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Reduction screening with endophytic fungi: Synthesis of homochiral secondary alcohols

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ABSTRACT

Twelve strains of endophytic fungi, isolated from various plants (i.e. *Eugenia hallii*, *Schinus molle*, *Crataegus monogyna*, *Juniperus communis* and *Sambucus nigra*) sampled in Amazonian forest and in Italy, were screened for their reduction activity with a cocktail of ketones **1–4**. The four most active strains [i.e. *Phomopsis* (FE86 and FE290), *Pestalotia* and *Epicoccum*] were chosen for the reduction of 5-hexen-2-one **1**, acetophenone **2**, *cis*-bicyclo[3.2.0]hept-2-en-6-one **3**, 2-methylcyclohexanone **4**, 6-methyl-5-hepten-2-one **5**, 2-furyl methyl ketone **6**, 1-indanone **7**, and 2,4,4-trimethyl-2-cyclohexen-1-one **8** and in all cases the *S*-alcohols were obtained with variable yields and enantiomeric excesses depending on the strains.

1. Introduction

Microbial reduction of prochiral carbonyl groups is wide spread and very efficient method for preparing enantiomerically pure secondary alcohols [1]. These compounds are useful as chiral auxiliaries in organic chemistry for both analytical and synthetic applications [2] and they are common as pheromones [3] aroma and flavour enhancing compounds. Baker's yeast [4,5] and other microorganisms [6–9] are currently used to reduce a full range of carbonyl compounds. Recently, differentiated and undifferentiated plant cells have been employed in the enantioselective reductions of various prochiral carbonyl groups [10], while endophytic fungi are still an unexplored or at least under-explored source for microbial biotransformation [11]. Endophytes [12] are bacterial or fungal microorganisms that colonize inter- and/or intracellularly the healthy plant tissue [13], without causing any apparent symptoms of disease. They have been isolated from almost every host plant studied so far and most of them are able to produce highly bioactive secondary metabolites [14–16]. A recent comprehensive study has indicated that 51% of bioactive substances isolated from endophytic fungi were previously unknown [17].

Only recently endophytic fungi were studied in the stereoselective oxidation of thioridazine [18], hydroxylation of flavans [19], and probably they are involved in the stereoselective reduction of ketones by *Daucus carota* root [20].

In this work 12 strains of endophytic fungi, isolated from different plants and sites, were tested for the enantioselective reductions of various ketones in order to obtain homochiral secondary alcohols (Scheme 1).

2. Materials and methods

2.1. Chemicals and culture media

5-Hexen-2-one **1**, acetophenone **2**, *cis*-bicyclo[3.2.0]hept-2-en-6-one **3**, 2-methylcyclohexanone **4**, 6-methyl-5-hepten-2-one **5**, 2-furyl methyl ketone **6**, 1-indanone **7**, and 2,4,4-trimethyl-2-cyclohexen-1-one **8** are commercially available.

Various culture media have been used:

- MEA (malt-extract-agar): malt extract (20 g/L), glucose (20 g/L), peptone (1 g/L), and agar (15 g/L).
- MA (malt-agar): malt extract (20 g/L), and agar (15 g/L).
- MYCOSEL (Mycosel-agar): papaic digest of soybean meal (10 g/L), dextrose (20 g/L), cycloheximide (0.4 g/L), and agar (15.5 g/L).
- TS (tryptic-soy-broth): bacteriological soy peptone (10 g/L), glucose (20 g/L), sodium acetate (1 g/L), sodium benzoate (0.05 mg/L), and bactoagar (15 g/L).
- PDA (potato-dextrose-agar): potato extract (4 g/L), glucose (20 g/L), and agar (15 g/L).
- PDB (potato-dextrose-broth): infusion from potatoes (20 g/L), and glucose (20 g/L).

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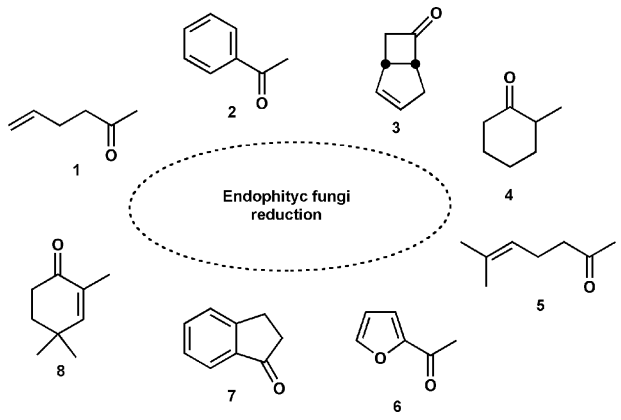


Scheme 1.

2.2. Sampled sites and plants

The endophytic fungi were isolated from the plants *Eugenia hallii*, *Schinus molle*, *Crataegus monogyna*, *Juniperus communis* and *Sambucus nigra*. The sampled sites were Amazonian forest (Quito, Ecuador), S. Giustina wood (Ferrara county, Italy) and Faedo mountain (Vicenza county, Italy) (Table 1).

2.3. Isolation, culture and identification of endophytic fungi

In order to isolate endophytic fungi, healthy plant tissues were washed in running tap water and processed as follows: samples were surface sterilized by sequentially dipping into 70% ethanol (1 min) and 5% sodium hypochlorite (5 min), rinsed with sterile water (10 min), and cut into about 0.5 cm^2 segments. The samples were put on four Petri dishes containing different media (MEA, MA, MYCOSEL, TS) and chloramphenicol (200 mg/L), incubated at 25 °C and checked daily for 21 days. The individual fungal colonies were placed in culture with PDA medium and chloramphenicol (200 mg/L).

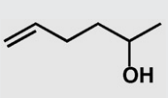
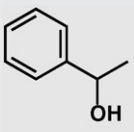
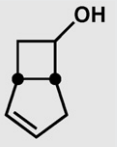
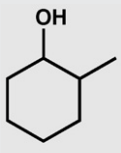
The isolated endophytic fungi were identified analysing their ribosomal DNA (ITS) sequence.

Genomic DNA was isolated as described by Griffin et al. [21] with the following modification. Fungal mycelia (about 50 mg) were placed in a sterile 1.5 mL microcentrifuge tube. 400 μL AP1 buffer (DNeasy Plant Mini Kit, Qiagen) were added to each tube and five freeze/thaw cycles were used to lyse fungal cells. PCR amplification of the ITS1 and ITS2 regions was performed with universal fungal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGA-TATG-3) [22], as described by Mares et al. [23]. Direct sequencing of the fragments was performed by MWG Biotech, using Big Dye terminator reaction chemistry. Sequences were determined both from strands using ITS1 and ITS4 as primers and they were submitted to GenBank database using an advanced BLAST search [24]. Alignments with more than 98% of identity were taken into account for the identification of endophytic fungi.

2.4. Reduction screening of endophytic fungi with the cocktail of the ketones **1–4**: general procedure

The ketone cocktail contains a mixture of substrates **1–4** (0.1 g of each substrate) in dimethyl sulphoxide (DMSO) (4.0 mL). Sterilized PDB (100 mL) was inoculated with a mycelium portion obtained from a culture of the selected endophytic fungus on PDA medium. The mixture was incubated for 4 days at 28 °C and 100 rpm. The ketone cocktail (1 mL) was added to the resulting suspension of grown cells and the incubation was continued for 7 days. Aliquots (1 mL) were withdrawn at 1, 3, 5 and 7 days. Samples were centrifuged to remove the cells (5000 rpm; 10 min), the supernatant was extracted with ethyl acetate (1 mL), dried over anhydrous Na_2SO_4 and analyzed by GLC on a chiral column with biphenyl as internal standard. GC analyses were performed on a Carlo Erba 6000 equipped with a fused capillary column. The reaction mixture of the ketone cocktail is analyzed on Megadex column (25 m × 0.25 mm) containing dimethyl-pentyl β-cyclodextrin in OV 1701; carrier gas: helium 80 kPa, temp 85–90 °C (0.5 °C/min), 90–100 °C (2 °C/min), 120–200 °C (10 °C/min), retention time (min): **1**, 4.89; (*S*)-**9**, 8.81; (*R*)-**9**, 9.03; (*S*)-**4**, 13.26; (*R*)-**4**, 13.48; (1*S*,5*R*)-**3**, 14.40; (1*R*,5*S*)-**3**, 15.50; (+/−)-*trans*-**13**, 18.24;

Table 1
Reduction screening of ketone cocktail **1–4** with endophytic fungi.

Endophytic fungus	Plant	Part	Origin	Reduction products[d]			
				 **9**	 **10**	 **11 and 12**	 **13 and 14**
FE86	*Schinus molle*	Leaves	Amazonian forest[a]	+++	+++	+++	++++
FE97	*S. molle*	Leaves	Amazonian forest[a]	–	–	++	–
FE114	*S. molle*	Bark	Amazonian forest[a]	++	+	+++	+++
Pestalotia	*Eugenia hallii*	Branches	Amazonian forest[a]	+++	++	+++	++++
FE180	*Crataegus monogyna*	Bark	Faedo wood[b]	–	+	–	++
FE452	*C. monogyna*	Branches	Faedo wood[b]	+	+	+++	+++
FE290	*C. monogyna*	Branches	S. Giustina wood[c]	+++	+++	+++	++++
FE360	*Juniperus communis*	Branches	S. Giustina wood[c]	+	–	+++	++
FE362	*J. communis*	Branches	S. Giustina wood[c]	–	–	–	–
FE521	*J. communis*	Bark	Faedo wood[b]	–	–	+	++
Epicoccum	*Sambucus nigra*	Branches	S. Giustina wood[c]	+++	+++	+++	++++

+, yield 10–20%; ++, yield 20–40%; +++, yield 40–70%; ++++, yield 70–90%.
[a] Quito, Ecuador.
[b] Vicenza, Italy.
[c] Ferrara, Italy.
[d] The best results are at 5/7 days of incubation.

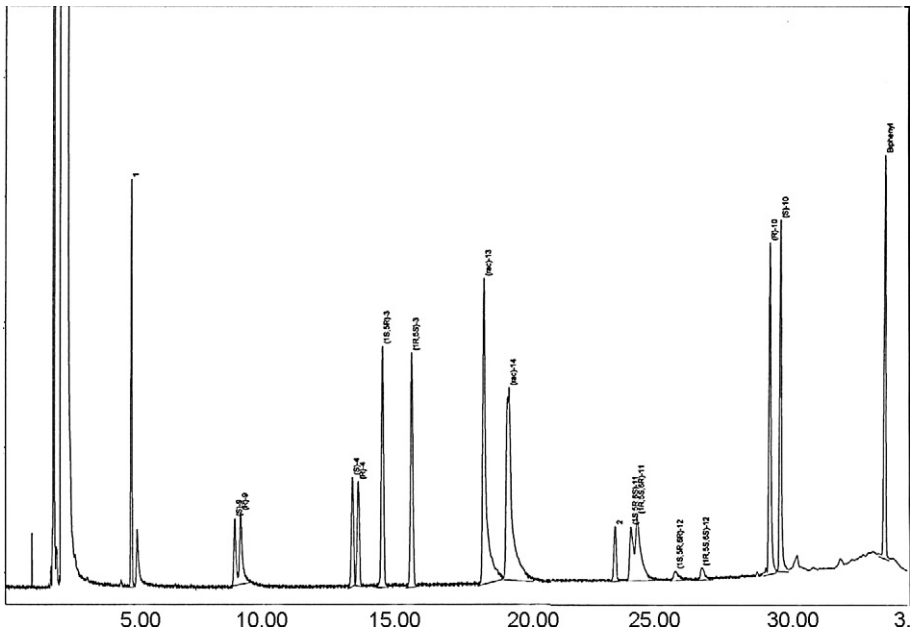


Fig. 1. Gaschromatographic analysis of the cocktail of ketones **1–4** with biphenyl as internal standard.

(+/−)-*cis*-**14**, 19.19; **2**, 23.31; (1*S*,5*R*,6*S*)-*endo*-**11**, 23.92; (1*R*,5*S*,6*R*)-*endo*-**11**, 23.92; (1*S*,5*R*,6*R*)-*exo*-**12**, 25.68; (1*R*,5*S*,6*S*)-*exo*-**12**, 26.71; (*R*)-**10**, 29.37; (*S*)-**10**, 29.79; biphenyl, 33.90 (Fig. 1).

The results are reported in Table 1.

2.5. Enantioselective reduction of ketones **1–8** with endophytic fungi: general procedure

The biotransformations were carried out as previously described. Sterilized PDB (200 mL) was inoculated with a mycelium portion obtained from the culture of the selected endophytic fungus on PDA medium. The mixture was incubated for 5 days at 28 °C and 80 rpm. To the resulting suspension of grown cells (dry weight 0.3 g for *Phomopsis* FE86, 0.45 g for *Phomopsis* FE290, 0.6 g for *Pestalotia* and 0.9 g for *Epicoccum*) a solution (2 mL) of the selected ketone (prepared dissolving 1 g of the ketone in 10 mL of DMSO) was added. The reaction was monitored withdrawing periodically aliquots (1 mL) that were centrifuged (5000 rpm; 10 min) to remove the biomass. The supernatant was extracted with ethyl acetate (1 mL), dried over anhydrous Na_2SO_4 and analyzed by GLC. The reaction mixtures, worked up as above, were chromatographed on silica (eluents were reported below) and the yields of the pure products **9–18** are reported in Table 2 together with the enantiomeric excesses determined by GLC on chiral column Megadex 5 (25 m × 0.25 mm) containing dimethyl-*n*-pentyl-β-cyclodextrin on OV 1701: helium as carrier gas (68 kPa).

For reduction of 5-hexen-2-one **1**, the chromatography (petroleum ether/diethyl ether 8:2 as eluent) afforded the (*S*)-5-hexen-2-ol **9**: $[\alpha]_D = 12.1$ (*c*, 4.6, $CHCl_3$) [25]. GLC analysis; temp 70–75 °C (0.5 °C/min) 75–200 °C (10 °C/min), retention time (min): **1**, 4.97; (*S*)-**9**, 9.8; (*R*)-**9**, 10.23.

For reduction of acetophenone **2**, the chromatography (cyclohexane/ethyl acetate 9:1 as eluent) afforded the (*S*)-1-phenylethanol **10**: $[\alpha]_D = -41$ (*c*, 5.1, $CHCl_3$) [26]. GLC analysis; temp 135–145 °C (2 °C/min) 145–200 °C (10 °C/min), retention time (min): **2**, 4.46; (*R*)-**10**, 5.83; (*S*)-**10**, 6.07.

For reduction of *cis*-bicyclo[3.2.0]hept-2-en-6-one **3**, the chromatography (petroleum ether/diethyl ether 7:3 as eluent) afforded the *endo*-(1*S*,5*R*,6*S*)-bicyclo[3.2.0]hept-2-en-6-ol **11** {$[\alpha]_D = 68$ (*c*, 1.1, $CHCl_3$)} [25] and the *exo*-(1*R*,5*S*,6*S*)-bicyclo[3.2.0]hept-2-en-6-ol **12** {$[\alpha]_D = -91$ (*c*, 2.6, $CHCl_3$)} [10]. GLC analysis; temp 100–120 °C (1.5 °C/min) 120–200 °C (10 °C/min), retention time (min): (1*S*,5*R*)-**3**, 5.49; (1*R*,5*S*)-**3**, 5.78; *exo*-(*1S,5R,6R*)-**12** (as acetyl derivative), 8.91; *exo*-(*1R,5S,6S*)-**12** (as acetyl derivative), 9.06; *endo*-(1*S*,5*R*,6*S*)-**11** (as acetyl derivative), 10.44; *endo*-(1*R*,5*S*,6*R*)-**11** (as acetyl derivative), 11.07.

For reduction of 2-methylcyclohexanone **4**, the chromatography (petroleum ether/diethyl ether 10:2 as eluent) afforded *trans*-(1*S*,2*S*)-2-methylcyclohexanol **13** {$[\alpha]_D = 38.2$ (*c*, 9.6, $CHCl_3$)} [25] and *cis*-(1*S*,2*R*)-2-methylcyclohexanol **14** {$[\alpha]_D = 18$ (*c*, 1.0, MeOH)} [25]. GLC analysis; temp 70–105 °C (1.5 °C/min) 105–200 °C (10 °C/min), retention time (min): (*R*)-**4**, 11.76; (*S*)-**4**, 11.96; *trans*-(*1S,2S*)-**13** (as acetyl derivative), 18.09; *cis*-(*1S,2R*)-**14** (as acetyl derivative), 18.62; *cis*-(*1R,2S*)-**14** (as acetyl derivative), 19.25; *trans*-(*1R,2R*)-**13** (as acetyl derivative), 19.40.

For reduction of 6-methyl-5-hepten-2-one **5**, the chromatography (petroleum ether/diethyl ether 8:2 as eluent) afforded the (*S*)-6-methyl-5-hepten-2-ol **15**: $[\alpha]_D = 14.5$ (*c*, 1.3, EtOH) [25]. GLC analysis; temp 90–115 °C (2 °C/min) 115–200 °C (10 °C/min) retention time (min): **5**, 9.31; (*S*)-**15**, 13.02; (*R*)-**15**, 13.44.

For reduction of 2-furyl methyl ketone **6**, the chromatography (cyclohexane/ethyl acetate 20:1 as eluent) afforded the (*S*)-1-(2-furyl)-ethanol **16**: $[\alpha]_D = -22$ (*c*, 2.7, $CHCl_3$) [27]. GLC analysis; temp 80–200 °C (1 °C/min), retention time (min): **6**, 12.94; (*R*)-**16**, 21.54; (*S*)-**16**, 21.68.

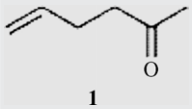
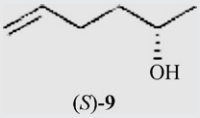
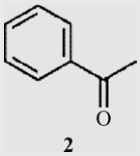
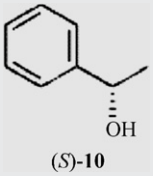
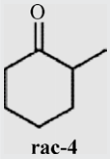
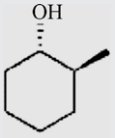
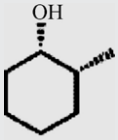
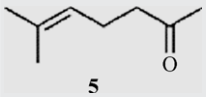
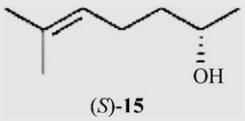
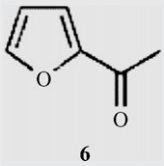
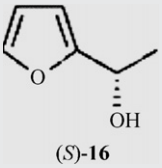
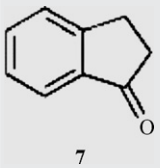
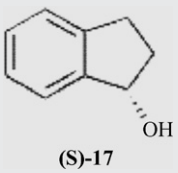
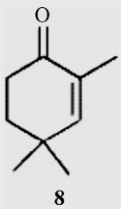
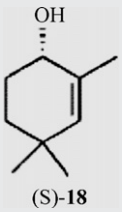
For reduction of 1-indanone **7**, the chromatography (cyclohexane/ethyl acetate 10/1 as eluent) afforded the (*S*)-indanol **17**: $[\alpha]_D = 31$ (*c*, 1, $CHCl_3$). The *R*-enantiomer is commercially available. GLC analysis; temp 100–200 °C (5 °C/min), retention time (min): **7**, 12.36; (*R*)-**17**, 12.74; (*S*)-**17**, 12.86.

For reduction of 2,4,4-trimethyl-2-cyclohexen-1-one **8**, the chromatography (cyclohexane/ethyl acetate 10/1 as eluent) afforded the (*S*)-2,4,4-trimethyl-2-cyclohexenol **18**: $[\alpha]_D = -88.3$ (*c*, 1.03, CH_3OH) [28]. GLC analysis; temp 100–200 °C (2 °C/min), retention time (min): **8**, 8.48; (*R*)-**18** 12.8; (*S*)-**18**, 13.3.

3. Results and discussion

The endophytic fungi were isolated from different parts (i.e. leaves, bark and branches) of various plants (i.e. *E. hallii*, *S. molle*,

Table 2
Reduction of ketones **1–8** with endophytic fungi.

Substrate	Endophytic fungi	Time (d)	Reduction products		Yield % (ee %)	
 **1**	*Phomopsis* (FE86)	7	 (*S*)-**9**	**41** (80)		
	Pestalotia	7		**33** (60)		
	Phomopsis (FE290)	7		**50** (70)		
	Epicoccum	3		**55** (62)		
 **2**	*Phomopsis* (FE86)	7	 (*S*)-**10**	**62** (66)		
	Pestalotia	7		**40** (25)		
	Phomopsis (FE290) *Epicoccum*	7		**35** (24)		
	Pestalotia	3		**50** (58)		
 **rac-3**	*Phomopsis* (FE86)	7	 (*1S,5R,6S*)-**11**	 (*1R,5S,6S*)-**12**	**30** (95)	**20** (>99)
	Pestalotia	7			**40** (75)	**7** (>99)
	Phomopsis (FE290)	7			**14** (72)	**46** (>99)
	Epicoccum	3			**55** (74)	**14** (>99)
 **rac-4**	*Phomopsis* (FE86)	7	 (*1S,2S*)-**13**	 (*1S,2R*)-**14**	**54** (76)	**35** (96)
	Pestalotia	2			**24** (>99)	**60** (>99)
	Phomopsis (FE290)	4			**39** (82)	**37** (90)
	Epicoccum	2			**51** (>99)	**44** (>99)
 **5**	*Phomopsis* (FE86)	7	 (*S*)-**15**	–		
	Pestalotia	7		**20** (59)		
	Phomopsis (FE290)	7		–		
	Epicoccum	7		**68** (40)		
 **6**	*Phomopsis* (FE86)	7	 (*S*)-**16**	–		
	Pestalotia	7		–		
	Phomopsis (FE290)	7		–		
	Epicoccum	7		**52** (25)		
 **7**	*Phomopsis* (FE86)	7	 **(S)-17**	–		
	Pestalotia	7		–		
	Phomopsis (FE290)	7		**23** (70)		
	Epicoccum	7		–		
 **8**	*Phomopsis* (FE86)	7	 (S)-**18**	–		
	Pestalotia	7		–		
	Phomopsis (FE290)	2		**70** (95)		
	Epicoccum	7		–		

C. monogyna, *J. communis* and *S. nigra)* which were sampled in different sites (i.e. Amazonian forest, S. Giustina wood and Faedo mountain) (Table 1).

Since fungi catalyzed biotransformations usually require long time, reductions were carried out using a recently proposed strategy which consists of a multi-substrate solution for the activity fingerprinting of various hydrolytic enzymes [29]. The strains isolated from *S. molle* and *C. monogyna* together with *Pestalotia* (from *E. hallii*) and *Epicoccum* (from *S. nigra*) showed a good potential in the prochiral ketones **1–4** reduction while the endophytic fungi FE360, and FE521 from the branches of *J. communis* poorly reduced the model substrates. Only the strain FE362, isolated from the bark of *J. communis*, showed no reduction activity.

The results obtained using a cocktail of substrates have been considered significant only for screening the biotransformation potential of an isolated organism. The ketones of the cocktail

were chosen in order to have a mixture with different chemical structures (i.e. aliphatic, cyclic and aromatic). However, when considering the chemical structure of ketones, all fungi showed a better activity in reducing the cyclic compounds **3** and **4** (40–70% yield of the alcohols) rather than the aliphatic and aromatic compounds **1** and **2**. Finally, it is worth of note that in all cases the enantioselectivity was appreciable towards the formation of the *S*-enantiomer.

The biotransformation procedure was very simple. A "cocktail" of ketones **1–4**, prepared dissolving an equal amount of each substrate in DMSO, was added to a PDB culture of the selected endophytic fungus, grown 5 days at 28 °C. The reaction mixture was periodically monitored by GLC till 7 days incubation. As shown in Table 1, the best reduction results were obtained when FE 86 (from *S. molle*), *Pestalotia* (from *E. hallii*), FE 290 (from *C. monogyna*) and *Epicoccum* (from *S. nigra*) were used.

Before proceeding with the enantioselective reduction of the ketones **1–8** on a preparative scale, the strains FE 86 and FE 290 were identified on the basis of their ribosomal DNA (ITS) sequences.

These sequences, namely FJ440699 (Genbank accession no.) for FE 86 and FJ440701 for FE 290, were highly similar to the *Phomopsis* sp. ITS (AY620999) with 98% and 100% identity, respectively. These highly related ITS strongly support the inclusion of the isolated strains in the *Phomopsis* genus [30]. From the sequences FE 86 and FE 290 strains were identified as *Phomopsis* sp.

On preparative scale biotransformations were carried out as described above replacing the multi-substrate solution with the pure ketones. As previously, the reaction mixtures were analyzed by GLC to determine the enantiomeric excesses of the obtained chiral alcohols **9–18**, subsequently purified and characterized in comparison with authentic samples. The results are reported in Table 2.

All fungi reduce the model substrates **1–4** with both good yields (33–95%) and enantiomeric excesses (24–99%) but there are some unexpected negative results varying the chemical structure of the ketones (i.e. compounds **5–8)**.

In particular, all four strains gave fairly good yields (33–55%) in the reduction of 5-hexen-2-one **1** to the corresponding (*S*)-hexenol **9** also with good enantiomeric excesses (ee 60–80%). On the other hand, *Phomopsis* (FE 86) produced the best enantiomeric excess (80%) with 41% yield in the reduction of 5-hexen-2-one **1**, while in the reduction of acetophenone **2** *P.* (FE86) gave the (*S*)-1-phenylethanol **10** both with the highest yield (62%) and lower ee (66%). The *S*-alcohol **10** was also obtained with the other strains but with lower yields (35–50%) and ees (25–58%).

Moreover the reduction of the racemic *cis*-bicyclo[3.2.0]hept-2-en-6-one **3**, however, afforded in all cases the (6*S*)-*endo*-alcohol **11** and the (6*S*)-*exo*-alcohol **12** with variable yields but always with excellent enantiomeric excesses. In particular, *Pestalotia* and *Epicoccum* and *P.* (FE 86) produced the *endo*-alcohol **11** as main product (30–55% yield, ee 74–95%), while *Phomopsis* (FE 290) gave the *exo*-alcohol **12** in good yields (46%, ee > 99%) with only a 14% yield of the *endo*-alcohol **11**.

Similar results were obtained in the reduction of the racemic 2-methylcyclohexanone **4**: in all cases the (1*S*)-*trans*-alcohol **13** and (1*S*)-*cis*-alcohol **14** were produced by endophytic fungi catalyzed reduction with excellent yields and enantiomeric excesses. The main product was the (1*S*,2*S*)-*trans*-2-methylcyclohexanol **13** with *Phomopsis* (FE86) (54% yield, ee 76%), *Epicoccum* (51%, ee > 99%), while *Phomopsis* (FE 290) afforded the *trans*- and *cis*-alcohols in quite equal amount (39% and 37%) with good ee (82% and 90%), respectively. On the contrary, the reduction of 2-methylcyclohexanone **4** with *Pestalotia* produced the *cis*-alcohol **14** as main product (60% yield, ee > 99%) together with a 24% yield (ee > 99%) of the *trans*-alcohol **13**.

Since data of the biotransformations carried out on preparative scale have substantially confirmed the preliminary qualitative results obtained in the screening, we utilized the same strains in the reduction of other structurally correlated ketones **5–8**.

Surprisingly in spite of the structural similarity to ketone **1**, 6-methyl-5-hepten-2-one (sulcatone) **5** has been reduced only by *Pestalotia* and *Epicoccum* obtaining the (*S*)-sulcatol **15** (20% and 68% yield) with quite good ees (59% and 40%), respectively.

A similar result was observed with 2-furyl methyl ketone **6** in comparison with acetophenone **2**. In the ketone **6** the replacement of a phenyl with a furyl ring drastically decreased the reductive activity of the endophytic fungi. In fact only *Pestalotia* produced quite good results in the reduction of **6** affording the (*S*)-2-furyl methyl carbinol **16** with good yield (52%) but with poor ee (25%).

Finally, only *Phomopsis* (FE 290) has reduced the 1-indanone **7** giving the (*S*)-1-indanol **17** (23%, ee 70%) and the 2,4,4-trimethyl-2-cyclohexen-1-one **8** affording the *S*-2,4,4-trimethyl-2-cyclohexen-1-ol **18** (70%, ee 95%). This last reduction is very interesting if compared with the expensive chemical methods that use ruthenium complexes for the chemoselective reduction of the carbonyl group [28,31].

4. Conclusions

This is the first report regarding the enantioselective reductions of prochiral ketones with endophytic fungi. The endophytic fungi were isolated from various plants sampled in Italy and in Amazonian forest (Ecuador). Initially the reduction potential was tested using a cocktail of model substrates **1–4** and the most active fungi (i.e. *Phomopsis* from *S. molle* and *Crataegus monogyna*, *Pestalotia* from *E. hallii* and *Epicoccum* from *S. nigra*) were used for reductions on preparative scale.

As most of the microorganisms these fungi follow the Prelog's rule in the reduction of the model substrates **1–4** affording the (*S*)-alcohols with good yields (33–95%) and enantiomeric excesses (24–99%). However, there are some unexpected negative results depending on the chemical structure of the ketones (i.e. compounds **5–8**). *Phomopsis* (FE290) is the only endophyte able to reduce 1-indanone **7** and 2,4,4-trimethyl-2-cyclohexen-1-one **8** affording 23% and 70% of the corresponding (*S*)-alcohols (ee 70% and 95%, respectively). The chemoselective and enantioselective reduction of **8** is very interesting if compared with the expensive chemical method currently used for this reaction. On the other hand *Phomopsis* (FE86) does not reduce any substrate, whereas *Pestalotia* reduces only 6-methyl-5-hepten-2-one **5** (sulcatone) with poor yield (20%, ee 59%) and *Epicoccum* reduces only 2-furyl methyl ketone (52%) with poor ee of the alcohol (25%). In conclusion the endophytes potential in reduction is worth examining because it could be interesting especially regarding the chemical constituents of the plants when compared with other microorganisms

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