Variability in oxygen tolerance among bacterial strains associated with the normal intestinal microbiota

Andrew Pridmore and Charlotte Austin / Don Whitley Scientific Limited, BD16 2NH, United Kingdom

ABSTRACT: Anaerobic incubation methods are widely used to cultivate pathogenic anaerobes, but recent years have seen increased interest in potential therapeutically species originating from the normal intestinal microbiota. We compared the abilities of selected anaerobic pathogens, "normal microbiota" and potentially therapeutic strains to grow on agar at 35°C in the presence of increasing oxygen concentrations, using a variable atmosphere workstation to control oxygen concentration in growth media. A panel of 12 anaerobes, encompassing potential pathogens, normal microbiota and potentially therapeutic species, was tested. Each strain was cultured in strict anaerobiosis and, simultaneously, in the presence of O₂ (0.1 - 2.5% v/v). Initial qualitative experiments used a high inoculum to establish the maximum O₂ concentration tolerated. Subsequent quantitative experiments determined the % recovery (compared with anaerobiosis) in the presence of various O₂ concentrations below the maximum tolerated. For each method, experimental details and results are presented diagrammatically below.

05A / Variability in oxygen tolerance among bacterial strains associated with the normal intestinal microbiota

Don Whitley Scientific (DWS) has worked with anaerobic culture equipment for over 40 years. Most anaerobic workstations are used for the culture of clinically significant species (pathogens). There have been recent increases in work with normal healthy microbiota and potentially therapeutic strains. Recently, characterized potential therapeutic anaerobes tend to be nutritionally fastidious. There is evidence that such strains are also fastidious with regard to oxygen tolerance. We wanted to establish how different they are from clinical anaerobes cultured readily for many years and whether they require special handling.

METHOD - INITIAL SCREEN

1. Whitley A35 Anaerobic Workstation / ANO2 / 48h / 35˚C

Cultures suspended in anaerobic (pre-reduced) Medium Recovery Diluent to density of 0.5 McFarland Standard. From each concentration (0.1 - 2.5% O₂) 3 x 1.5 cm plates were used to check replicate plates (n = 1.5 cm plates per strain). Cultures grown at 35°C in the presence of increasing oxygen concentrations, using a Whitley Hypoxystation with oxygen concentrations well below the maximum tolerated. For each method, experimental details and results are presented diagrammatically below.

METHOD - QUANTITATIVE RECOVERY

1. Whitley A35 Anaerobic Workstation / ANO2 / 48h / 35˚C

Each strain cultured anaerobically in liquid Medium Recovery Diluent (Medium Recovery Diluent + Anaerobic Medium). Each strain was cultured in liquid recovery for 24h. Cultures were prepared to grow under oxygen containing (1% O₂) and non-oxygen containing (0% O₂) conditions. Percentage recoveries compared with anaerobiosis were determined in the presence of various O₂ concentrations. Each strain was cultured at 2.5% v/v. Oxygen concentration in growth media was calculated as follows: Colonies on anaerobic plate / Colonies on O₂ exposure plate x 100.

Colonies on anaerobic plate / Colonies on O₂ exposure plate x 100.

Maximum % O₂ compatible concentration at 0.1%.

Each strain was cultured anaerobically in liquid Medium Recovery Diluent (Medium Recovery Diluent + Anaerobic Medium). Each strain was cultured in liquid recovery for 24h. Cultures were prepared to grow under oxygen containing (1% O₂) and non-oxygen containing (0% O₂) conditions. Percentage recoveries compared with anaerobiosis were determined in the presence of various O₂ concentrations. Each strain was cultured at 2.5% v/v. Oxygen concentration in growth media was calculated as follows: Colonies on anaerobic plate / Colonies on O₂ exposure plate x 100.