Effect of temperature on growth of *Pseudomonas protegens* Pf-5 and *Pseudomonas aeruginosa* PRD-10 in LB Lennox medium

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Abstract

Temperature affects growth of bacteria by influencing enzyme and growth kinetics. Specifically, evolution selects for specific temperature range in which a microbe could thrive, and thus fix the temperature range in which biomolecule structure and function are finely tuned for coping with the thermal conditions prevailing within a cell at a particular temperature. Using aerobic culture in LB Lennox medium in shake flasks, this study aimed to understand the growth of *Pseudomonas protegens* Pf-5 (ATCC BAA-477) and *Pseudomonas aeruginosa* PRD-10 (ATCC 15442) at 25, 30 and 37 ^{o}C. Experiment results revealed that *P. protegens* Pf-5 grew very poorly at 37 ^{o}C (with maximal optical density of 0.66), while better growth was observed at 25 and 30 ^{o}C. Specifically, *P. protegens* Pf-5 appeared to be better adapted to growth at 25 ^{o}C, where the maximal optical density obtained was 5.3 compared to 4.6 at 30 ^{o}C. More importantly, two phase growth behaviour was observed during growth at 30 ^{o}C where a faster initial phase of growth was followed by a slower one. Growth at 25 and 30 ^{o}C exhibited similar pH trend, which suggested similar metabolic processes was activated during growth. On the other hand, *P. aeruginosa* PRD-10 demonstrated a more efficient conversion of LB Lennox medium into biomass where the maximal optical density obtained at all three growth temperatures were higher than those of *P. protegens* Pf-5. More importantly, growth of *P. aeruginosa* PRD-10 exhibited a clear adaptation to growth at 25 and 37 ^{o}C, while growth at 30 ^{o}C resulted in a lower biomass yield compared to that of 25 and 37 ^{o}C. On the other hand, pH variation during culture revealed that *P. aeruginosa* PRD-10 likely activated similar metabolic processes at all three growth temperatures, where a higher growth temperature would result in the net secretion of more alkaline metabolites. Collectively, *P. protegens* Pf-5 and *P. aeruginosa* PRD-10 demonstrated clear temperature adaptation at an evolutionary level. In addition, experiment data suggested that *P. aeruginosa* PRD-10 might have co-evolved with humans on a substantial time scale resulting in a temperature preference of 37 ^{o}C over 30 ^{o}C.

Keywords: enzyme kinetics, growth kinetics, growth temperature, *Pseudomonas protegens*, *Pseudomonas aeruginosa*, metabolic processes, biomass formation, cellular maintenance, adaptation, pH variation,

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

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Highlights

1) *Pseudomonas protegens* Pf-5 (ATCC BAA-477) grew well at 25 and 30 °C but poorly at 37 °C

2) On the other hand, *Pseudomonas aeruginosa* PRD-10 (ATCC 15442) demonstrated more efficient conversion of LB Lennox medium into biomass compared to *P. protegens* Pf-5 at all three growth temperatures.

3) Moreover, *P. aeruginosa* PRD-10 was better adapted to growth at 25 and 37 °C compared to 30 °C.

Introduction

Microbes occupy diverse ecological niches on Earth, where environmental conditions significantly differ; thereby, highlighting that microbes could adapt to a variety of environmental conditions such as temperature difference. However, temperature range at which specific microbes grow is typically narrow, due possibly to the inability of the metabolic machinery of the cell to cope with extreme fluctuations in temperature. While heat shock proteins help protect cells against sudden fluctuation in temperature, the temperature range at which a microbe could grow remain narrow and evolutionary constrained.

Using three growth temperatures of 25, 30 and 37 °C, this study seeks to characterize the growth performance of *Pseudomonas protegens* Pf-5 (ATCC BAA-477) and *Pseudomonas aeruginosa* PRD-10 (ATCC 15442) in LB Lennox medium under aerobic cultivation in 250 mL glass shake flasks under different incubation temperatures. Optical density and pH were measured to determine, quantitatively, the effect of different growth temperature on the culture of the different bacteria in LB Lennox medium. While optical density provided a measure of cell concentration obtained, pH provided a lens into the net amount of metabolites secreted into the culture broth during growth, which, in turn, suggests possible metabolic changes.

Materials and Methods

Materials

Composition of LB Lennox medium (Difco) in [g/L]: Tryptone, 10.0; Yeast extract, 5.0; NaCl, 5.0.

Growth of bacteria in LB Lennox medium

Stock cultures of the bacteria were kept in glycerol at – 70 °C until use. Seed cultures were inoculated with glycerol stock cultures of the respective bacterium in LB Lennox medium and cultured at the following temperatures: *Pseudomonas protegens* Pf-5 (30 °C) and *Pseudomonas aeruginosa* PRD-10 (37 °C). Seed cultures were prepared in 100 mL of LB Lennox medium in 250 mL glass shake flask with cotton plugs and shaken at 230 rpm in a temperature controlled

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incubator (Yih Der LM 570RD, Taiwan). After 14 hours of incubation, 1 mL of the seed cultures were used in inoculating three biological replicates of experiment cultures in 100 ml of LB Lennox medium contained in 250 mL glass shake flask with cotton plugs at the respective growth temperature. Non temperature controlled orbital shaker was used for culture at 25 ^{o}C (room temperature) with rotational speed of 250 rpm. Air temperature controlled incubator shaker was used for culture at 30 and 37 ^{o}C with rotational speed of 230 rpm. Three biological replicates were performed for each experiment.

Measurement of optical density and pH

At appropriate time points, aliquots of culture were withdrawn for optical density measurement via Shimadzu's Biospec Mini spectrophotometer with a quartz cuvette of pathlength 10 mm (volume: 3.5 ml). If the optical density was higher than 1, deionized water was used for dilution. pH measurement was performed using an Orion BNWP 9156 pH probe fitted to a Mettler Toledo Delta 320 pH meter.

Results and Discussion

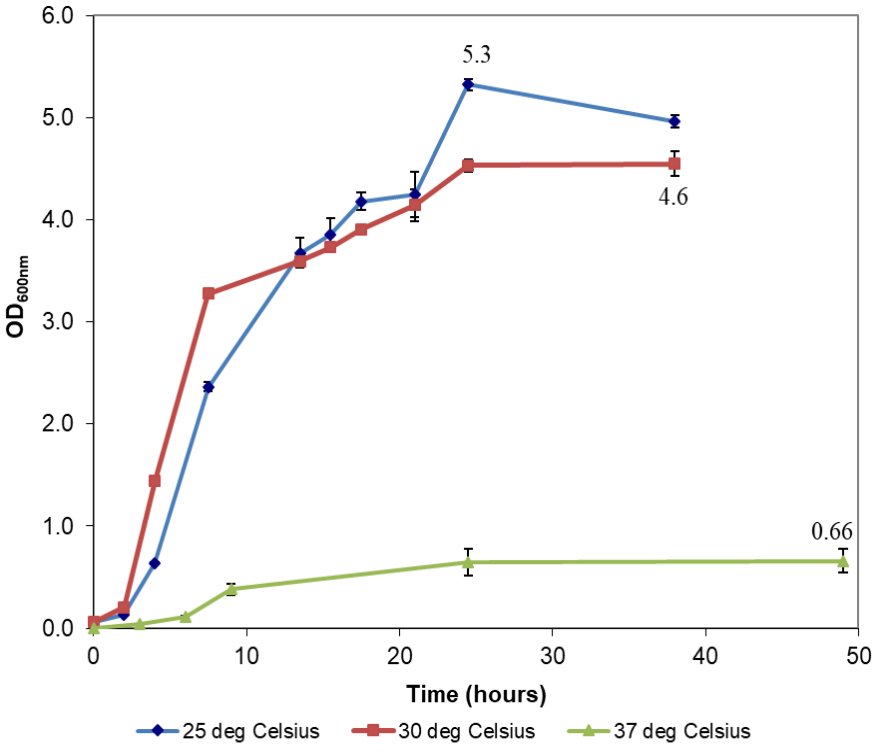

Figure 1: Aerobic growth of *P. protegens* Pf-5 in LB Lennox medium contained in shake flask. The bacterium exhibited very poor growth at 37 ^{o}C compared to optimal growth at 25 ^{o}C.

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Aerobic growth of *Pseudomonas protegens* Pf-5 (ATCC BAA-477) in LB Lennox medium revealed very poor growth at 37 ºC, with maximal optical density of 0.66 after 49 hours of cultivation (Figure 1a). This is different from report of other strains of *P. protegens* forming biofilm at 37 ºC.[1] Growth of *P. protegens* Pf-5 at 25 and 30 ºC, on the other hand, resulted in higher maximal optical density. However, the biomass yield at 25 and 30 ºC was not the same; thus, highlighting that the two different growth temperatures induced differential metabolism that resulted in different partition of energy from nutrients into cell maintenance and biomass formation. In particular, growth at 25 ºC resulted in more energy channelled to biomass formation (maximal optical density of 5.3) compared to growth at 30 ºC (maximal optical density of 4.6). With increase in growth temperature from 25 to 30 ºC, faster rate of optical density increase was observed. In addition, *P. protegens* Pf-5 appeared to preferentially consume specific metabolites in LB Lennox medium compared to other medium components that resulted in two phase growth behaviour of the species. Specifically, rate of growth decreased significantly after 7.5 hours of cultivation, which suggested possible preferential use of one carbon source in the complex medium prior to the secondary carbon source. Interestingly, such preferential use of carbon sources did not result in diauxic growth and a characteristic lag phase.

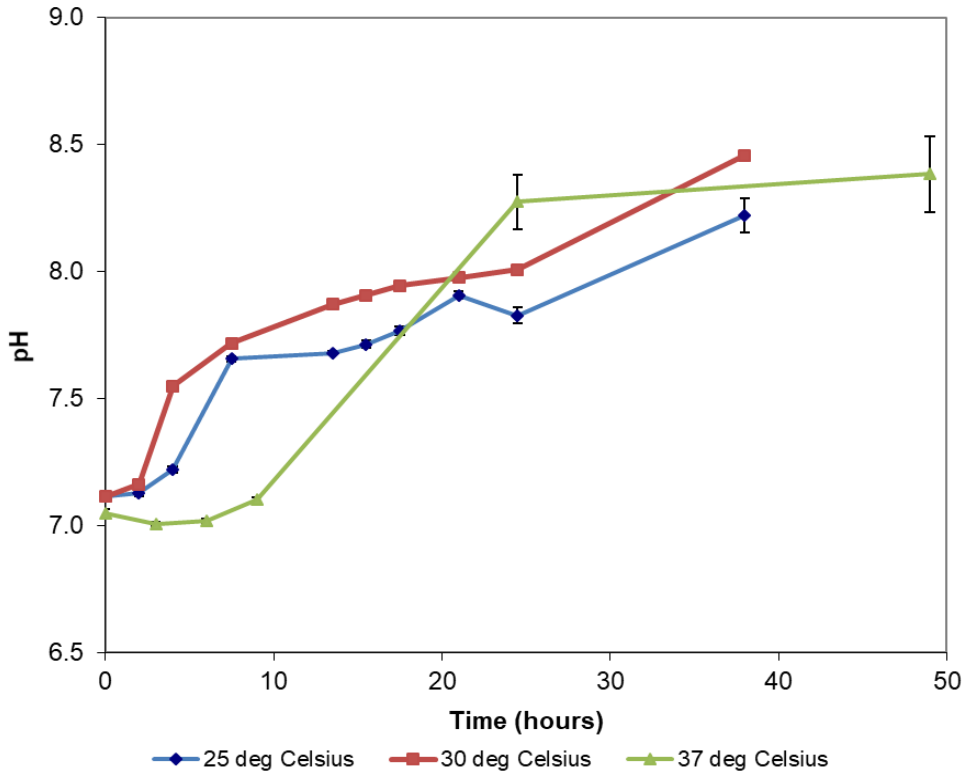

Figure 1b: pH variation in LB Lennox medium during growth of *P. protegens* Pf-5 at different growth temperatures. Note that cells cultivated at 37 ºC are clearly metabolizing and secreted significant amounts of alkaline metabolites into the culture broth while biomass formation was retarded, given the need to divert most of the nutritional energy into cellular maintenance at an unfavourable growth temperature.

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Analysis of pH variation during cultivation of *P. protegens* Pf-5 in LB Lennox medium at different growth temperatures revealed that substantial alkaline metabolites were secreted into the culture broth even with low biomass formation at 37 ^{o}C. Specifically, broth's pH increased from 7.05 to 8.38 after 49 hours of cultivation at 37 ^{o}C (Figure 1b); thereby, indicating significant amount of metabolic energy derived from metabolizing nutrients was devoted to cellular maintenance during growth at the unfavourable temperature of 37 ^{o}C, which likely resulted in substantial cell damage requiring repair. Growth of the bacterium at 25 and 30 ^{o}C, on the other hand, generated pH profiles similar in trend, but more alkaline metabolites were secreted during growth at 30 ^{o}C. pH increased with increase in cell density during growth of *P. protegens* Pf-5. Specifically, pH increased from 7.11 to 8.46 after 38 hours of cultivation at 30 ^{o}C. Finally, the optimal growth temperature for *P. protegens* Pf-5 from the perspective of maximal biomass formation is 25 ^{o}C.

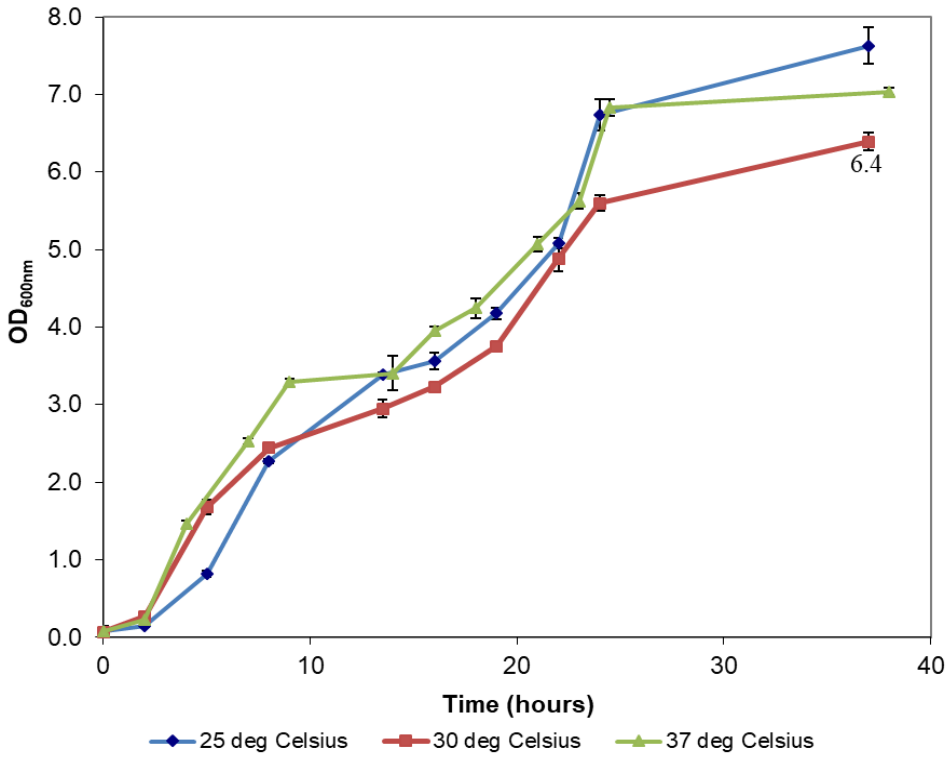

Figure 2a: Aerobic growth of *P. aeruginosa* PRD-10 in LB Lennox medium at different growth temperatures in shake flask. Note that the bacterium may be adapted for growth at 25 and 37 ^{o}C given the higher biomass formation at these temperatures.

Pseudomonas aeruginosa PRD-10 (ATCC 15442) exhibited different growth characteristics from that of *P. protegens* Pf-5 during aerobic growth in LB Lennox medium at 25, 30, and 37 ^{o}C (Figure 2a). Specifically, higher biomass formation was observed for growth at 25 and 37 ^{o}C, where the maximal optical density attained were 7.6 and 7.0, respectively. This was higher than the maximal optical density of 6.4 attained during growth at 30 ^{o}C. Thus, experiment

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results revealed that *P. aeruginosa* PRD-10 may be adapted for growth at 25 and 37 ºC, where more energy derived from nutrients was channelled to biomass formation rather than cellular maintenance relative to the case for growth at 30 ºC. Evidence of the non adaptation for growth at 30 ºC for *P. aeruginosa* PRD-10 could be seen in the higher growth rates during culture at 25 and 37 ºC compared to 30 ºC. Hence, the growth performance of *P. aeruginosa* PRD-10 fits its description as a soil bacterium that later became adapted for growth at 37 ºC as an opportunistic pathogen. This is corroborated by other studies indicating global transcriptomic changes in metabolism as *P. aeruginosa* grew at different temperatures of 22 and 37 ºC.[2] Growth at 30 ºC, on the other hand, may entail the activation of a different metabolism that utilized different metabolic pathways, which were less efficient at converting nutrients into biomass. Such inefficiency in biomass formation at an intermediate temperature between two temperatures where the bacterium had adapted for growth is an interesting phenomenon. Given that enzymes adapted for function at 37 ºC should also be operative at 30 ºC, possible temperature sensors linked to different metabolic programmes may be present in *P. aeruginosa* PRD-10, where temperature changes led to switches between metabolic programmes. Such temperature sensors might operate in similar ways as an RNA thermometer.[3]

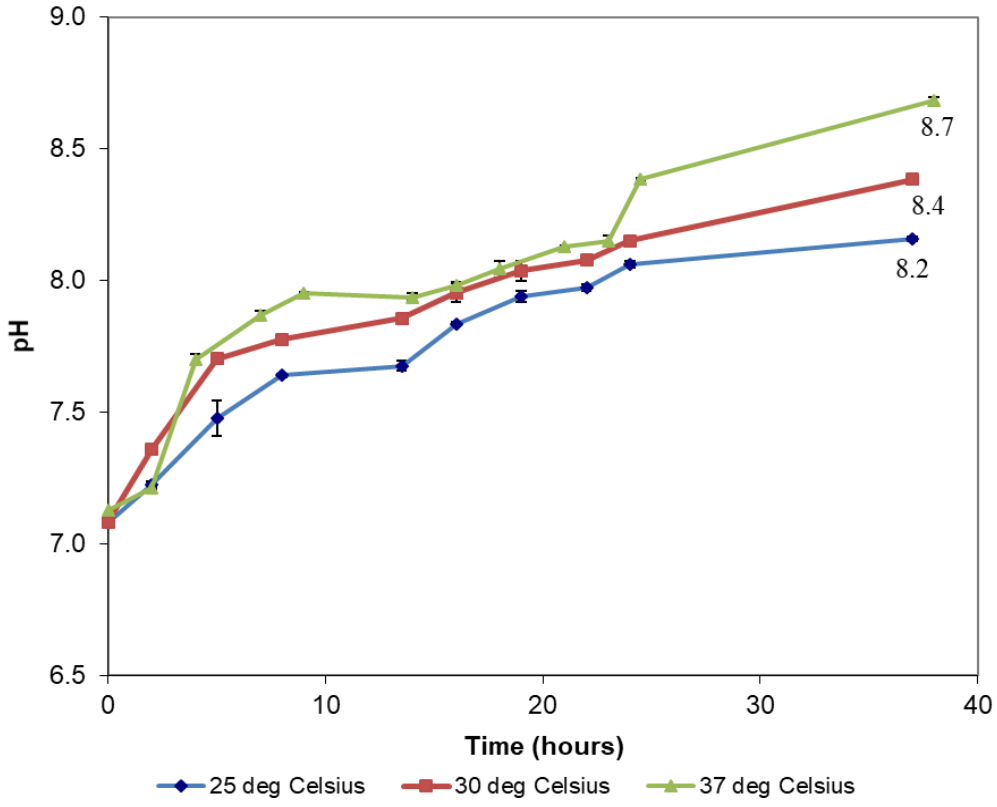

Figure 2b: pH variation for aerobic growth of *P. aeruginosa* PRD-10 in LB Lennox medium at different growth temperatures. In general, the higher the growth temperature, the greater the amount of secreted alkaline metabolites.

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Observation of the pH variation in the culture broth of *P. aeruginosa* PRD-10 cultures revealed there was no net secretion of acidic metabolites into the culture broth during growth in LB Lennox medium. Specifically, pH increased from 7.1 at the start of the cultivation to 8.2, 8.4 and 8.7, for growth at 25, 30 and 37 ºC, respectively (Figure 2b). More importantly, the higher the growth temperature, the greater was the amount of alkaline metabolites secreted into the culture broth. Finally, the same trend in pH variation was observed during growth of *P. aeruginosa* PRD-10 at the three growth temperatures.

Conclusions

P. protegens Pf-5 grew very poorly at 37 ºC where the maximal optical density obtained was 0.66 after 49 hours of cultivation. Growth at 25 ºC resulted in the highest optical density obtained of 5.3. On the other hand, *P. protegens* Pf-5 demonstrated significant adaptation for growth at 30 ºC given a maximal optical density of 4.6 obtained at the growth temperature. However, distinct two phase growth behaviour was observed during exponential growth of *P. protegens* Pf-5 in LB Lennox medium at 30 ºC, which suggested that the bacterium could preferentially utilized one component of the medium relative to another. Additionally, growth at 30 ºC likely utilized metabolic pathways that were not as efficient as those utilized during growth at 25 ºC for converting nutrients into biomass. Observation of pH variation with culture time during growth at 25 and 30 ºC revealed no net secretion of acidic metabolites into the culture broth. Finally, growth of *P. protegens* Pf-5 at 37 ºC revealed substantial cellular maintenance with the secretion of significant amounts of alkaline metabolites that resulted in a high pH of 8.4 after 38 hours of cultivation during growth at an unfavourable temperature, which the bacterium was not adapted to.

Overall, *P. aeruginosa* PRD-10 was more efficient in converting nutrients in LB Lennox medium into biomass given that its growth at all three growth temperatures resulted in maximal optical density higher than that of *P. protegens* Pf-5. Growth data further revealed that *P. aeruginosa* PRD-10 was adapted to growth at 25 and 37 ºC and less so at 30 ºC, which opens up interesting questions concerning how cellular enzymatic machinery adapts to temperature. Specifically, given that biomass formation was lower at an intermediate growth temperature between two adapted ones, enigmatic details remain to be elucidated concerning how temperature response was encoded by the behaviour of enzymes to different growth temperatures. For example, interesting questions such as: is there a temperature sensor in *P. aeruginosa* PRD-10, and how does it sense and respond to temperature changes? Furthermore, biomass formation was more efficient and cell growth faster at 25 and 37 ºC compared to 30 ºC, which provided more evidence that the soil bacterium *P. aeruginosa* PRD-10 was originally adapted for growth at 25 ºC, but later became accustomed to growth at 37 ºC. Observation of pH variation during culture revealed that with higher growth temperatures, more alkaline metabolites were secreted into the culture broth. Finally, the general trend of pH variation of the bacterium was similar for all three growth temperatures used.

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Conflicts of interest

The author declares no conflicts of interest.

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