FRIDAY-
321

Evaluation of Oxyrase® Agar Media for Growth and Selection of Anaerobic Isolates

L. M. Koeth[1], J. DiFranco-Fisher[1], A. R. Windau[2]
[1]Lab. Specialists, Inc., Westlake, OH, [2]Microtech Lab., Westlake, OH

Contact information:
Laura Koeth
Laboratory Specialists, Inc.
1651 A Crossings Parkway
Westlake, OH 44145
Tel: +1 440 835 4458
Fax: +1 440 835 5786
E-mail: info@labspec.org

Abstract

Background: Oxyrase® (Oxyrase, Inc., Mansfield, OH) is an enzyme system that removes dissolved oxygen from liquid, gas or semisolid products. Oxyrase® is included in agar media that are typically used to grow and select for anaerobic organisms. Oxyrase allows the plate to remain reduced on the bench up to 2 hours. This study was performed to evaluate the growth of anaerobic bacteria on Oxyrase Brucella and Oxyrase Schaedler agar plates compared to conventional media. Oxyrase selective media was also evaluated for growth of specific anaerobic organisms from mixed isolate cultures. **Methods:** The study isolates were stock strains from clinical sources that included various Gram-positive and Gram-negative aerobes and anaerobes. Within the anaerobe chamber, inocula were prepared and plated onto control media (Remel brucella agar w/5% sheep blood, hemin, vitamin K and Becton Dickinson tryptic soy agar with 5% sheep blood). In addition, inocula were plated onto Oxyrase media (Schaedler OxyPlate, Brucella OxyPRAS, BBE, KVL, AnaSelect and PEA) outside the anaerobe chamber. All plates were incubated under anaerobic conditions for 48 hours. Phase I consisted of single isolate cultures plated onto control media. Phase II consisted of 3 separate mixed isolate cultures (anaerobic and aerobic) plated onto various selective media. **Results:** As shown in the table, bacteria concentration was similar on all tested media. The colony sizes were also comparable. In Phase II, the selective media also showed good growth and selection/inhibition of specified organisms according to manufacturer specifications.

		CFU/mL Range		
Organism	n	Remel Brucella	Brucella OxyPras	Schaedler OxyPlate
***Bacteroides* spp.**	4	2 - 5.4 x 10^7	8 x 10^7 - 2.6 x 10^8	1.8 x 10^7- 1.5 x 10^8
***Clostridium* spp.**	3	1 x 10^6 - 9.9 x 10^7	7 x 10^6 - 7.6 x 10^7	1 x 10^6 - 1.5 x 10^7
Eubacterium lentum	1	3.8 x 10^8	4.4 x 10^8	2.1 x 10^8
Fusobacterium nucleatum	1	6.8 x 10^7	4.4 x 10^7	4.8 x 10^7
***Peptostreptococcus* spp.**	2	4 x 10^6- 5 x 10^7	2.6 - 3.6 x 10^7	6 - 8.0 x 10^6
***Prevotella* spp.**	2	1.6 x 10^8 - 3.1 x 10^8	1.4 - 2.1 x 10^8	1.9 x 10^{8a}
Propionibacterium acnes	1	2 x 10^6	1.2 x 10^7	1.4 x 10^7
***One isolate (*Prevotella bivia*) had no colonies on Schaedler OxyPlate after 48 hours incubation**				

Conclusions: Count and size of colonies on the Schaedler OxyPlate and the Brucella OxyPRAS plate (inoculated aerobically and incubated anaerobically) were similar to control plates (inoculated and incubated anaerobically). The Oxyrase selective media (BBE, KVL, AnaSelect and PEA) allowed for growth of specified organisms.

Introduction

- Oxyrase® (Oxyrase, Inc., Mansfield, OH) is a versatile product that utilizes an enzyme system capable of removing dissolved oxygen from liquid, gas or semisolid products by selectively capturing oxygen and with a substrate converting it to water. The substrates and reactants are all-natural and designed to work efficiently over wide ranges of pH and temperature. Oxyrase is incorporated into agar media that are typically used for the growth and selection of anaerobic organisms and allows the media to remain reduced on the bench up to 2 hours. All Oxyrase media include PRAS (PreReduced Anaerobically Sterilized) and are available in two formats: OxyPRAS Plus® Plate and OxyPlate™. Both formats include Oxyrase and have the advantage of plating and protecting anaerobic specimens in an open aerobic environment. OxyPlate is an anaerobic media made using the patented OxyDish™ which creates its own self–generating anaerobic environment allowing plates to be inoculated and incubated aerobically, eliminating the need for bags, jars and chambers. OxyPRAS Plus Plate media utilizes a standard petri dish that can be inoculated aerobically but requires incubation in anaerobic conditions.
- The purpose of this study was to evaluate the growth of anaerobic bacteria on Oxyrase Brucella blood agar (OxyPRAS PlusPlate) and Schaedler Blood Agar (OxyPlate) compared to conventional media. Oxyrase selective media was also evaluated for growth of specific anaerobic organisms from mixed isolate cultures.

Methods

Bacterial Strains

- Study isolates were stock strains from clinical sources including various Gram-positive and Gram-negative aerobes and anaerobes as shown in Table 1.

Phase I – single cultures

- Inocula for 14 anaerobic bacterial strains was prepared under anaerobic conditions inside the anaerobe chamber using pre-reduced Brucella broth.
- Control plates (Remel Brucella agar w/5% sheep blood, hemin, vitamin K and Becton Dickinson tryptic soy agar with 5% sheep blood) were inoculated anaerobically inside the anaerobe chamber. After 48 hours incubation inside the anaerobe chamber, colony counts and sizes were determined and recorded.
- Oxyrase plates (Schaedler Blood Agar OxyPlate™ and Brucella Blood Agar OxyPRAS Plus® Plate) were inoculated aerobically on the bench and incubated anaerobically inside the anaerobe chamber. An additional set of Schaedler Blood Agar OxyPlates™ were also incubated aerobically on a separate testing day. After 48 hours of incubation, colony counts and sizes were determined and recorded.

Phase 2 – mixed cultures

- Inocula for bacterial strains in Mix 1 culture (*Bacteroides fragilis, Clostridium perfringens, Eubacterium lentum, Prevotella bivia, Staphylococcus aureus* and *Escherichia coli*), Mix 2 culture (*B. fragilis, C. perfringens, Fusobacterium nucleatum, Peptostreptococcus* spp., *P. bivia, S. aureus*, *E. coli* and *Proteus mirabilis)* and Mix 3 culture (*B. fragilis, C. perfringens, S. aureus*, *E. coli* and *P. mirabilis*) were prepared inside the anaerobe chamber using pre-reduced Brucella broth. Oxyrase and control agar plates were inoculated aerobically on the bench. Presence or absence of growth of each bacterial species was assessed after 48 hours anaerobic incubation inside the anaerobe chamber.
- Mix 1 was inoculated to KVL OxyPlates, KVL OxyPRAS, BBE OxyPRAS, BBE/KVL OxyPRAS Bi-Plate and Remel Brucella agar. Mix 2 was inoculated to ANASelect OxyPRAS and Remel Brucella agar. Mix 3 was inoculated to PEA OxyPRAS and Remel Brucella agar.

Results

Phase I– single cultures

- As shown in Table 1, the concentration of bacteria (CFU/mL) was similar on all tested media. The CFU/mL on the Schaedler OxyPlates was also comparable on plates incubated aerobically and anaerobically. In addition, there were no significant differences in colony size between the various selective media as compared to the control agar plates.

Phase 2– mixed cultures

- As shown in Figures 1-3, the Oxyrase selective media (KVL OxyPRAS, KVL OxyPlate, BBE/KVL OxyPRAS Bi-plate, ANASelect OxyPRAS and PEA OxyPRAS) showed good growth and selection/inhibition of anaerobic and aerobic bacterial species according to the manufacturer specifications.
- For Mix 1 culture (Figure 1), the Oxyrase selective media inhibited the growth of *S. aureus* and *E. coli*, allowing for the isolation of the anaerobic species (*B. fragilis, C. perfringens, E. lentum, P. bivia*) included in the mixed bacterial culture. All aerobic and anaerobic bacterial species in Mix 1 showed good growth on the control plates.
- For Mix 2 culture (Figure 2), the ANASelect OxyPRAS agar plates inhibited the growth of *S. aureus*, *E. coli* and *Proteus mirabilis* allowing for the isolation of the anaerobic species (*B. fragilis, C. perfringens, F. nucleatum, Peptostreptococcus* spp., *P. bivia)* in the mixed culture. All aerobic and anaerobic bacterial species in Mix 2 showed good growth on the control plates.
- For Mix 3 culture (Figure 3), the PEA agar plates inhibited the swarming of *P. mirabilis* allowing for the isolation of the anaerobic species (*B. fragilis, C. perfringens*) in the mixed culture. All bacterial species in Mix 3 grew on control plates and *P. mirabilis* showed swarming over entire plate.

Conclusions

- **The evaluation of the growth on the Brucella OxyPRAS plate and Schaedler OxyPlate (inoculated aerobically and incubated anaerobically) as compared to the control agar plates showed similar bacterial concentrations and colony sizes.**
- **Schaedler OxyPlates incubated in aerobic conditions showed similar bacterial concentrations and colony sizes as compared to Schaedler OxyPlates incubated anaerobically.**
- **The Oxyrase selective media (ANASelect, BBE, KVL and PEA) allowed for the growth of specific bacterial species while inhibiting the growth of others according to the expected performance as outlined in their respective product inserts.**

Table 1. Evaluation of Anaerobic Bacterial Growth On Oxyrase Agar Plates Compared to Conventional Agar

		CFU/mL Range				
Organism	n	Remel Brucella (ANO_2)	Brucella OxyPRAS (ANO_2)	Schaedler OxyPlate (ANO_2)	Schaedler OxyPlate (O_2)[a]	BD BAP (ANO_2)[a]
Bacteroides spp.	4	2 - 5.4 x 10^7	8 x 10^7 - 2.6 x 10^8	1.8 x 10^7- 1.5 x 10^8	4 x 10^7 - 2.2 x 10^8	9.8 x 10^7 - 5.2 x 10^8
Clostridium spp.	3	1 x 10^6 - 9.9 x 10^7	7 x 10^6 - 7.6 x 10^7	1 x 10^6 - 1.5 x 10^7	2 x 10^7 - 4.4 x 10^8	8 x 10^6 - 7.5 x 10^8
Eubacterium lentum	1	3.8 x 10^8	4.4 x 10^8	2.1 x 10^8	NT	NT
Fusobacterium nucleatum	1	6.8 x 10^7	4.4 x 10^7	4.8 x 10^7	9.6 x 10^7	8.8 x 10^7
Peptostreptococcus spp.	2	4 x 10^6- 5 x 10^7	2.6 - 3.6 x 10^7	6 - 8.0 x 10^6	4.8 - 9 x 10^7	3 x 10^7 - 2.2 x 10^8
Prevotella spp.	2	1.6 x 10^8 - 3.1 x 10^8	1.4 - 2.1 x 10^8	1.9 x 10^{8b}	1 x 10^7 - 1.9 x 10^8	2.8 - 5 x 10^8
Propionibacterium acnes	1	2 x 10^6	1.2 x 10^7	1.4 x 10^7	NT	NT

Abbreviations: ANO_2 = Anaerobic, O_2= Aerobic; BD BAP = Becton Dickinson blood agar plate; CFU = colony forming unit; NT=Not Tested
[a]Plates inoculated on a different test date than other plates in this table
[b]One isolate (*Prevotella bivia*) had no colonies on Schaedler OxyPlate after 48 hours anaerobic incubation

Figure 1: Bacterial culture (Mix1) on Oxyrase selective media compared to control agar

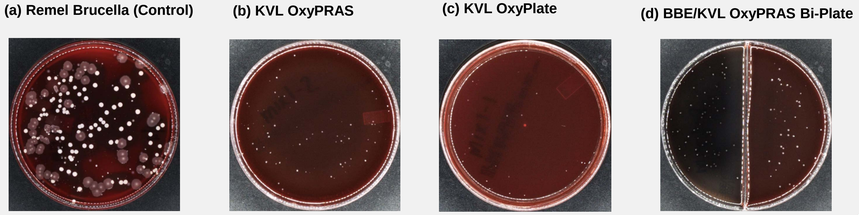


Figure 2: Bacterial culture (Mix2) on Oxyrase selective media compared to control agar

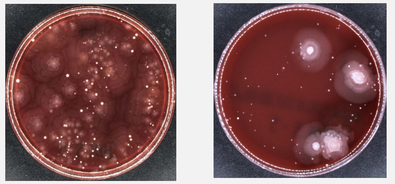
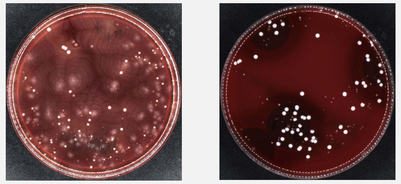


Figure 3: Bacterial culture (Mix3) on Oxyrase selective media compared to control agar



References:

1. Clinical and Laboratory Standards Institute. 2015. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 10th ed. Approved standard, CLSI M7-10, Wayne, PA.
2. Oxyrase product inserts 2015. Available at www.oxyrase.com/technical-information/product-inserts.[Accessed June 2, 2016]

Acknowledgement:

Supplies for this research were provided by Oxyrase, Inc..