**Co-biomass degradation of fluoranthene by marine-derived fungi; *Aspergillus aculeatus* and *Mucor irregularis*: comprehensive process optimization, enzyme induction and metabolic analyses**

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*Molecular identification and phylogenetic relationship between AA and MI*

Internal Transcribed Spacer (ITS) 1 and 4 sequences were used in the characterization of the marine fungi; *A. aculeatus* and *M. irregularis*. The Basic Local Alignment Search Tool (BLAST) analysis of the nucleotide sequences revealed that the molecular data on *A. aculeatus* had homologous identities with *A.* *aculeatus* (MH368116) by 99.47%, *A.* *aculeatus* (KJ958359) by 99.61%, *A.* *aculeatus* (KJ439163) by 96.55%, *A.* *aculeatus* strain 4-4 (MF196878) by 95.00%, and *A.* *aculeatus* (AJ279995) by 92.78% (Fig. SM1). Similarly, the BLAST query revealed that *M. irregularis* sequence data had homologous similarities with *M.* *irregularis* strain LZ02 (MN533715) by 93.67%, *M.* *irregularis* strain LZ04 (MN533717) by 91.77%, *M.* *irregularis* (MH102381) by 90.87%, *M.* *irregularis* strain Bpf-19 (MH397495) by 97.21% and *M.* *irregularis* strain E22F (KY425732) by 90.54%.

**Table SM1**. GC-MS data of obtained fluoranthene metabolites after degradation by *Aspergillus* *aculeatus*, *Mucor* *irregularis* and AA+MI co-culture

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Peak | R*t*  (min) | m/z | Mol. Weight  (g moL-1) | Description | Mass spectrum |
| aI | 15.794 | 225 | 224.21 | 2-formylacenaphthylene-1-carboxylic acid |  |
| aII | 12.511 | 182 | 182.22 | 9*H*-fluoren-9-ol |  |
| aIII | 11.324 | 171 | 170.21 | 1,2-dihydroacenaphthylen-1-ol |  |
| aIV, bI | 9.122 | 155 | 154.21 | 1,2-dihydroacenaphthylene |  |
| bII | 8.679 | 186 | 186.32 | 1,2-dihydroacenaphthylene-1,2-diol |  | 11.34 |  |
| bIII | 7.999 | 181 | 180.55 | 9*H*-fluoren-9-one |  |  |  |
| cI | 7.924 | 237 | 238.28 | 1,2- dihydroxyfluoranthene |  |  |  |
| cII | 7.672 | 256 | 254.23 | 9*H*-fluorene-1,9-dicarboxylic acid |  |  |  |
| cIII | 7.101 | 211 | 210.14 | benzene-1,2,4-tricarboxylic acid |  |  |  |
| cIV | 6.907 | 166 | 166.13 | benzene-1,3-dicarboxylic acid |  |  |  |
| cV | 4.966 | 121 | 122.12 | benzoic acid |  |  |  |

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*Aspergillus* *aculeatus* (KJ439163)

*Aspergillus* *aculeatus* (KJ958359)

*Aspergillus* *aculeatus* (MH368116)

***Aspergillus* *aculeatus* strain bpo2 (MT492456)**

*Aspergillus* *aculeatus* (AJ279995)

*Aspergillus* *aculeatus* strain 4-4 (MF196878)

*Aspergillus* *aculeatus* strain KU20018.78 (MT487837)

*Aspergillus* *japonicus* (KF493862)

*Aspergillus* sp. strain 79-B7 (MT594360)

*Aspergillus* *aculeatus* (KF493861)

*Mucor* *irregularis* strain E22F (KY425732)

*Rhizomucor* sp. UFMGCB 3507 (JQ418437)

*Mucor* *irregularis* strain Bpf-19 (MH397495)

*Mucor* sp. (MN968723)

*Rhizomucor* sp. JJP-2009a (FJ210508)

*Rhizomucor* sp. JJP-2009a (FJ210515)

*Mucor* *irregularis* (MH102381)

*Mucor* sp. (MN853752)

*Mucor* *irregularis* strain LZ04 (MN533717)

*Mucor* *irregularis* strain LZ02 (MN533715)

*Aspergillus* *aculeatus* strain AN5 (KY859793)

***Mucor* *irregularis* bpo1 (MK373020)**

*Aspergillus* sp. (MK332589)

*Aspergillus* *aculeatus* strain A1.9 (EU833205)

*Aspergillus* *aculeatus* strain MR10 (KY320594)

*Aspergillus* *aculeatus* (EU326206)

*Aspergillus* *japonicus* (KX621981)

*Mucor* *circinelloides* strain SD-8 (KY807767)

*Rhizomucor* sp. strain SH-16 (MN521812)

*Mucor* *circinelloides* strain LZ27 (MN533740)

99

98

98

90

98

98

90

96

94

96

91

97

94

95

90

96

99

100

96

91

90

93

90

90

87

99

0.5

**Fig. SM1.** Evolutionary relationships of the marine-derived fungi, *A. aculeatus* bpo 2 and *M. irregularis*bpo 1. The tree was constructed by bootstrap analysis and inferred through the Neighbor-Joining tree method using MEGA X Software.

|  |  |  |
| --- | --- | --- |
| **a**     |  | | --- | | **c** | | **b** |

**Fig. SM2**. Pareto chart showing the influence of (a) 11 standard variables (b) 6 standard variables (c) 4 standard variables (Plackett-Burman Designs) in fluoranthene degradation by AA+MI co-culture (p≤0.05)



**Fig. SM3.** Validation of the FFD using growth-linked experiment of the optimized culture conditions

|  |  |
| --- | --- |
|  |  |
|  |  |

**Fig. SM4.** HPLC Chromatogram of fluoranthene (a) untreated (b) after degradation by AA (c) after degradation by M1 (d) after degradation by AA+MI