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# *Aspergillus* genus as a source of new catalysts for sulfide oxidation

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ABSTRACT

This work extends the present knowledge about the ability of filamentous fungi to selectively transform sulfur-containing compounds. Here, it has been demonstrated that several species of the *Aspergillus* genus are able to perform chemo- and stereoselective oxidation of organic sulfides. Two substrates were chosen as models, the aliphatic cyclohexyl(methyl)sulfide and the alkyl aryl sulfide, thioanisole. All the tested strains showed the ability to oxidize cyclohexyl(methyl)sulfide, whereas most of them oxidized thioanisole. In all cases *R*-stereoselectivity was observed and full chemoselectivity was verified since no sulfone was detected. Several biotransformation parameters such as the employment of growing or resting cell, different head-space volumes, age of the cultures, bio-reaction times and biocatalyst/substrate ratios resulted crucial in the optimization of the processes. Thus, when using isopropyl alcohol as co-solvent in growing cell systems, chemo- and stereoselectivity could be modulated. Among the studied strains, *Aspergillus japonicus* ICFC 744/11 was found to be a promising whole cell biocatalyst to prepare enantioenriched sulfoxides since after optimization, either conversion and optical purity of (*R*)-cyclohexyl(methyl)sulfoxide were excellent (*c*: 100%, *ee* > 99%).



## 1. Introduction

Non-racemic sulfoxides are recognized as valuable reagents. They are extensively used as chiral auxiliaries since they present configurational stability and exert high asymmetric induction [1]. Furthermore, the stereoselective oxidation of organic sulfides has particular interest since chiral sulfoxides are valuable synthons, being precursors of bioactive compounds [2], such as omeprazole, for which optical purity is essential.

A vast number of bioactive sulfur-containing metabolites are found in plants belonging to Cruciferae and Alliaceae families [3–6]. It is well known that among the fungal genus involved in pathogenesis of such sulfur-rich plants, one of the most important is *Aspergillus* [7–9]. At first glance, it is easy to assume that *Aspergilli* contain the enzymatic machineries to detoxify such defense compounds [10,11], transforming them into more hydrophilic and -often- less toxic metabolites. Such ability to metabolize xenobiotic chemicals might be suitably exploited by organic chemists to generate interesting derivatives [12,13].

Some fungi have been tested for the oxidation of sulfides in order to prepare sulfoxides with good optical purities, such as *Helminthosporium*, *Rhizopus*, *Trichaptum* and *Trametes* species, *Mortierella isabellina*, *Botrytis cinerea*, *Trichoderma viride* and *Eutypa lata* [14–21].

*Aspergillus* genus has been historically used to perform biotransformations due to its immense metabolic potential. However, its ability to carry out sulfoxidation of prochiral sulfides has been scarcely explored. At the best of our knowledge, only a few previous works have been reported dealing with *Aspergillus* capability to oxidize prochiral sulfides. In the 60–70s, Auret's group studied the fungal oxidation of diaryl and aryl alkyl sulfides employing mainly *A. niger* with certain success albeit the enantiomeric excesses ($ee_s$) and optical rotation signs resulted rather variable and substrate over-oxidation (sulfone formation) was always detected [14,22–24]. More recently, it has been studied the possibility of performing sulfoxidations using *A. terreus* [25,26].

Among the enzymes reported to catalyze sulfides oxidation, the most common are Baeyer-Villiger monooxygenases (BVMOs) [27] and peroxidases [28]. Their presence in *Aspergillus* strains, along with the occurrence of other enzymes competent for sulfoxidation such as cytochrome P450 monooxygenases [29] make this fungal genus a good candidate to be investigated in the selective preparation of enantioenriched sulfoxides.

Hence, in the frame of our studies on the biotransformation of prochiral compunds [30–33] we have undertaken a survey study of different *Aspergillus* strains with the aim of applying them as

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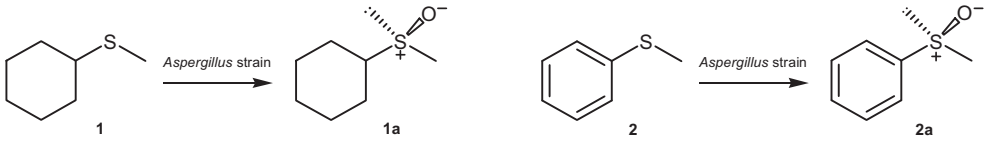


**Fig. 1.** Biotransformation of prochiral sulfides by *Aspergillus* strains.

biocatalysts in selective sulfoxidation reactions toward an aliphatic and an aromatic sulfide as model substrates (Fig. 1).

## 2. Experimental

Sulfides **1** and **2** were purchased from Alfa Aesar. Sulfoxides and sulfones were prepared by chemical oxidation from the corresponding sulfides ($H_2O_2$/MeOH and *m*-CPBA/$CH_2Cl_2$) and exhibited physical and spectral properties in agreement with those reported [34].

Microorganisms

Fungal strains were purchased from different collections: American Type Culture Collection (*Aspergillus niger* ATCC 11394), Instituto Malbran collection (*Aspergillus terreus* INM0 31783), Facultad de Ciencias Exactas-Universidad de Buenos Aires (*Aspergillus flavus* UBA 294), Departamento de Microbiología e Inmunología-Universidad Nacional de Rio Cuarto (*Aspergillus fumigatus* RC 78; *Aspergillus nidulans* (*Emericella*) RC 36; *Aspergillus ochraceus* RC 33), Laboratorio de Micología-Universidad Nacional del Litoral (*Aspergillus candidus* LMFIQ 081; *Aspergillus clavatus* LMFIQ 089; *Aspergillus parasiticus* LMFIQ 133), IIB-INTECH Collection of Fungal Cultures (*Aspergillus japonicus* ICFC 744/11). Stock cultures of all fungal strains were stored in Petri dishes on solid PDA or Czapeck media at 4 °C.

### 2.1. General procedures for biotransformation

#### 2.1.1. Growing cells

A two-step-process was conducted. Spores of *Aspergillus* strains were inoculated into 50 mL Czapeck medium in 250 mL baffled Erlenmeyer flasks and incubated at 28 °C for 2 days on orbital shaker (150 rpm). Following incubation, fungal cells were harvested and subcultured in 20 mL fresh medium in 50 mL Erlenmeyer flasks. Substrates **1** and **2** were added dissolved in DMSO and the reaction mixtures were incubated at 28 °C for 6 days on orbital shaker (150 rpm). Biotransformation progress was monitored every 2 days by GC-FID. Blank assays without substrates and without fungi were carried out in parallel. Experiments were performed in triplicate.

#### 2.1.2. Resting cells

Pre-cultures of selected *Aspergillus* strains were prepared as described above. After incubation, fungal cells were harvested by centrifugation and washed with phosphate buffer. Cells (1 g per flask) were re-suspended in phosphate buffer (30 mL, pH 6.0, 0.1 M) in Erlenmeyer flasks (50 mL). Substrate **1** (1 mM, dissolved in 25 μM of DMSO) was added and bio-reaction was incubated in orbital shaker (150 rpm) at 28 °C for 6 days. Biotransformation progress was monitored every 2 days by GC-FID analysis. Blank assays without substrate and without fungi were carried out in parallel. Experiments were performed in triplicate.

### 2.2. Optimization of the biotransformation process

To determine best culture ages and bio-reaction times, biotransformation of **1** using *A. candidus* resting cells was conducted in three different conditions. A: a 3-day-old pre-culture was used and biotransformation was monitored for 2 and 4 days; B: a 3-day-old pre-culture was used, then cells were harvested and re-suspended in buffer for 2 days and then substrate was added, reaction was monitored every 24 h for 2 days; C: a 5-day-old pre-culture was used and biotransformation was monitored every 24 h for 2 days (Fig. 2).

To evaluate the effect of co-solvents, biotransformation of **1** with *A. japonicus* growing cells was performed. In each case the substrate (1 mM) was dissolved in *n*-hexane, toluene, methanol, *tert*-butanol, *iso*-propanol or DMSO (0.5% (v/v) final concentration), and added to the reaction mixture. To evaluate the effect of increasing substrate concentration, biotransformation of **1** employing *A. japonicus* growing cells was performed. Substrate concentrations were 1, 10, 25, 50 and 100 mM (dissolved in 25 μL of DMSO). In both assays reaction was incubated for 5 days. Blank assays without fungi were carried out in parallel. Experiments were performed in duplicate.

### 2.3. Analytical methods

Biotransformation samples were extracted with ethyl acetate (3× 15 mL). No internal standard was added. The organic phases were combined and dried over anhydrous $Na_2SO_4$ and solvent removed in vacuo. Conversion and *ee* of substrate **1** were determined by chiral GC-FID with a β-DEX-column. Temperature setting: 140 °C, 0.5 °C/min, 150 °C (10 min), 0.5 °C/min, 160 °C (10 min); injector: 200 °C, carrier gas $N_2$: 25 cm/seg; FID: 300 °C; $r_t$ (**1**) 9.7 min; $r_t$ (*S*)-**1a**: 50.3 min; $r_t$ (*R*)-**1a**: 50.8 min; $r_t$ **1b**: 52.9 min. Absolute configuration of enantiomers was determined by optical rotation of the isolated products purified by column chromatography (CC) over silica gel (70–230 mesh) eluted with $CH_2Cl_2$:$CH_3OH$ mixtures. For substrate **2**, conversion was determined by GC-FID equipped with a 007 methyl 5% phenyl silicone column. Temperature setting: 100 °C (2 min), 2 °C min, 130 °C; injector: 200 °C; carrier gas $N_2$: 25 cm/seg; FID: 300 °C; $r_t$ **2**: 6.2 min; $r_t$ **2a**: 14.37 min. The *ee* was determined by chiral HPLC-DAD with a Chiralcel OD column; *n*-hexane: IPA (9:1); 0.8 mL/min; 25 °C; 210 nm; $r_t$ (*R*)-**2a**: 16.8 min; $r_t$ (*S*)-**2b**: 18.1 min.

## 3. Results and discussion

### 3.1. Screening procedure

Previous works have reported the sulfoxidation of thioanisole to (*R*)-methyl(phenyl)sulfoxide employing fungi such as *M. isabellina* and *T. viride*, which furnished optical purities of 65% and 70%,

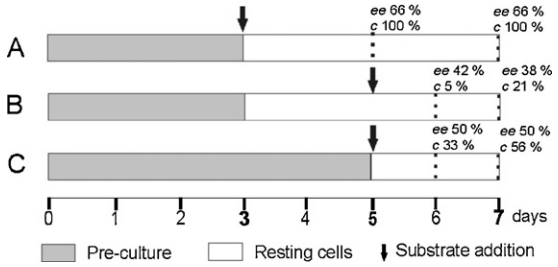


**Fig. 2.** Culture age and bio-reaction time using *A. candidus* resting cells. Arrows indicate the time when substrate **1** was added. Dotted lines show the day when samples were taken. *c* (%) and *ee* (%) determined by chiral GC analysis.

**Table 1**
Screening results for the oxidation of sulfides (**1**, **2**) employing *Aspergillus* strains as biocatalysts.

| Entry | Strain | Biotransformation time (days) | (*R*)-1a | | (*R*)-2a | |
|---|---|---|---|---|---|---|
| | | | *c* (%) | *ee* (%) | *c* (%) | *ee* (%) |
| 1 | *Aspergillus* | 2 | 29 | 62 | 10 | *rac* |
| 2 | *candidus* | 4 | 71 | 58 | 55 | *rac* |
| 3 | | 6 | 100 | 58 | 55 | *rac* |
| 4 | *Aspergillus* | 2 | 29 | 22 | 2 | *rac* |
| 5 | *clavatus* | 4 | 60 | 22 | 4 | *rac* |
| 6 | | 6 | 100 | 22 | 7 | *rac* |
| 7 | *Aspergillus* | 2 | 31 | 66 | 33 | 17 |
| 8 | *flavus* | 4 | 96 | 57 | 98 | 12 |
| 9 | | 6 | 100 | 59 | 100 | 9 |
| 10 | *Aspergillus* | 2 | 85 | 58 | 42 | 38 |
| 11 | *fumigatus* | 4 | 95 | 61 | 92 | 52 |
| 12 | | 6 | 100 | 62 | 100 | 72 |
| 13 | *Aspergillus* | 2 | 87 | 60 | 80 | 50 |
| 14 | *japonicus* | 4 | 89 | 62 | 85 | 53 |
| 15 | | 6 | 90 | 97 | 88 | 55 |
| 16 | *Aspergillus* | 2 | 5 | *rac* | 2 | 9 |
| 17 | *nidulans* | 4 | 98 | *rac* | 18 | 20 |
| 18 | | 6 | 100 | *rac* | 64 | 15 |
| 19 | *Aspergillus* | 2 | 93 | 32 | 58 | 42 |
| 20 | *niger* | 4 | *nd* | *nd* | 95 | 54 |
| 21 | | 6 | 100 | 37 | 100 | 57 |
| 22 | *Aspergillus* | 2 | 82 | 48 | 3 | 25 |
| 23 | *parasiticus* | 4 | 100 | 54 | 22 | 11 |
| 24 | | 6 | 100 | 55 | 36 | *rac* |
| 25 | *Aspergillus* | 2 | *nd* | *rac* | *nd* | *nd* |
| 26 | *terreus* | 4 | *nd* | *rac* | *nd* | *nd* |
| 27 | | 6 | 100 | *rac* | 100 | *rac* |

*c* (%): Conversion of substrate into sulfoxide. In all cases no sulfone formation was detected. *ee* (%): enantiomeric excess determined by chiral GC-FID for **1a** and by chiral HPLC-DAD for **2a**.
*nd*: not determined; *rac*: racemic.

respectively [35]. Conversely, *Helmintosporum* sp. and *B. cinerea* showed a slight preference to the *S*-enantiomer with $ee_s$ of 48% and 50% [19,20]. Also, the basidiomycetes *Irpex lacteus*, *Pycnoporus sanguineus*, *Trametes rigida* and *T. versicolor* were able to transform thioanisole and other aromatic sulfides to the *S*-sulfoxides [21]. Nevertheless, we consider that the use of a sole substrate as molecular probe to detect selective sulfoxidation in general screenings might guide to misleading conclusions, since false negatives may pass through. Thus, by using two substrates of different chemical nature the chance to detect a biocatalyst will certainly increase. Therefore a dialkyl and an alkyl aryl sulfide, namely cyclohexyl(methyl)sulfide (**1**) and thioanisole (**2**), were selected as model substrates (Fig. 1).

In the present work, nine different *Aspergillus* species as growing cells were screened for their sulfoxidation ability. At different biotransformation times (2, 4 and 6 days) samples were withdrawn and the relative amounts of the corresponding sulfide and sulfoxide were measured (Table 1). In cases in which stereoselection was achieved, the formation of the *R*-enantiomer was observed. Remarkably, no sulfone was detected in all the experiments.

Overall it was noticed that **1** was better substrate than **2**, concerning conversion and stereoselection. Conversions of dialkyl sulfide were almost completed at 6 days of incubation with all the tested fungi. It is noticeable that some strains display poor or moderate (if any) stereoselection toward thioanisole but good to excellent selectivities for sulfide **1**. This observation gives a good reason for the use of at least two different substrates for general screenings. Thioanisole was totally oxidized by *A. flavus*, *A. niger* and *A. fumigatus* after 4 days of bio-reaction but, the $ee_s$ did not remain constant during the process. Specifically in the case of *A. flavus*, the *ee* decreased during incubation similarly to what occurred with *A. parasiticus* although this fungus did not fully transform the substrate (entries 22–24, Table 1). On the contrary, with *A. niger* and *A. fumigatus* the $ee_s$ increased during the bio-reaction time, more significantly for the latter (entries 10–12 and 19–21, Table 1). Collado et al. [20] observed the same phenomena by using *B. cinerea* as biocatalyst and tried to rationalize this fact by suggesting that one enantiomer of the sulfoxide is transformed into sulfone. This explanation could not be applied in our system since no over-oxidation was noticed. Besides, as it happened with substrate **1**, when using *A. nidulans*, *A. candidus* and *A. clavatus* in the oxidation of **2**, no enantioselectivity was displayed throughout the process (entries 1–3 and 4–6, Table 1).

*A. terreus* INM 031783 did not show stereoselection. In contrast, other authors have reported that a Brazilian strain (*A. terreus* CCT 3320) oxidized **2** into the *S*-sulfoxide, but with low chemoselectivity, since an appreciable amount of sulfone was reported, either using free cell or immobilized on chrysotile systems [25].

Interestingly, *A. japonicus* resulted an excellent biocatalyst to perform the oxidation of **1**. In this case even before optimization, optical purity of the obtained (*R*)-cyclohexyl(methyl)sulfoxide was excellent (*ee* 97%) and conversion reached 90% in this preliminary screening assay. Subsequently, in order to isolate the product without changing oxygenation conditions, the experiment was performed increasing the number of batches to 12. The isolated yield obtained after purification by CC was 21% (97% *ee* by chiral GC analysis). The $[\alpha]_D$ −27.87 (*c* = 2.44, acetone) is in agreement with the optical rotation sign, reported by Capozzi et al., for the *R*-enantiomer [36]. This experiment allowed us to isolate and purify the biotransformation product and unambiguously assign the sulfur absolute configuration. A hypothesis to explain this yield might be the probable degradation of the substrate and/or product into more polar molecules which were not extracted from the reaction medium. Consequently the time-course *ee* values could also be affected (e.g. entries 13–15, Table 1).

### 3.2. Optimization of biotransformation conditions

#### 3.2.1. Avoiding non-enzymatic oxidation

Substrate **1** was chosen to optimize biotransformation conditions.

It was observed by the analysis of the blank assays that around 3–10% of the sulfoxide is produced by non-enzymatic oxidation as already reported [37]. This fact worsens the overall stereoselectivity of the process lowering the *ee*. Therefore, we chose the sulfoxidation catalyzed by *A. flavus* to set up conditions in order to minimize this effect since with this strain the product *ee* remained unaltered along the reaction times (entries 7–9, Table 1). The strategy consisted of varying the availability of oxygen, by modifying the head-space volume of the reaction batch using different volumes of culture media in Erlenmeyer flasks of the same size. Oxygenation proved to be a critical parameter. As depicted in Table 2, best conditions resulted with 30 mL of media in 50 mL flasks, since with smaller volume of medium (entries 1 and 2) non-enzymatic oxidation was higher, and with less oxygen availability sulfoxide formation was very poor (entry 5).

#### 3.2.2. Biotransformations with resting cells

An advantage of using resting cells is that the process is generally cleaner and work-up is much easier. Based on the results of the growing cell biotranformations, we selected *A. flavus*, *A. fumigatus*, *A. parasiticus*, *A. candidus* and *A. japonicus* to explore the influence of resting cell on the process (Table 3). Under these

**Table 2**
Effect of head-space volume in non-enzymatic oxidation.

| Entry | | Culture medium volume (mL) | *c* (%) | *ee* (%) |
|---|---|---|---|---|
| 1 | *Biotransformation* | 20 | 12 | 64 |
| 2 | *Non-biocatalyzed reaction* | 20 | 17 | *rac* |
| 3 | *Biotransformation* | 30 | 24 | 76 |
| 4 | *Non-biocatalyzed reaction* | 30 | <3 | *nd* |
| 5 | *Biotransformation* | 40 | 4 | *nd* |
| 6 | *Non-biocatalyzed reaction* | 40 | <3 | *nd* |

Biotransformation of substrate **1** carried out using *A. flavus* growing cells. Different volumes of medium were assessed in 50 mL Erlenmeyer flasks. As control non-biocatalyzed reactions were performed in different volumes of medium.
*nd*: not determined.

conditions, best results were achieved employing *A. candidus* as biocatalyst. After 5 days, (*R*)-**1a** was recovered with 66% *ee*. For the other *Aspergilli* tested, complete conversions were obtained after 5–6 days, while optical purities of (*R*)-**1a** were slightly lower. By using this methodology the expression and/or the activity of enzymes may vary and consequently, reaction rates as well as stereoselection could be significantly affected. This fact is particularly important in these studies since not only the enzymes responsible of the sulfide oxidation are involved but also the ones that play a role in the regeneration of the redox cofactors. Besides, the results of the screening procedure suggested that the expression and/or the activity of the enzyme/s involved in the desired reaction depend on the developmental stage of the fungal culture, since conversions and $ee_s$ vary along the incubation times in several cases. By comparing these results with those of the growing cell experiments, it is observed that in general, the sulfoxide $ee_s$ slightly improved by using resting cells. Unfortunately, *A. japonicus*, our best biocatalyst in growing cell systems, resulted severely affected by this starving condition, in which the *ee* of the product dropped drastically from 97% to 57% (entries 13–15, Table 3). A possible explanation of this fact could be the inhibition/impaired expression of cofactor regeneration enzymes that affects the overall sulfoxidation performance, as it was discussed above.

### 3.2.3. Age of the culture and reaction times in resting cell systems

Since *A. candidus* was the best biocatalyst as resting cells (Table 3), it was chosen to analyze the influence of using fungal cultures of different ages and reaction times. Three different experiments (A–C) were run in parallel using *A. candidus*, as shown in Fig. 2. In experiment A, conversion rates (100%) and sulfoxide *ee*

**Table 3**
Biotransformation of **1** employing *Aspergillus* resting cells.

| Entry | Microorganism | Incubation time (days) | *c* (%) | *ee* (%) |
|---|---|---|---|---|
| 1 | *A. fumigatus* | 2 | 81 | 55 |
| 2 | | 5 | 100 | 58 |
| 3 | | 6 | 100 | 60 |
| 4 | *A. parasiticus* | 2 | 83 | 48 |
| 5 | | 5 | 100 | 69 |
| 6 | | 6 | 100 | 63 |
| 7 | *A. flavus* | 2 | 10 | 49 |
| 8 | | 5 | 55 | 52 |
| 9 | | 6 | 99 | 56 |
| 10 | *A. candidus* | 2 | *nd* | – |
| 11 | | 5 | 100 | 66 |
| 12 | | 6 | 100 | 66 |
| 13 | *A. japonicus* | 2 | *nd* | – |
| 14 | | 3 | 98 | 50 |
| 15 | | 5 | 99 | 57 |

*c* (%) and *ee* (%) determined by chiral GC-FID analysis. *nd*: not determined.

**Table 4**
Effect of substrate/biocatalyst ratio in the oxidation of substrate **1** using *A. japonicus*.

| Entry | [S] (mM)[a] | *c* (%) | *ee* (%) |
|---|---|---|---|
| 1 | 1 | 100 | 97 |
| 2 | 10 | 99 | 70 |
| 3 | 25 | 27 | 30 |
| 4 | 50 | 14 | 30 |
| 5 | 100 | <3 | *nd* |

*c* (%) and *ee* (%) determined by chiral GC-FID analysis. *nd*: not determined.
[a] Final substrate concentration. Bio-reaction time 5 days.

(66%) resulted constant along all reaction times. These results are comparable to those observed on the sixth day in growing cells methodology where the optical purity was a little lower (*ee* 58%) although conversion was 100%. The conversions and $ee_s$ decreased significantly in experiment B compared to experiment A. When resting cell systems were performed with "old cultures" (experiment C) results were intermediate. In the last two experiments, conversion increased along with the bio-reaction time, although maximal values were lower than 60%.

### 3.2.4. Substrate/biocatalyst ratio

Since the best results were observed employing *A. japonicus* as growing cells toward susbtrate **1**, this system was chosen in order to continue with the process optimization.

To improve the substrate/biocatalyst ratio, increasing substrate concentrations were evaluated. Working conditions were similar to those already described for the screening test, varying only the substrate concentration between 1 and 100 mM. Samples were withdrawn at the fourth day of bio-reaction. Results are depicted in Table 4.

For the two lowest concentrations (1 and 10 mM) no significant changes were evident in the biomass dry weight at the end of the experiment, in comparison with blank assays without substrate addition (data not shown). At concentrations higher than 25 mM, the conversion and stereoselectivity decreased, although the chemoselectivity was not affected since no sulfone was detected. This fact could be attributed to a toxic effect caused by the substrate, since there was no biomass development after substrate addition. These results are in agreement with those reported for a bacterial strain, *Rhodococcus sp*. ECU0066. Despite this microorganism was isolated from soil using substrate 2 as a sole carbon source, its viability was affected by high substrate concentration [38,39].

### 3.2.5. Use of co-solvents

Although in nature enzymes work in aqueous medium, many substrates of interest for organic chemists are hydrophobic, requiring the addition of organic solvents to increase their solubilities. Moreover, the use of biphasic systems with whole cell biocatalysts offers the possibility to xenobiotic substrates and/or their products may be partitioned between the two phases, reducing their toxic effects on the living biocatalyst and improving the overall performance of the process. In this regard, it was reported the use of organic-aqueous biphasic systems to overcome the poor substrate tolerance and improve enantioselectivity for the above mentioned *Rhodococcus* strain in the asymmetric sulfoxidation of 2 [39]. The effect of six different co-solvents in the biooxidation of 1 was studied (Table 5). Among the most notable results, the use of DMSO in concentration of 0.5% (v/v) significantly diminished cell development and viability, being affected the conversion, as well as the stereoselectivity. Something similar occurred when toluene was used as co-solvent since the substrate was oxidized in 47% with poor stereoselectivity (*ee* 25%). With methanol and *tert*-butanol, although the conversions were excellent, the $ee_s$ dropped significantly and, more importantly, the formation of sulfone was observed for the first time. Undoubtedly, the best results were

**Table 5**
Co-solvent influence on the biotransformation of substrate **1** using *A. japonicus*.

| Entry | Co-solvent [0.5%, v/v] | Sulfide 1 (%) | Sulfoxide 1a (%) | (*R*)-1a *ee* (%) | Sulfone 1b (%) |
|---|---|---|---|---|---|
| 1 | DMSO | 94 | 6 | 42 | 0 |
| 2 | *iso*-Propanol | 0 | 100 | >99 | 0 |
| 3 | Methanol | 0 | 93 | 64 | 7 |
| 4 | *n*-Hexane | 5 | 95 | 80 | 0 |
| 5 | *tert*-Butyl alcohol | 13 | 80 | 77 | 7 |
| 6 | Toluene | 60 | 40 | 25 | 0 |

Determined by chiral GC-FID analysis. Substrate concentration 1 mM. Bio-reaction time 5 days.

recorded with isopropyl alcohol, reaching a 100% substrate conversion and excellent optical purity (*ee* > 99%).

## 4. Conclusion

The ability of several *Aspergillus* species of performing enantioselective sulfoxidations toward an aryl alkyl and a dialkyl sulfide was screened. All the strains that showed selectivity favored the (*R*)-enantioner. Remarkably, no sulfone was detected in the tested standard conditions, demonstrating the chemoselectivity of the process. The results of the optimization studies indicated that *A. japonicus* presents a huge potential to be used as whole cell biocatalyst for symmetric sulfoxidations. In fact its growing cell cultures with the addition of isopropyl alcohol as co-solvent resulted a good alternative for the preparation of (*R*)-cyclohexyl(methyl)sulfoxide since the conversion (100%) and the optical purity (*ee* > 99%) were excellent.

Further studies aimed at the cloning and characterization of the involved enzyme/s, as well as their use for sulfoxidation of different substrates, are in progress in our laboratory.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molcatb.2012.05.003.

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