A. laidlawii Filtration Through a 0.1 µm Rated Filter at Elevated Pressures

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ABSTRACT

Nine 47 mm 0.1 µm rated filter discs (Pall Fluorodyne® EX Grade EDT) were each challenged with ≥ 10^7 CFU/cm² of *Acholeplasma laidlawii* (ATCC 23260) in 1 L of Mycoplasma Broth Base at 30 and 45 psi. An additional set of nine filters were again challenged at 45 psi with a challenge > 10^8 CFU/cm², allowing for the demonstration of a high titer reduction even at elevated pressure. No penetration was observed at ≥ 10^7 CFU/cm²/30 psi and only 1 cell penetrated 3 out of 9 filters tested at >10^8 CFU/cm²/45 psi. At the excessive > 10^8 CFU/cm² challenge level, only 2 to 7 cells penetrated 4 out of 9 filters. This resulted in a minimum titer reduction of 10^8 (log reduction value, LRV > 8) in all cases. These results along with Fluorodyne EX Grade EDT filters higher filtration capacity and good scale-up provide for an improvement in process economics for filtering a cell culture media batch.

INTRODUCTION

Due to their unique characteristics, mycoplasma are particularly adept at penetrating 0.2 µm rated sterilizing grade filters. As a result, protection from mycoplasma contamination requires the use of 0.1 µm rated filters. We previously reported on the effect of culture media on the generation of cells with an enhanced ability to penetrate 0.2 µm rated filters[1]. We used these cells to challenge 0.1 µm rated filters at elevated pressures (30 and 45 psi).

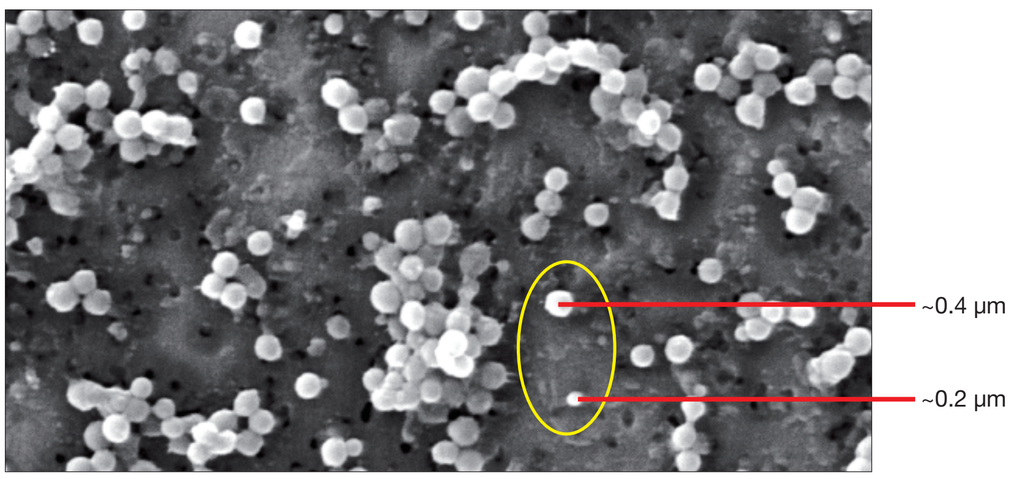
Mycoplasma

- Bacteria that have no cell wall.
- Cells are pleomorphic (assume various shapes from cocci to rods to filaments)
- Cells vary in size from < 0.2 to ≥ 0.5 µm
- Consistently capable of penetrating 0.2 µm rated filters, but are retained by 0.1 µm rated filters.
- The extent of the retention by 0.1 rated filters varies with different filters and depends on the challenge conditions.

Why are Mycoplasma a challenge for filtration?

- Very small procaryotes (bacteria)
- Compare to *Brevundimonas diminuta* at 0.3 – 0.4 µm x 0.6 – 1.0 µm
- Potentially flexible due to absence of rigid cell wall

Figure 1
Acholeplasma laidlawii (mycoplasma)



METHODS AND MATERIALS

Test Conditions

- Test organism: *Acholeplasma laidlawii* ATCC 23206 (*A. laidlawii*)
- Culture media: Mycoplasma broth with 10% horse serum, incubated at 37 °C for 72 hours
- Minimum challenge level: > 1 x 10^7 CFU/cm²
- Challenge volume: 1 L
- Test Filters: 47 mm discs (13.8 cm² EFA) Fluorodyne EX (P/N FTKEDT)
- Test set up: Pressure vessel at 30 and 45 psi connected to sterile, stainless steel, 47 mm disc holders
- All test filters were bubble point tested (IT) in 60:40 IPA:DI water before and after bacterial challenge testing
- All filters passed pre and post IT.

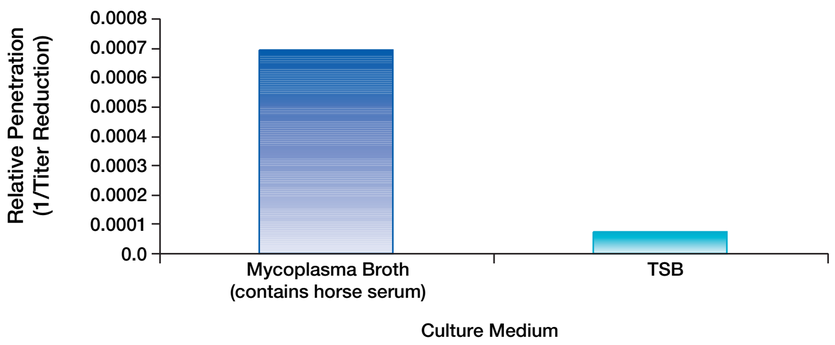
Test Culture Evaluation

- Measurement of penetrative ability of the test mycoplasma culture = degree to which the test culture penetrates a 0.2 µm rated filter
- Test organism is cultivated in test broth
- The titer reduction through a 0.2 µm rated filter is measured
- Relative penetration = 1/Titer Reduction (T_R).

Equation 1
Definition of Titer Reduction

$$TR = \frac{\text{Upstream Titer x Volume = Total Influent}}{\text{Downstream Titer x Volume = Total Effluent}}$$

Figure 3
Relative Penetrative Ability of the Culture Through a 0.2 µm Rated Filters used for Testing of 0.1 µm Rated Filters at Elevated Pressures



RESULTS

Table 1
Results of Testing at 30 psi

Filter (Filter Rating)	Lot Number	Average Flow Rate (mL/min)	Challenge Level/cm² (CFU)	Total Recovery (CFU)	Titer Reduction
FTKEDT (0.1)	00788-030	114.3	5.1E+07	0	7.1E+08
FTKEDT (0.1)	00788-030	90.4	5.1E+07	0	7.1E+08
FTKEDT (0.1)	00788-030	119.3	5.1E+07	0	7.1E+08
FTKEDT (0.1)	00788-027	85.5	5.1E+07	0	7.1E+08
FTKEDT (0.1)	00788-027	95.7	5.1E+07	0	7.1E+08
FTKEDT (0.1)	00788-027	64.8	5.1E+07	0	7.1E+08
FTKEDT (0.1)	02388-012	63.2	5.1E+07	0	7.1E+08
FTKEDT (0.1)	02388-012	97.1	5.1E+07	0	7.1E+08
FTKEDT (0.1)	02388-012	86.5	5.1E+07	0	7.1E+08
FTKNR (0.2)	YA1601	116.0	5.1E+07	7.7E+04	9.2E+03
FTKNT (0.1)	IA5900	28.4	5.1E+07	0	7.1E+08

Table 2
Results of Testing at 45 psi

Filter (Filter Rating)	Lot Number	Average Flow Rate (mL/min)	Challenge Level/cm² (CFU)	Total Recovery (CFU)	Titer Reduction
FTKEDT (0.1)	00788-027	191.7	1.9E+07	0	3.2E+09
FTKEDT (0.1)	00788-027	173.9	1.9E+07	0	3.2E+09
FTKEDT (0.1)	00788-027	175.4	1.9E+07	1	3.2E+09
FTKEDT (0.1)	00788-030	182.9	1.9E+07	0	1.1E+09
FTKEDT (0.1)	00788-030	196.7	1.9E+07	1	6.0E+08
FTKEDT (0.1)	00788-030	181.3	1.9E+07	1	2.1E+09
FTKEDT (0.1)	02388-012	140.8	1.9E+07	0	3.2E+09
FTKEDT (0.1)	02388-012	135.4	1.9E+07	0	3.2E+09
FTKEDT (0.1)	02388-012	152.7	1.9E+07	0	3.2E+09
FTKNR (0.2)	IV257	176.5	1.9E+07	> 200	ND
FTKNT (0.1)	IA5980	40.3	1.9E+07	0	3.8E+08

Elevated Challenge

- The required minimal challenge level for a sterilizing grade filter is defined as 1 x 10^7 CFU/cm² of effective filter area.
- For 0.1 µm rated filters, we use the same minimal challenge level

However, as an even more difficult challenge:

- A 10-fold increase in minimum challenge level
- As before, utilizing a culture grown under conditions that generate mycoplasma cells with an enhanced penetrative ability.

Table 3
Results of Testing at 45 psi with a > Ten-fold Excess Challenge Level

Filter (Filter Rating)	Lot Number	Average Flow Rate (mL/min)	Challenge Level/cm² (CFU)	Total Recovery (CFU)	Titer Reduction
FTKEDT (0.1)	00788-030	90.0	2.3E+08	1	3.2E+09
FTKEDT (0.1)	00788-030	97.9	2.3E+08	0	3.2E+09
FTKEDT (0.1)	00788-030	107.7	2.3E+08	0	3.2E+09
FTKEDT (0.1)	00788-027	101.9	3.0E+08	4	1.1E+09
FTKEDT (0.1)	00788-027	107.0	3.0E+08	7	6.0E+08
FTKEDT (0.1)	00788-027	84.2	3.0E+08	2	2.1E+09
FTKEDT (0.1)	02388-012	60.7	2.3E+08	0	3.2E+09
FTKEDT (0.1)	02388-012	59.7	2.3E+08	0	3.2E+09
FTKEDT (0.1)	02388-012	57.3	2.3E+08	0	3.2E+09
FTKNR (0.2)	NG0257	116.1	3.0E+08	1.3E+06	3.2E+03
FTKNT (0.1)	IA5980	28.4	1.0E+08	4	3.8E+08

CONCLUSIONS

- *A. laidlawii* mycoplasma challenges of 47 mm discs of 0.1 µm rated Fluorodyne EX Grade EDT membrane at 30 and 45 psi resulted in titer reductions of > 10^8 (log reduction value, LRV > 8)
- Mycoplasma with an enhanced penetrative ability can be effectively retained by 0.1 µm rated Fluorodyne EX Grade EDT filters at elevated pressures.
- These results coupled with Fluorodyne EX Grade EDT filters higher filtration capacity[3] and good scale-up[4,5] provide for an improvement in process economics[6].

REFERENCES

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