國立臺灣海洋大學
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題目：使用修正的病毒稀釋法同時估計病毒裂解和原生動物攝食對細菌死亡率的影響

Simultaneous estimation of viral lysis and protozoan grazing on bacterial mortality using a modified virus-dilution method

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Abstract

A modified dilution method designed to simultaneously estimate bacterial mortality due to viral infection and protozoan grazing was developed and compared with the standard dilution protocol. Various fractions of original seawater (non-filtered) and 1.0 μm-filtered seawater (grazer-free) collected from coastal waters in Hokkaido, Japan, were diluted with 10 kDa filtered seawater (virus- free) to set up 4 gradients of predator–prey interaction, and monitored every 12 h for bacterial abundance during a 48 h incubation. In more diluted fractions, bacterial abundance increased rapidly, and a good linear fit with a negative slope (as mortality) was found between the apparent growth rate and fractions of original water. The resulting slopes of the regression in samples prepared from the original seawater were significantly higher than grazer-free samples, which denotes both viral lysis and protozoan grazing in the former, and only viral lysis in the latter. Ranges of specific lytic and grazing rate were 0.53 to 0.98 and 0.05 to 0.13 d^{-1}, respectively, and lytic pressure accounted for 87 to 91% of the total mortality. Comparison of our method (using virus-free diluent) with the standard dilution protocol (using 0.2 μm diluent) showed significant differences in slope and y-intercept (potential growth rate without mortality) found at 0.26 d^{-1} and 0.88 d^{-1}, respectively. The above results suggest that using the standard dilution protocol might underestimate instantaneous growth rate, particularly in environments where lytic pressure is relatively high.

摘要

本實驗開發研究了一種修正的稀釋方法，旨在同時估計病毒感染和原生動物攝食對細菌死亡率的影響程度，並與先前標準稀釋培養法進行比較。本實驗由日本北海道沿海水域收集的原始海水（未過濾）和 1.0 μm 過濾海水（無攝食者存在）各用 10 kDa 過濾海水（無病毒存在）來進行稀釋，以此建立 4 個不同稀釋梯度的捕食者—餌料相互作用進行培養，培養時間為 48 小時並以每 12 小時採取培養水樣來觀測細菌數量變化。在較高稀釋的培養組中，細菌數量迅速增加，其結果發現細菌淨成長速率與各稀釋比之間呈現明顯負斜率（視為細菌死亡率）的迴歸關係。其中未過濾原始海水所造成的稀釋培養組(代表病毒裂解和原生動物攝食影響)其迴歸線的斜率顯著高於 1.0 μm 過濾海水（只有病毒裂解）的培養組。

培養結果顯示病毒裂解率和攝食率的變化範圍各分別為 0.53 至 0.98 和 0.05 至 0.13 d^{-1} 之間，可知病毒裂解率佔細菌總死亡率的 87% 至 91%。如以標準稀釋培養方法（使用 0.2 μm 過濾液稀釋）來比較，發現迴歸線斜率和 y 截距（無死亡率存在的原始成長率）各為 0.26 d^{-1} 和 0.88 d^{-1}，其值與本實驗所推估的數值有明顯的差異。由上述結果表明，特別是在病毒裂解能力對細菌死亡壓力相對高的環境中，使用標準稀釋培養方式可能會造成細菌原始成長率的低估。