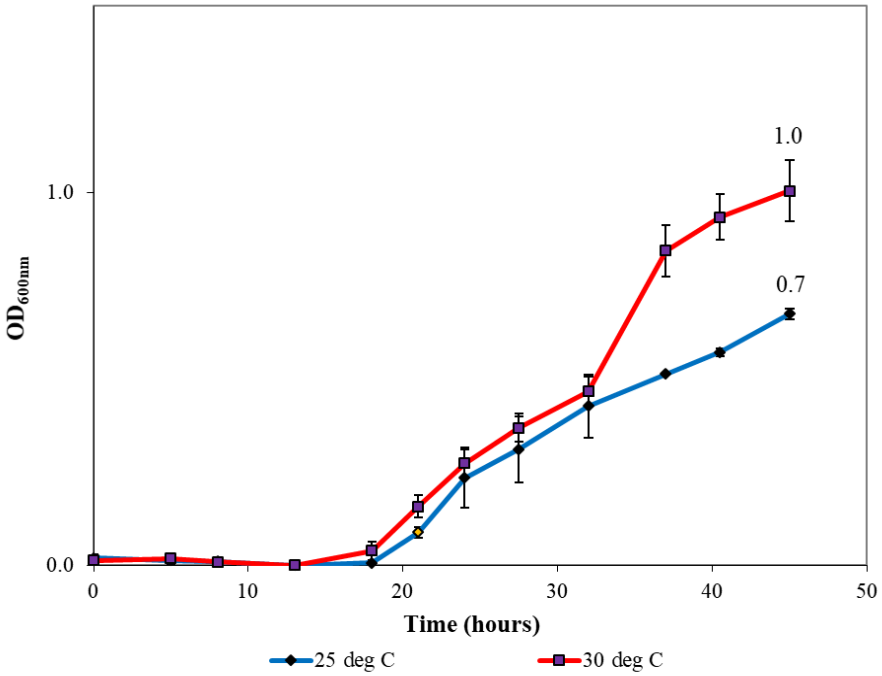

Figure 1a: Aerobic growth of *Pseudomonas protegens* Pf-5 in M9 medium at 25 and 30 ºC in a 250 mL shake flask culture system. Results indicated *P. protegens* Pf-5 could grow in M9 minimal salts medium without supplementation of yeast extract. Biomass formation increased with growth temperature.

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Observations of aerobic growth of *P. protegens* Pf-5 in M9 minimal medium revealed reasonable growth of the bacterium in the medium with optical density of 0.7 and 1.0 at 25 and 30 ^{o}C, respectively after 45 hours of incubation (Figure 1a). Thus, increase in growth temperature resulted in higher biomass formation and optical density. Lag phase in M9 minimal salts medium was relatively long at 18 hours for growth at both 25 and 30 ^{o}C.

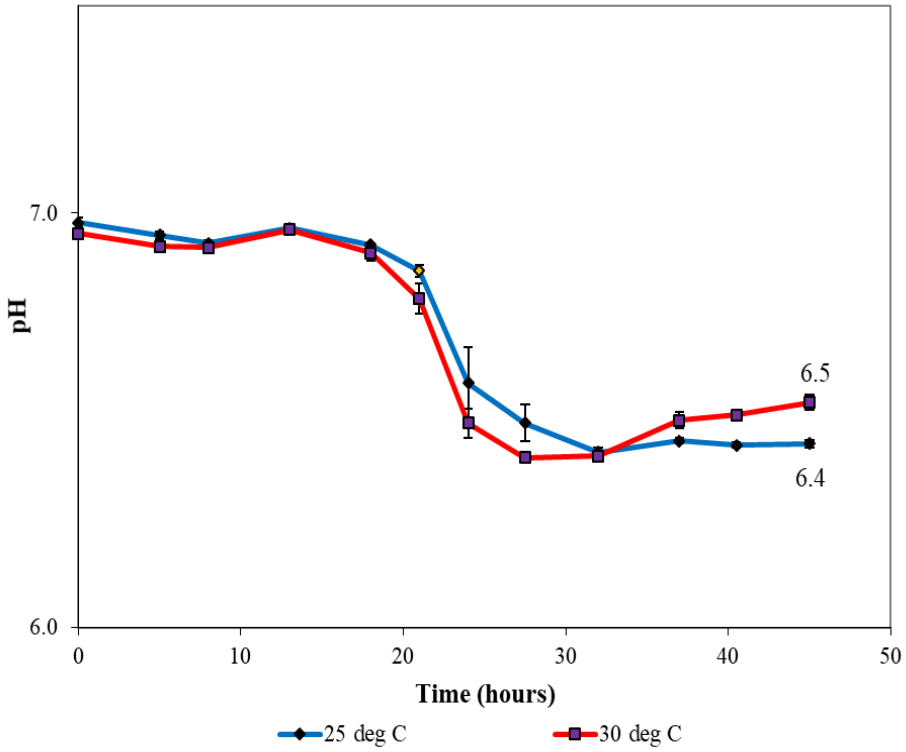

Figure 1b: Variation of pH in culture broth of *P. protegens* Pf-5 in M9 minimal medium grown at 25 and 30 ^{o}C. Note that despite differences in maximal optical density obtained at different growth temperatures, pH profile for growth at 25 and 30 ^{o}C tracked each other closely.

pH profile, on the other hand, revealed that *P. protegens* Pf-5 likely utilized the same type of metabolism during growth at 25 and 30 ^{o}C in M9 minimal medium (Figure 1b). Specifically, after lag phase ended at 18 hours post inoculation, there was net secretion of acidic metabolites into M9 medium. However, there was no continuous decline in pH, which stabilized at between 6.4 and 6.5 after 32 hours of incubation at both temperatures. Overall, pH profile for growth at 25 and 30 ^{o}C were similar to each other, which was in contrast to the difference in maximal optical density obtained.

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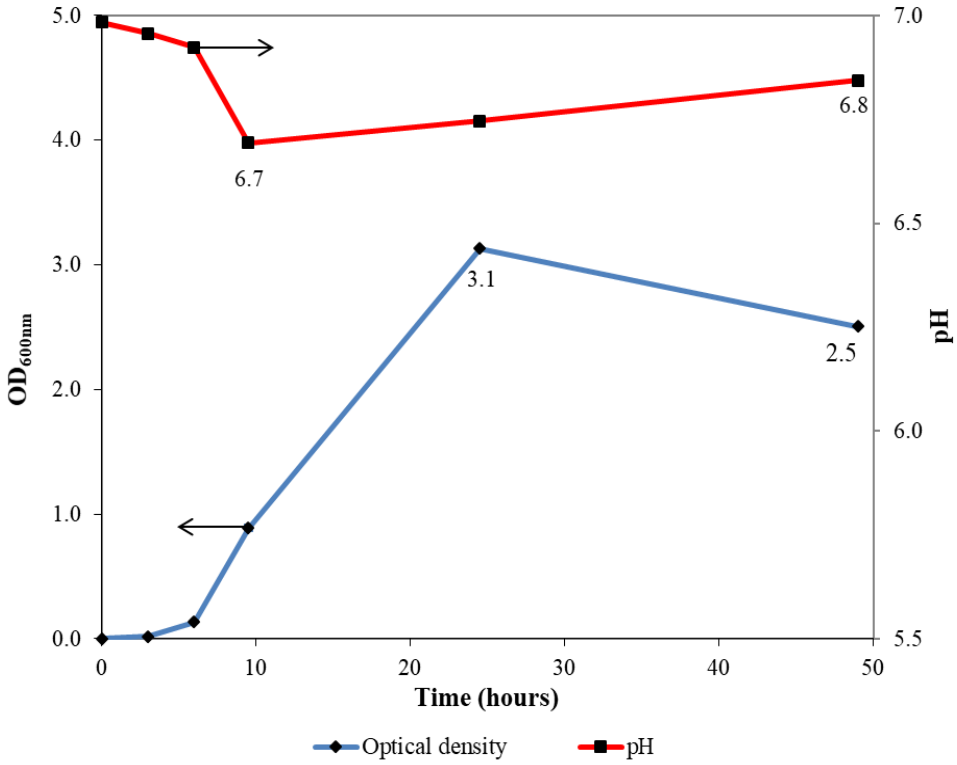

Figure 2: Growth of *P. protegens* Pf-5 in M9 medium supplemented with 1 g/L yeast extract at 25 ºC. Supplementation of yeast extract reduced the lag phase substantially and improved maximal optical density obtained.

P. protegens Pf-5 was subsequently cultivated at 25 ºC in M9 medium with 1 g/L of yeast extract supplementation. Growth performance data (Figure 2) indicated a significant reduction in lag phase to 3 hours and a maximal optical density of 3.1, which was substantially higher than that obtained without yeast extract supplementation. pH variation, on the other hand, revealed a decrease in pH from 7.0 at the start of the cultivation to a minimum of 6.7 after 9.5 hours of culture. This subsequently increased to 6.8 at the end of the cultivation. Overall, there was net secretion of acidic metabolites into the culture broth during the initial phase of growth where various building blocks were synthesized for biomass formation. But, the pH gradually increased to 6.8 at the end of the cultivation.

Conclusions

Ability to grow in minimal medium is somewhat of a grand challenge in microbial cell culture. From a natural selection perspective, it is the endowment in a microbe of a profound competitive advantage in the ability to grow at a new habitat with low availability of vitamins and growth factors. Thus, while growth may be slow due to the channelling of nutritional energy into

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biosynthetic pathways for generating, *de novo*, all the building blocks for biomass formation, the ability to grow without uptake of vitamins and growth factors from the environment is a critical survival advantage both in colonizing new habitats as well as surviving nutritional stressors.

Growth experiments in M9 minimal medium showed that *Pseudomonas protegens* Pf-5 was able to grow in the medium without yeast extract supplementation at both 25 and 30 ^{o}C. Although the lag phase was 18 hours, *P. protegens* Pf-5 achieved optical density of 0.7 and 1.0 at 25 and 30 ^{o}C respectively, after 45 hours of incubation, which indicated that the bacterium could grow in minimal salts medium without supplementation of vitamins and growth factors. Overlap in pH profiles for both temperatures indicated that differences in growth temperature did not induce differential metabolism for growth in M9 minimal salts medium.

Supplementation of M9 medium with 1 g/L of yeast extract improved growth of *P. protegens* Pf-5 at 25 ^{o}C, where the lag phase was reduced to 3 hours and optical density of 3.1 was obtained. Concomitant with growth was a decline in pH from 7.0 to 6.7 at 9.5 hours post-inoculation prior to a small increase to 6.8 at 49 hours of incubation. Collectively, *P. protegens* Pf-5 was able to grow in minimal salts medium without supplementation of vitamins and growth factors, which is an important competitive advantage for colonization of new habitats or for the preservation of the clonal population during nutritional stress.

Conflicts of interest

The author declares no conflicts of interest.

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