Oxygen-requiring molecular reporters of gene expression for anaerobic microorganisms

Matti Karp*[1] and Simone Guglielmetti*[2]

[1]Laboratory of Chemistry and Bioengineering, Tampere University of Technology (KEB, TUT) – Korkeakoulunkatu 8, FI-33101 Tampere, Finland, Finland

[2]Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente (defens) – Via Mangiagalli, 25 20133 - Milano (MI), Italy

Abstract

Many genetic reporter systems need molecular oxygen and therefore reporter genes used in studies for understanding molecular mechanisms in anaerobic microorganisms have hampered the lack of convenient and real-time performance. These microbes represent 98 % of all living material on earth and therefore it is of utmost importance to generate reliable and sensitive reporters of gene expression. We describe here whole cell-based reporter gene systems that are based on luciferase genes and the oxygen-needing enzymes thereof. We show here by using two different oxygen-needing reporters, insect and bacterial luciferases, and two bacterial hosts, gram-(+) *Bifidobacterium longum* and gram-(-) *Escherichia coli*, respectively, that surprisingly those systems can be used in gene expression studies of anaerobic bacteria. *E. coli* being a facultative anaerobe was grown both in aerobic and anaerobic conditions with an arabinose inducible expression system. It was noticed that short treatment time of five minutes in ambient atmosphere is enough to get light emission from living cells that is directly proportional to the amount of cells, dynamic measurement range of 6 orders of magnitude, and inducer concentration. The induction levels were same both in aerobically and anaerobically grown cells. Similar results, except that the incubation in ambient oxygen conditions of 40 min, were obtained in case of *B. longum* grown in anaerobic conditions. Compared to previously used reporter genes such as fluorescent proteins or enzymes utilizing chromogenic substrates, luciferases are superior in overall performance.

Keywords: bioluminescence, gram, negative, gram, positive, luciferase, arabinose induction, Photorhabdus, Pyrophorus, real, time

*Speaker