

## Aging and Respiration in the Marine Bacteria, *Pseudomonas nautica*

Vanesa Romero Kutzner<sup>1</sup>, Theodore Train Packard<sup>1</sup>, Elisa Berdalet<sup>2</sup>, Silvie O. Roy<sup>3</sup>, Jean Pierre Gagné<sup>4</sup> & May Gómez<sup>1</sup>

<sup>1</sup> Marine Ecophysiology group (EOMAR), IU-ECOQUA, Universidad de Las Palmas de Gran Canaria 35017, Spain

<sup>2</sup> Institut de Ciències del Mar (CSIC), Passeig Marítim de la Barceloneta, 37-49, 08003 Barcelona, Spain

<sup>3</sup> 10 Ryan Court, Embrun, Ontario K0A 1W0, Canada

<sup>4</sup> Université du Québec à Rimouski, Rimouski, Québec G5L 3A1, Canada

### ABSTRACT

The effect of aging and substrate limitation on respiratory metabolism was compared during two different physiological states of acetate-grown *Pseudomonas nautica* cultures. In this study, we analyze 30 h and 170 h time-course experiments in which cell-protein, acetate, respiratory CO<sub>2</sub> production (RCO<sub>2</sub>) and respiratory O<sub>2</sub> consumption (RO<sub>2</sub>) were measured and respiratory quotients (RQs= RCO<sub>2</sub>/RO<sub>2</sub>) were calculated. RQs are commonly used as a constant, over a range of 0.8 to 1, in ocean studies, to evaluate many factors such as the autotrophic-heterotrophic nature of the ocean and the vertical carbon flux. In the laboratory we observed that RQ can rise an order of magnitude above this constant. In the early well-fed growth stage of our short term experiment RO<sub>2</sub> rates were higher than RCO<sub>2</sub> rates (RQ=0,4), nevertheless in senescence and under limited carbon-substrate conditions this ratio shifted up to 9,6. We conclude that the culture aging and substrate limitations affected the RQ of *P. nautica* culture, in a way that the use of a constant RQ for biological calculations is inadvisable. Therefore, these results argue that oceanic measurements which are influenced by bacterial metabolism and nutrient limitation need to be reconsidered in face of this strong RQ variability. Lastly, because our microbial biomass remained relatively constant in face of nutrient-limitation, our results suggest a link between substrate deprivation, senescence and possibly, longevity.

### INTRODUCTION

The oligotrophic ocean has a direct influence on the metabolism of different marine organisms. This metabolism is mainly controlled by food availability and respiration. Respiration in the ocean has many facets, but in the most of the world's ocean it consumes oxygen and produces water and CO<sub>2</sub>. Knowing respiration rates in the water column is essential to quantify carbon fluxes [1]. The respiration quotient, or RQ, is essential in calculating either organic carbon consumption or CO<sub>2</sub> production (RCO<sub>2</sub>) from respiratory O<sub>2</sub> consumption (RO<sub>2</sub>) measurements, whether in the laboratory or in the ocean [2,3,4]. In the present study we measure the respiration in cultures of the marine bacteria, *Pseudomonas nautica*, in two different aging experiments in which the bacteria pass from carbon substrate sufficiency to carbon substrate limitation. We expected that the RQ variability would be related to culture aging and to carbon consumption.

### MATERIAL & METHODS

To investigate respiration in different bacterial growth stages, time-course experiments were run on batch cultures at 22°C, maintained on acetate as described by [5] and [6]. A short-term experiment was run for 30 h (strain 2906), and long-term experiment (strain 1204) was run for 2 weeks. *P. nautica* was grown in 500 ml cotton-plugged Erlenmeyer flasks with 100 ml of culture media on an orbital shaker (100 rpm). The following measurements were carried out: RCO<sub>2</sub> and RO<sub>2</sub> were measured by a respirometer (Oxymax), growth by optical density [OD550], turbidity was measured as absorbance at 550 nm,

protein and acetate (substrate) as the cultures grew exponentially, reached steady state, and passed into senescence.

### RESULTS & DISCUSSION

In this study, we observed a rising RQ after the stage of nutrient depletion. The RQ ranged from a low of 0.3 during exponential growth to a high of 9.6 during senescence and nutrient depletion in short term experiments (Fig. 1).

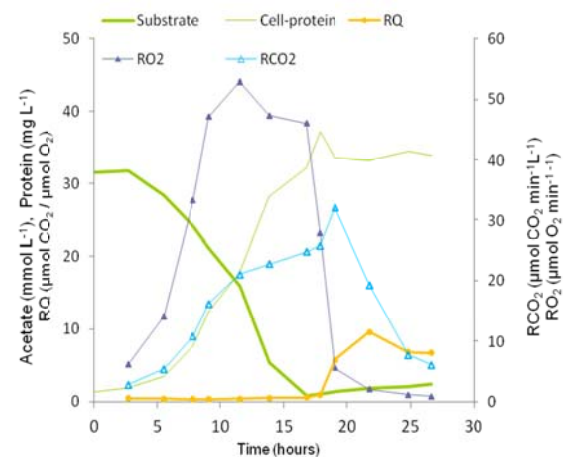


Fig. 1. Short term experiment. Effect of aging and substrate limitation on the RQ in *Pseudomonas nautica* (strain 2906). For display-clarity, the cell-protein data have been divided by 10.

In comparison, the RQs from fresh water bacteria of Quebec ranged from 0.25 to 2.26 [7]. Those authors also found that the common use as a constant RQ value (between 0.8 and 1.0) was not justified. In our study, it is not easy to explain how RQ could rise to 9.6, but it did. In Fig. 1 the  $RO_2$  and  $RCO_2$  curves are out of phase, meaning that their peaks are displaced in time.  $RO_2$  was higher than  $RCO_2$  in substrate sufficiency. During starvation  $RO_2$  fell rapidly,  $RCO_2$ , on the other hand, first peaked and then descended. As in the short term experiment, in long term experiment,  $RCO_2$  and  $RO_2$  decreased in the first 15 h (Fig. 2).

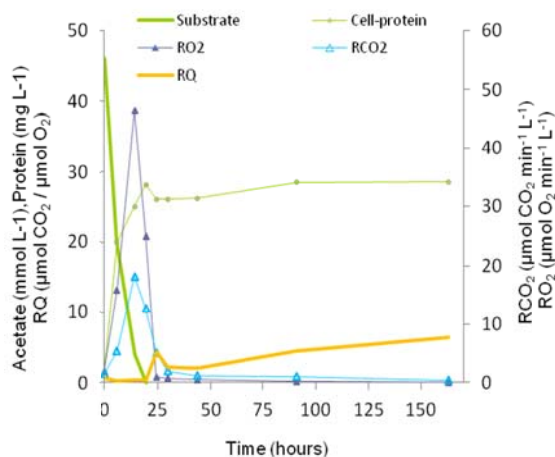


Fig. 2. Long term experiment. Effect of aging and substrate limitation on the RQ in *Pseudomonas nautica* (strain 1204). For display-clarity, the cell-protein data have been divided by 10.

Here (Fig. 2) the RQ presents a peak (5.3) at hour 25, then it decreases and starts to increase again until it reaches a higher value, 7.8 at 163 h. Which reactions are involved in this process is unknown. As shown in Fig. 1 and Fig. 2, cell-protein content shifts from a gradual increase with substrate sufficiency to a steady state with substrate deficiency conditions. This is because the starvation response in aquatic bacteria switches cell physiology from growth to metabolic maintenance. Specifically, growth is limited by ATP deficiency. In addition, our recent research has shown that starvation decreases  $RO_2$  without decreasing the activity of ETS [8], this same behavior was shown in a nematode aging study [9]. In this cited study, the impact was traced to increases in the alpha-keto glutarate ( $\alpha$ -KG) concentration, which is related to longevity.  $\alpha$ -KG and  $CO_2$  are products of IDH activity, nevertheless in [8] an increase in  $RCO_2$  and IDH was not observed. But then, could prolonged substrate limitation have caused an increase in  $\alpha$ -KG concentration in our aging culture? To address this question further experimentation is needed.

-Conclusions:

- (1) In early growth and when the culture is well-fed, no RQ changes are observed. However, under continuing substrate-limitation conditions, RQs are higher.
- (2) In senescence, RQs rise by nearly a factor of 10 (Fig. 1). Limited nutritional conditions of bacteria should be considered, when selecting an RQ for calculation purposes.
- (3) Respiration under well-fed cultures of *P.nautica* show that  $RO_2$  and  $RCO_2$  during substrate-sufficiency parallels the biomass increase (Fig. 1 & 2), but after the carbon-substrate is exhausted, both respiration rates fall rapidly in starved conditions.
- (4) If this RQ variability is found in oceanic studies, then predictions of ocean's metabolic state and carbon flux calculations could be affected.

#### ACKNOWLEDGEMENTS

This work was supported by BIOMBA project CTM 2012-32729/MAR granted to M.G., the Canarian Government (Gobierno de Canarias) thesis/2015 granted to V.R.K.

#### REFERENCES

- 1 - Osma N, Aristizabal M, Fernández-Urruzola, I, Packard TT, & Gómez M, 2016. Influence of Starvation on Respiratory Metabolism and Pyridine Nucleotide Levels in the Marine Dinoflagellate *Oxyrrhis marina*. *Protist*, 167 (2):136-147.
- 2 - Boucher G, Clavier J, Garrigue C, 1994. Oxygen and carbon dioxide fluxes at the water-sediment interface of a tropical lagoon. *Mar Ecol Prog Ser* 107:185-193
- 3 - Bergström I, 2011. Carbon gas fluxes from boreal aquatic sediments. *Monogr Boreal Environ Res* 38
- 4 - Giering SLC, Sanders R, Lampitt RS, Anderson TR and others, 2014. Reconciliation of the carbon budget in the ocean's twilight zone. *Nature* 507:480-483
- 5 - Berdalet E, Packard TT, Legacé B, Roy S, St-Amand L, Gagné JP, 1995.  $CO_2$  production,  $O_2$  consumption and isocitrate dehydrogenase in the marine bacterium *Vibrio natriegens*. *Aquat Microb Ecol* 9:211-217
- 6 - Packard TT, Berdalet E, Blasco D, Roy SO and others, 1996. Oxygen consumption in the marine bacterium *Pseudomonas nautica* predicted from ETS activity and bisubstrate enzyme kinetics. *J Plankton Res* 18:1819-1835
- 7 - Berggren M, Lapierre J, Del Giorgio PA, 2012. Magnitude and regulation of bacterioplankton respiratory quotient across freshwater environmental gradients. *ISME J* 6:984-993
- 8 - Romero-Kutzner V, Packard TT, Berdalet E, Roy SO, Gagné JP, Gómez M, 2015. Respiration quotient variability: bacterial evidence. *Mar Ecol Prog Ser* 519:47-59
- 9 - Chin RM, Fu X, Pai MY, Vergnes L and others, 2014. The metabolite  $\alpha$ -ketoglutarate extends lifespan by inhibiting ATP synthase and TOR. *Nature* 510: 397-401