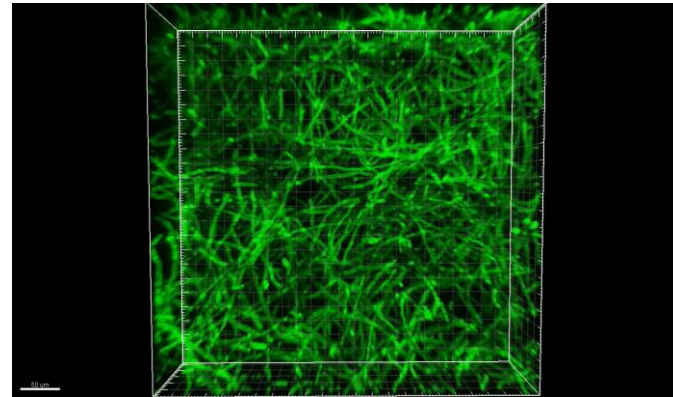
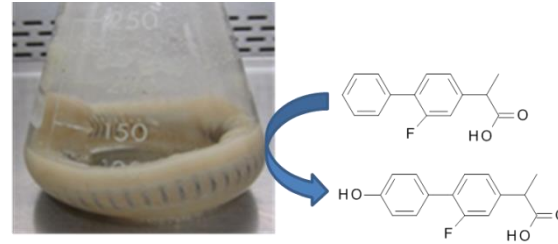


# Fungal biofilms and organofluorine compounds

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# Fungal biofilms and organofluorine compounds

## *Cunninghamella elegans*

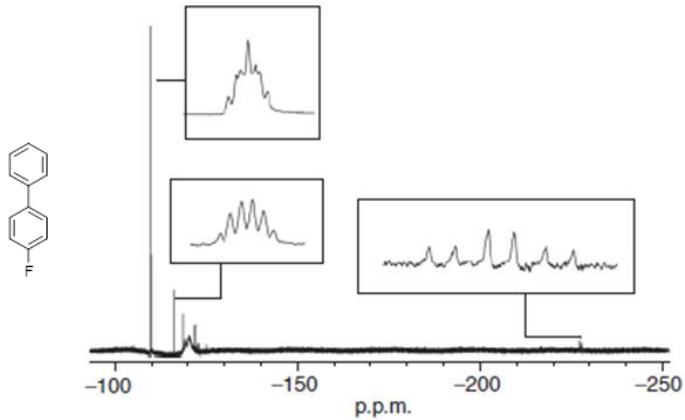
- Filamentous fungus
- Xenobiotic metabolism
- Cytochrome P450

## Organofluorine compounds

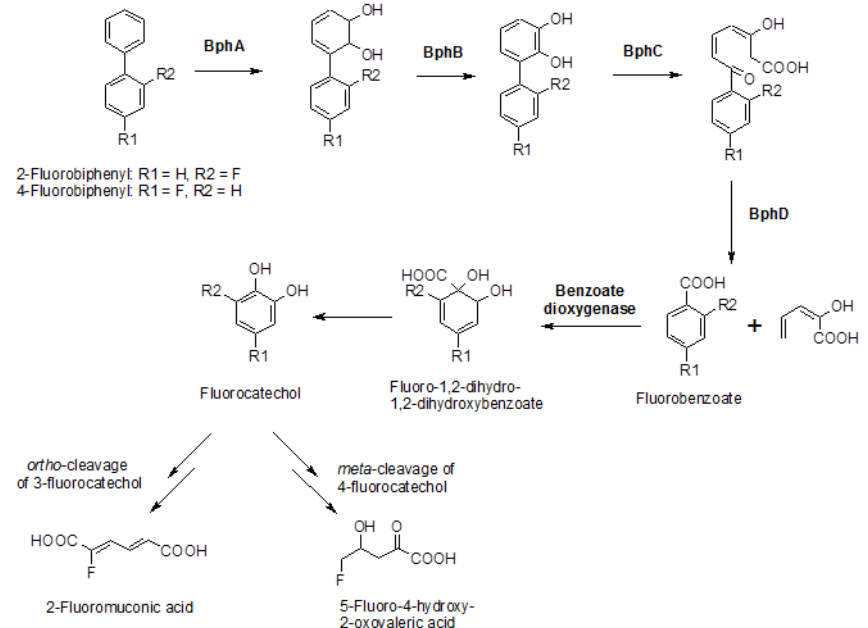
- Pharmaceuticals (>20 %)
- Agrochemicals (>30 %)
- <10 natural organofluorines
- $^{19}\text{F}$  NMR

# Fluorobiphenyl biodegradation

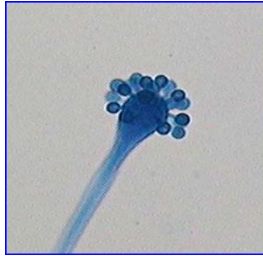
## *Pseudomonas pseudoalcaligenes* KF707



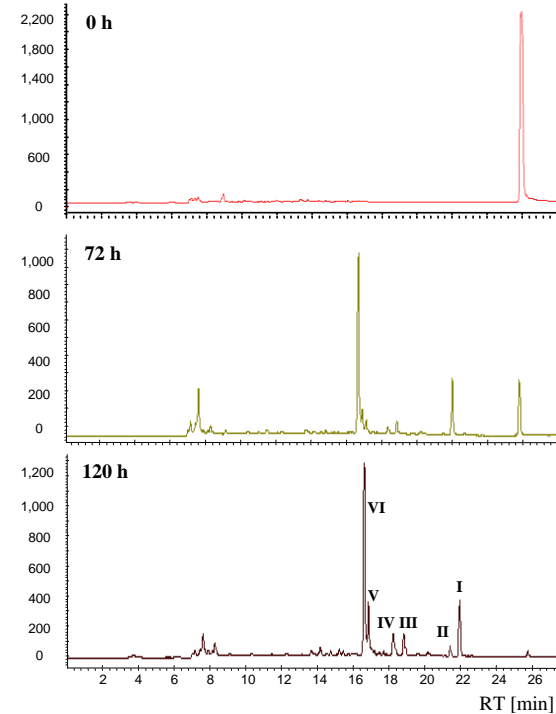
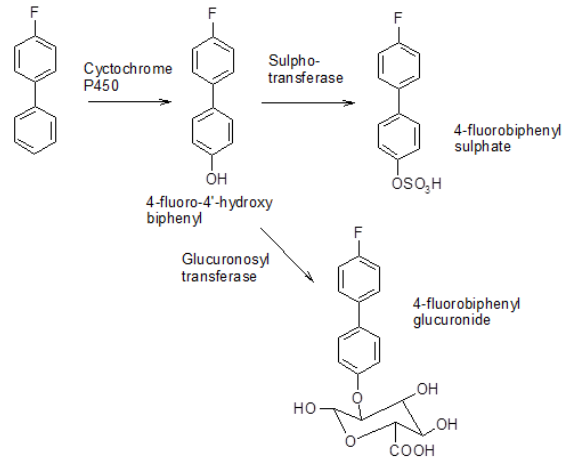
Growth substrate	2,3-dioxygenase (nmol/min/mg wet cell weight)	1,2-dioxygenase (nmol/min/mg wet cell weight) <sup>a</sup>
Benzoate	51	N.D. <sup>b</sup>
2-Fluorobiphenyl	0.5	0.7
4-Fluorobiphenyl	1.8	N.D.



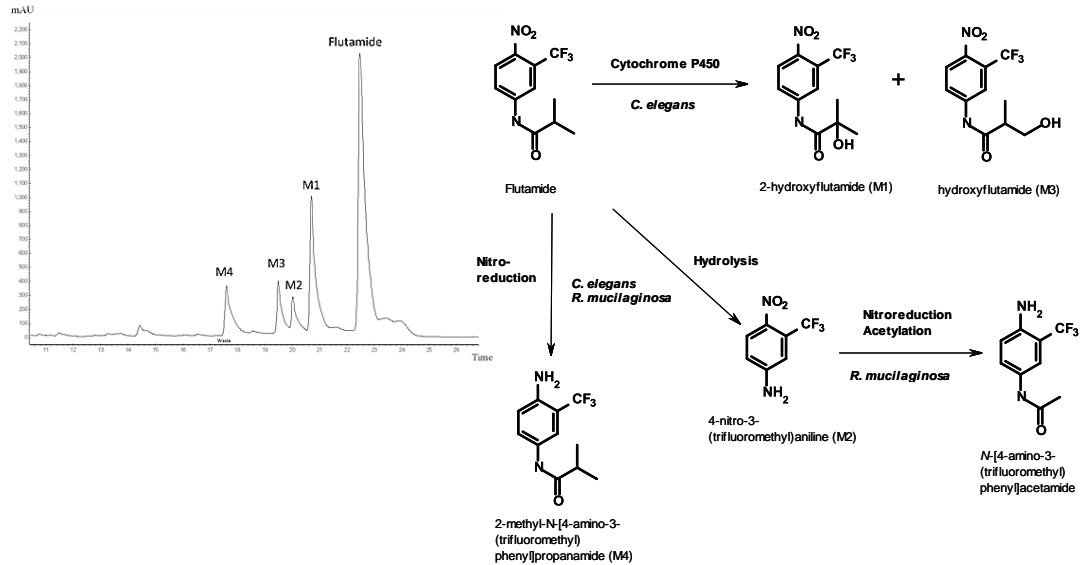
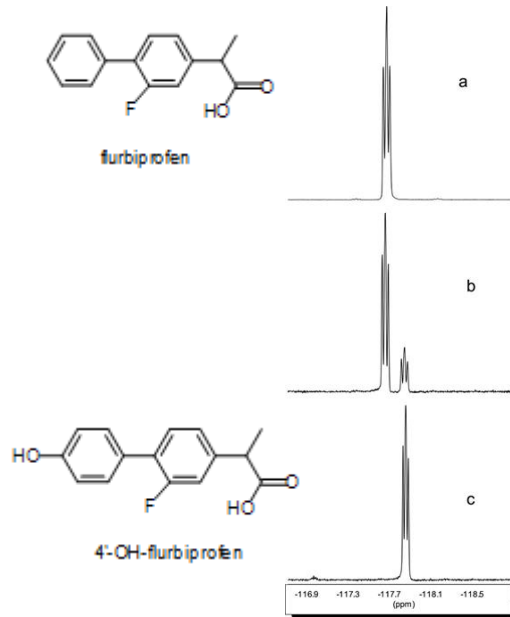
# Fluorinated drugs



*Cunninghamella elegans*

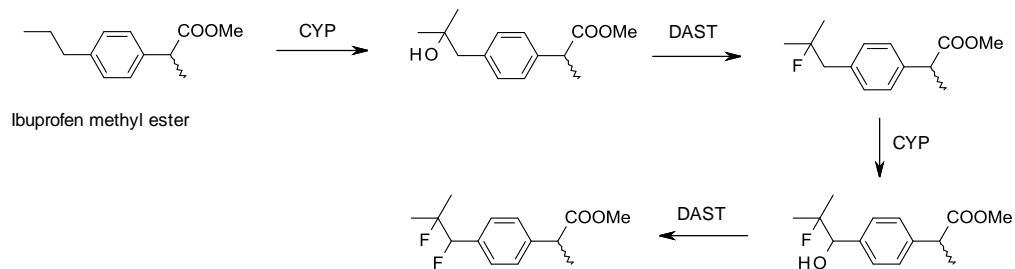


# Fluorinated drugs



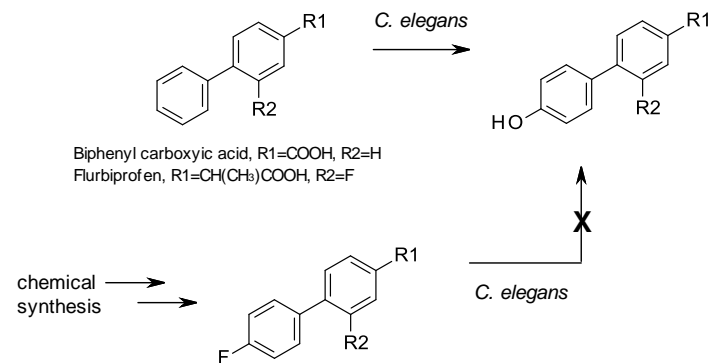
Amadio and Murphy (2011) Biotechnol Lett 33, 321-326  
 Amadio et al. (2010) Appl Environ Microbiol, 76, 6299-6303

# Fluorinated drug design



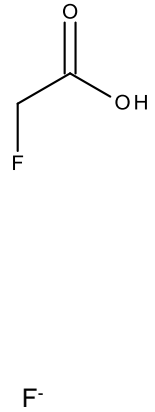
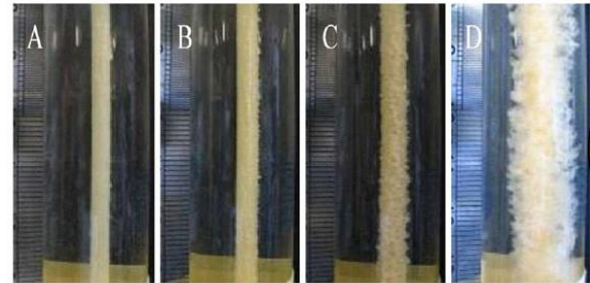
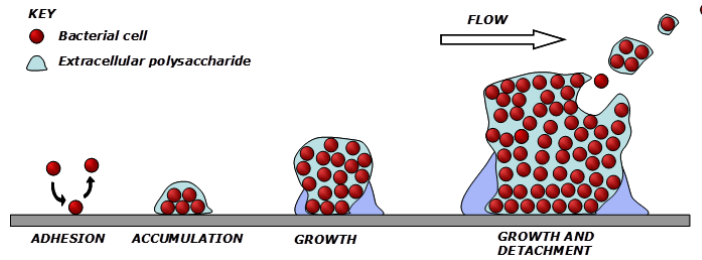
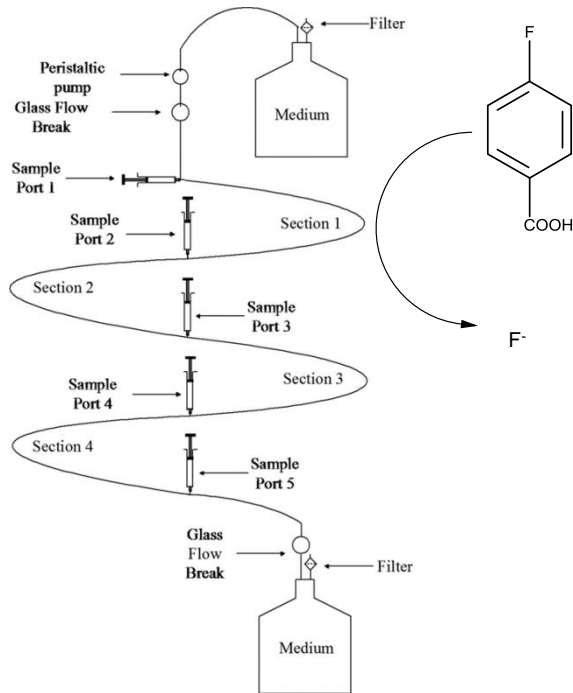
Ibuprofen methyl ester

Rentmeister et al. (2009) Nat Chem Biol, 5, 26



Bright et al. (2013) Org Biomol Chem, 11, 1135  
Shaughnessy et al. (2014) ChemMedChem, 9, 733

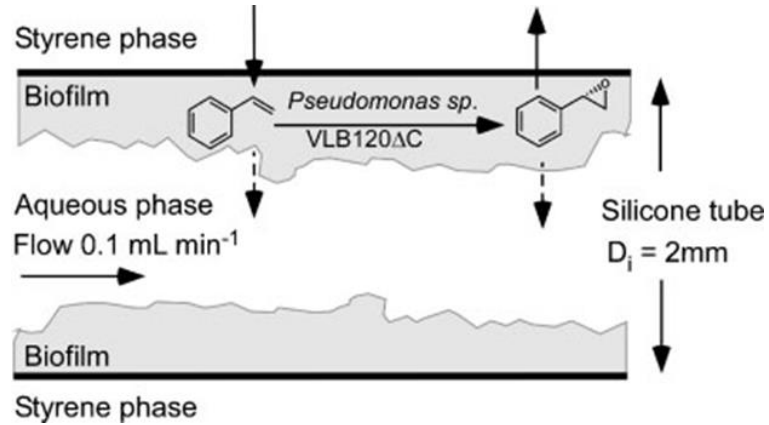
# Fluoroorganic degradation in *Pseudomonas* biofilms



Misiak et al. (2011) Water Res, 45, 3512

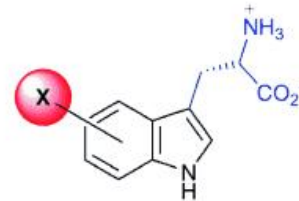
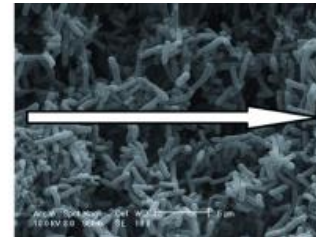
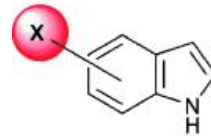
Heffernan et al. (2009) Environ Sci Technol, 43, 6776

# Productive biofilms



Gross et al. (2007) *Biotechnol Bioengin* 98, 1123

Tsoligkas et al. 2011 *ChemBioChem* 12, 1391



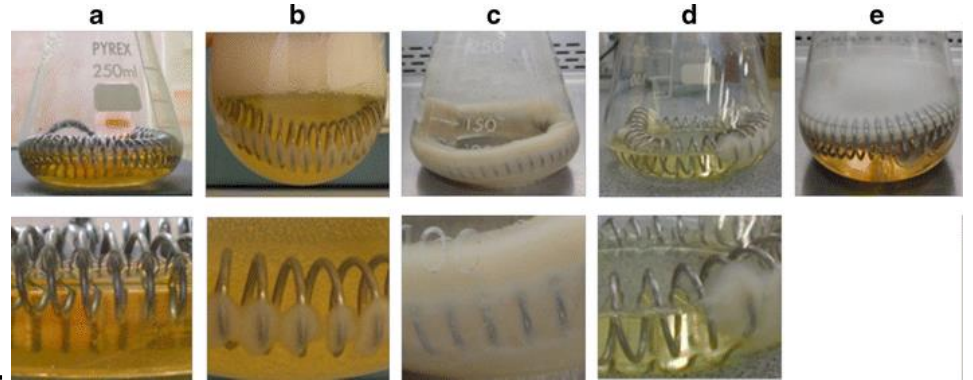


# Criteria for biofilm formation by filamentous fungi

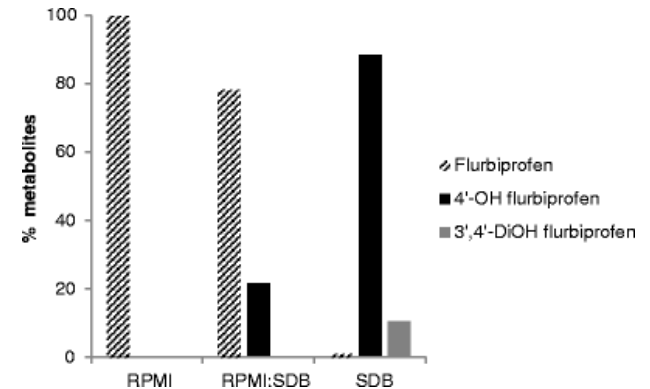
- Structural
  - ▣ Hyphal bundles and/or layers
  - ▣ Surface associated growth
  - ▣ Extracellular polymeric matrix
- Phenotypic
  - ▣ Enhanced tolerance to xenobiotics
  - ▣ Changes in enzyme/metabolite production
  - ▣ Physiological changes

# Fungal biofilm for biocatalysis

- Continuous biocatalysis
- Specific conditions required for biofilm formation
  - ▣ 96-well plate
  - ▣ Erlenmeyer flask
- Biotransformation dependent on medium
- Polysaccharide EPS
- Recyclable



Amadio et al. (2013)  
Appl Microbiol Biotechnol,  
97, 5955



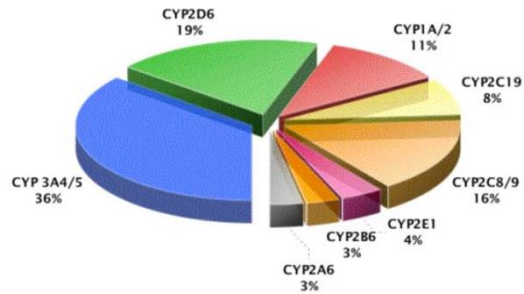
# Semi-continuous production of drug metabolites

- Convenient removal of supernatant
  - ▣ Biotransformation conducted in water
- Rejuvenation
  - ▣ Biofilm thickness
    - Diffusion through EPS

Immobilisation method	Productivity (mg L <sup>-1</sup> h <sup>-1</sup> )			
	4'-OH diclofenac		4'-OH flurbiprofen	
	Max	Average	Max	Average
Alginate	1.16	0.71	1.40	1.03
Biofilm I	1.02	0.46	0.77	0.60
Biofilm II	1.63	1.13	0.83	0.69
Biofilm III	1.80	0.99	1.26	0.75
Biofilm IV	4.12	2.66	2.58	0.58

# CYP

Proportion of Drugs Metabolized by P450 Enzymes



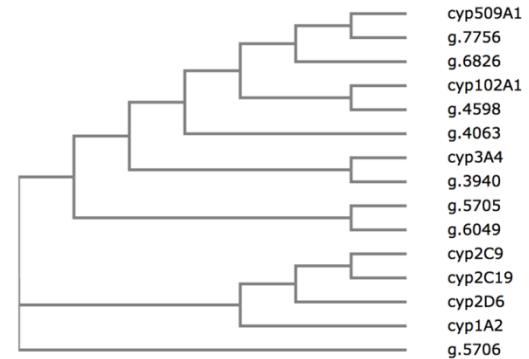
- *C. elegans* CYP509A1
  - Cloned, sequenced, immunoassay  
FEMS Microbiol Lett (2000), 188, 55
  - No activity measured
- *C. elegans* genome
  - Numerous CYP homologues

**cyp509A1**  
g. 7756\*  
g. 7756

```
ETFDPDRWASNGEAEQLAGK-----MAWVFSNGNRQCIGMNFSLLEQRVILGSLLR 436
ETFNDRWASNGEAEQLAGK-----MAWVFSNGNRQCIGMNFSLLEQRVILGSLCR 436
ETFNDRWASNGEAEQLAGK-----MAW-----MAW----- 467
```

g. 6826  
**cyp102A1**  
g. 4598  
cyp2C9  
cyp2C19  
cyp2D6  
g. 6049  
cyp1A2  
**cyp3A4**  
g. 3940  
g. 4063  
g. 5706

```
ETFNDRFAPGGEAEQNERSC-----LSWAPFASGSRMCIGMNFSLAEQRVMVSCLLR 501
EEFRPERFE-----NPSAIPQ-----HAFKPPFNGGQRACIGQQFALHEATLVLGMLK 420
ERFDPDFRSP---EEEQKRSR-----FAWLPPSTGTGRACIGMAPALQEAKTVLAMFLH 509
EMFDPHHFLDEGGNFKSK-----YFMPFSAGKRCVGEALAGMELFLFLTSILQ 454
EMFDPRHFLDEGGNFKSKN-----YFMPFSAGKRCVGEGLARMELFLFLTIFLQ 454
FRFHPEHFLDAQGHFVKPE-----AFLPFSAGRRAICGEPLARMELFLFFTSLLQ 462
TSFNDRFLDHTRTISASANGS---IDTRDQFNFGGRRICPGIYLAEMEMPHIVTRMVA 467
SEFRPERFLTADGTAINKPLS-----EKMLFMGMKRRICGEVLAKWEIFLFLAILLQ 477
EKFLPERFSKKNKDIDPY-----IYTPFGSGPRNCIGMRFALMNMKLLAIRVLQ 461
SSFIPERWLEGGISANASPND-----LMNFSLGSRNCIGKQFALMEMRLVLATMVK 474
LEFNDRWNHAPAKNISPYDY-----APFLYPGPRTICGNRFAMMEMKVLALAILK 547
EKFIPERHWDYVKNHTTTEQRFSQSVEDRPHLAFSTIGRVCVCIHLAERSIFMAISALIA 477
* * *
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Engineering

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Prof Ken Wolfe, UCD Conway Institute

