Testing selective agents for the Icthyosporeans *Abeoforma whisleri, Pirum gemata* and Spaheroforma *artica*

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1 *Works for me* dx.doi.org/10.17504/protocols.io.z5nf85e

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ABSTRACT

Minimal protocol for testing selective agents for *Abeoforma whisleri, Sphaeroforma arctica, Pirum gemmata*

MATERIALS

NAME	CATALOG #	VENDOR
Benomyl		
Phleomycin		
100 mg Nourseothricin Sulfate	RC-188	G-Biosciences
1 g Puromycin Dihydrochloride	RC-270	G-Biosciences
Marine Broth 2216	279110	BD Biosciences

MATERIALS TEXT

Carboxin, Fluorotic Acid

1 Seed cells on 24 well/plate. Density depends on the organism but should allow to screen for growth and death easily
Seed the same density to test four or five different dilutions of each drug
Seed enough wells to have 3X replicates for each condition
Seeded cells for *Abeoforma whisleri, Pirum gemata and Spaheroforma artica* 100 cells/well 500 microliters of medium.

2 Add to each well the corresponding amount of drug. Following the amounts tested in our case for *Abeoforma whisleri, Pirum gemmata, Sphaeroforma arctica*

Nourseothricin	10-50 µgr/ml
Phleomycin	10-300 µgr/ml
Fluorotic Acid	250-30ugr/ml
Benomyl Carboxine Puromycin	1-20 µgr/ml 20-300µgr/ml 100-500 µgr/ml

3 Spin down cells every 24 hours and refresh media with new antibiotic.
Observe for up to a week.
If effects on growth are suspected repeat experiment with the apropriate concentration of drug and count cells every 24 hours.

4 Summary of our results

Abeoforma whisleri	Nourseothricin	10-50 µgr/ml (not sensitive)
	Phleomycin	10-300 µgr/ml (not sensitive)
	Fluorotic Acid	250-30 µgr/ml (not sensitive)
	Benomyl Carboxine Puromycin	1-20 (500) µgr/ml 20-300 µgr/ml 100-500 µgr/ml (cytostatic)
Pirum gemmata	Nourseothricin	10-50 µgr/ml (not sensitive)
	Phleomycin	10-300 µgr/ml
	Fluorotic Acid	250-30ugr/ml (not sensitive)
	Benomyl Carboxine Puromycin	1-20 µgr/ml 20-300µgr/ml 100-500 µgr/ml (cytostatic)

Sphaeroforma arctica	Nourseothricin	10-50 µgr/ml (not sensitive)
	Phleomycin	10-300 µgr/ml
	Fluorotic Acid	250-30 µgr/ml (not sensitive)
	Benomyl	1-20 µgr/ml (cytostatic)
	Carboxine	20-300 µgr/ml
	Puromycin	100-500 µgr/ml (cytostatic)