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

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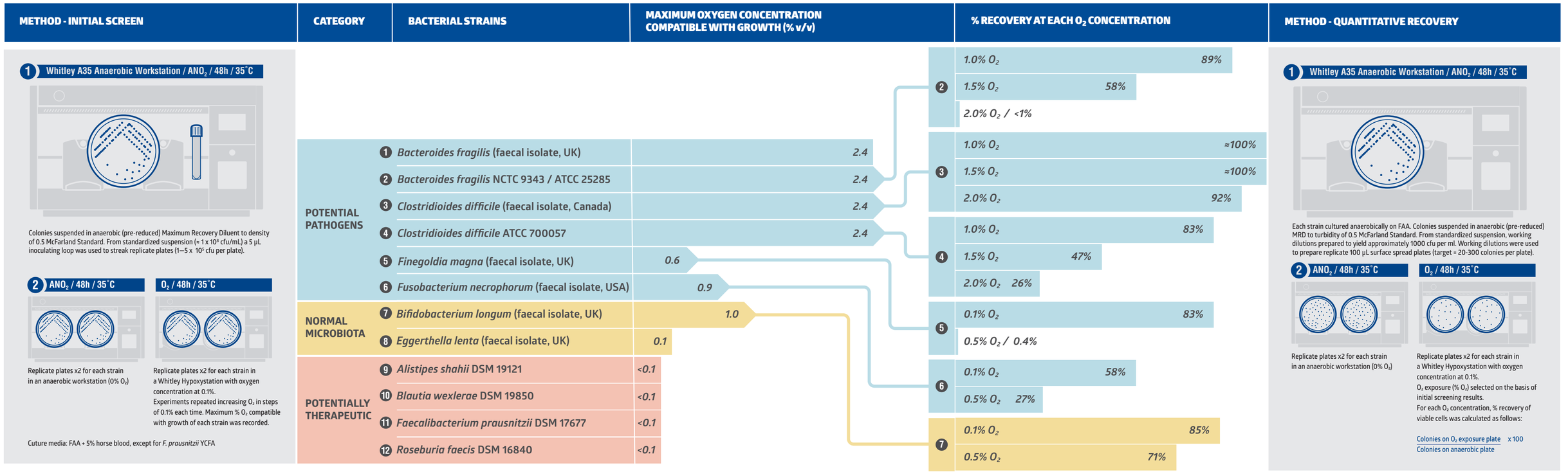
BACKGROUND: Don Whitley Scientific (DWS) has worked with anaerobic culture equipment for over 40 years. Most anaerobic workstations are used for the culture of clinically significant species (pathogens). There have been recent increases in work with normal healthy microbiota and potentially therapeutic strains. Recently characterized potentially 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.

METHODS: A panel of 12 anaerobes, encompassing potential pathogens, normal microbiota and potentially therapeutic species, was tested. Each strain was cultivated in strict anaerobiosis and, simultaneously, in the presence of O_2 (0.1 – 2.5% v/v). Initial qualitative experiments used a high inoculum to establish the maximum O_2 concentration tolerated. Subsequent quantitative experiments determined the % recovery (compared with anaerobiosis) in the presence of various O_2 concentrations below the maximum tolerated. For each method, experimental details and results are presented diagrammatically below.



ABSTRACT: Anaerobic incubation methods are widely used to cultivate pathogenic anaerobes, but recent years have seen increased interest in potentially therapeutic species originating from the normal intestinal microbiota.

We compared the abilities of selected anaerobic pathogens, "normal microbiota" and more recently characterized 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 increments of 0.1%.

In initial screening with a high inoculum of 10^5 to 10^6 cfu on streak plates, *Bacteroides fragilis* and *Clostridioides difficile* strains grew in the presence of up to 2.4% v/v oxygen. *Bifidobacterium*, *Fusobacterium* and *Finegoldia* strains tolerated 0.5 – 1.0% and *Eggerthella lenta* tolerated 0.1%. Strains of *Roseburia*, *Alistipes*, *Blautia* and *Faecalibacterium* grew only in strictly anaerobic conditions (<0.01% oxygen) and not in 0.1% oxygen.

For strains tolerating >0.1% oxygen in initial experiments, percentage recoveries of smaller inocula (100 – 300 cfu on surface spread plates) were determined in atmospheric oxygen concentrations increasing by 0.1% increments, in comparison with strictly anaerobic colony counts. In 2.0% v/v oxygen, inoculum recovery for 2 × *B. fragilis* and 1 × *C. difficile*, was >80%, while recovery of a second *C. difficile* strain was 26%. For *F. magna*, inoculum recovery ranged from approximately 83% in 0.1% oxygen to <1% in 0.5% oxygen.

These findings demonstrate the variable oxygen tolerance of obligately anaerobic bacteria and emphasize the need for stringent anaerobiosis when culturing the more recently characterized strains currently being developed as live biotherapeutic products.

DISCUSSION: Initial experiments with high inoculum densities revealed marked variability between anaerobe strains with regard to maximum atmospheric O_2 compatible with growth. *Bacteroides and C. difficile* strains grew on agar in >2% O_2. *Roseburia, Alistipes, Blautia* and *Faecalibacterium* strains were unable to grow from dense inocula in 0.1% O_2.

These observations suggest that strict anaerobic conditions and techniques are required when working with these strains.

Quantitative recovery experiments (low inoculum densities) were performed with anaerobe strains able to grow in O_2 concentrations > 0.1% (mostly potential pathogens). There was a marked reduction in recovery of low inocula at concentrations well below the maximum tolerated in initial screen.

In addition, bacterial cell density affects apparent oxygen tolerance. The effects of oxygen may not be evident if sufficient cells are present. Our results demonstrate that the "normal microbiota" and potentially therapeutic strains are especially intolerant to O_2 exposure.