environmental microbiology reports

Environmental Microbiology Reports (2012) **4**(1), 105–112 doi:10.1111/j.1758-2229.2011.00310.x

Dissemination of wine-related yeasts by migratory birds

Nicola Francesca,[1] Domenica E. Canale,[2] Luca Settanni[1] and Giancarlo Moschetti[1]*

[1]*DEMETRA Department, Viale delle Scienze 4, 90128, Palermo, Italy.*

[2]*Department of Animal Biology, University of Palermo, Via Archirafi 18, 90123, Palermo, Italy.*

Summary

The present work was undertaken to evaluate the contribution of migratory birds in the environmental dissemination of yeasts. Four sites (Mazara del Vallo, Lampedusa, Ustica and Linosa), representing the main stop-over points in Sicily, were analysed during spring and autumnal bird migration and 349 birds (belonging to 10 families) were ringed and analysed for yeast presence. A total of 125 yeasts were isolated and identified by a multiple genotypic approach, consisting of restriction fragment length polymorphism (RFLP) of 5.8S rRNA gene and 26S rRNA and sequencing of D1/D2 domain of the 26S rRNA gene, which resulted in the recognition of 18 species, including the technological relevant *Saccharomyces cerevisiae* which were characterized at strain level applying three techniques (interdelta analysis, minisatellite analysis based on the separate amplification of three genes and microsatellite multiplex PCR of polymorphic microsatellite loci). The evaluation of the persistence of living *S. cerevisiae* in birds for about 12 h from ingestion of inoculated feed allowed the conclusion that yeasts with technological potential are disseminated during migration.

Introduction

The microbial habitat associated with birds represents the object of several ecological surveys (Maul *et al.*, 2005). The interest towards these investigations is on the increase for the evaluation of the health state and/or risk of diseases of birds (Silvanose *et al.*, 2001), but especially to deepen the knowledge about the human infections associated with wild birds (Omenn, 2010). Free-living birds, including migratory species, have been reported as long-distance vectors of microorganisms that can be transmissible to humans (Nuttall, 1997). Indeed, associations between wild birds and microorganisms have been studied mainly focusing on bacteria, whereas limited studies on yeasts are available (Cafarchia *et al.*, 2006).

The monitoring of bird movements allows the investigation about behavioural and demographical responses to a given environment (Burton *et al.*, 2006). The migration of birds includes a round trip to the resting areas and a return to the territories of nesting, which occur in autumn and spring respectively. These movements follow the seasonality of food resources. The main energy source during flight is the body fat. The birds with low fat reserves have the necessity of stopping in some resting sites (stop-over) along the route. The phenomenon of migration is typical in the islands of the Mediterranean Sea where birds stop to increase fat lost during flight.

Since birds act as microorganism vectors, the analysis of the microflora they host may be important to evaluate the microbial diversity of the sites visited. From the application perspective, yeasts carried out by birds have not been deeply investigated. Nowadays, there is a growing interest of wine producers to perform winemaking employing 'autochthonous' strains which may ensure typical terroir characteristics (Terroir Viticoles, 2006). At this regard, Francesca and colleagues (2010) recently reported on the dissemination of oenological yeasts by vineyard inhabiting birds, mainly Black birds (*Turdus merula*), although no yeast with technological relevant traits was found in the few migratory birds analysed. Those authors evidenced an issue related to the autochthonous status of yeasts, since they may not be indigenous in a given environment. The species *Saccharomyces cerevisiae* represents the most important oenological yeast, whose origin is quite hard to retrieve (Naumov, 1996; Mortimer, 2000). This species is not an air-borne contaminant and needs a vector (Mortimer and Polsinelli, 1999).

Yeasts may be transported at different distances depending on the vector. Some studies provided evidences that insects such as honey bees disseminate *S. cerevisiae* strains at a distance of approximately 10 km (Goddard *et al.*, 2010), thus the investigation of migratory birds may better clarify the movements of yeasts with technological relevance. During migration, several sites

Received 21 September, 2011; accepted 27 October, 2011. *For correspondence. E-mail giancarlo.moschetti@unipa.it; Tel. (+39) 091 23896050; Fax (+39) 091 6515531.

are visited by birds because they represent important stop-over points. In case of migration from Africa to Europe and vice versa, Lampedusa and Ustica are visited in spring when the direction is from sub-Saharan areas to North Europe, while Linosa and Mazara del Vallo are visited in autumn when the movement is opposite (Svensson, 1992).

For the above reasons, the main aims of the present work were: to evaluate the potential of birds in disseminating yeasts at long distances, analysing the main migration stop-over points in the Mediterranen Sea (1, Riserva Naturale Integrale Lago Preola and Gorghi Tondi – Mazara del Vallo; 2, Lampedusa island; 3, Ustica island; and 4, Linosa island); to identify yeast species; and to determine the permanence of yeasts in birds after ingestion.

Results and discussion

Characterization of the sites

The four experimental sites located within Sicily region (Riserva Naturale Integrale Lago Preola and Gorghi Tondi, Lampedusa island, Ustica island and Linosa island) and the period (spring and autumnal seasons) for bird collection were strategic for the evaluation of the role of several birds in the dissemination of yeasts through North Africa and Europe. The experimental procedure included the collection of samples in the first stop-over points visited during each migration. In order to avoid any contamination of birds with local materials, the capture has been performed in the proximity of the woody areas in each site of ringing just at the moment of landing. The sampling size could not be designed because affected by species (and /or individuals per species) variability.

In the present study, 349 birds were captured (Table 1). All birds were quickly ringed, identified at species level by phenotypic analysis (Mullarney *et al*., 1999), classified according to their migration strategy (trans-saharan or partial) (Svensson, 1992) and subjected to visual biometric measurement of subcutaneous fat amount (SFA) of the abdominal region (Kaiser, 1993). The body fat is the first energy source dissipated by birds during migration and when SFA reaches a value of 0 or 1, a stop is necessary (Goymann *et al*., 2010).

Isolation and identification of yeasts

Yeasts were isolated from bird cloacae, where they have reasonably arrived after gastric transit. Hence, the primary source of yeast contamination has to be imputable to the food ingested.

Yeast presence was found in 32.66% of birds (Table 1) that was a higher percentage of yeast isolation than that reported by Cafarchia and colleagues (2006) who analysed a similar number of birds (421). The bird families showing the highest frequencies of isolation were Muscicapidae, Passerideae and Turdidae and except the birds belonging to the Fringillidae and Passerideae families, which are insectivores in spring and granivorous in autumn, all other birds captured in this study are obligate insectivores (Snow and Perrins, 1998).

The process of isolation resulted in the collection of 125 yeasts, which were genetically identified. After restriction analysis of 5.8S-ITS region, the isolates were clustered in 18 groups; eight of these groups were recognized at species level (*Aureobasidium pullulans*, *Candida albicans*, *Cryptococcus magnus*, *Debaryomyces hansenii*, *Hanseniaspora guillermondii*, *Pichia terricola*, *Metschnikowia pulcherrima* and *Rhodotorula mucilaginosa*) but 10 were not. They were characterized by atypical restriction profiles of 5.8S-ITS that is not surprising, since many authors observed this behaviour in several yeasts (Fernandez-Espinar *et al*., 2000; Kurtzman and Robnett, 2003; Solieri *et al*., 2007; Tofalo *et al*., 2009).

Yeast identification continued with 26S rRNA gene digestion which confirmed the 18 groups, but identified less species then the previous methods, included in the eight above cited. Moreover, the identification at species level was concluded by sequencing of the D1/D2 domain of the 26S rRNA gene, which was successful for 17 out of the 18 groups (Table 2). Yeasts of group V were only recognized at genus level, since a low percentage (95%) of identity was found with *Candida* spp. An even lower similarity level (92%) with the same genus was obtained from the sequencing of the region 5.8S-ITS (Acc. No. JF292448). It is worth of note that the digestion products obtained from ITS fragment of group III (*Candida glabrata*), IV (*Candida inconspicua*), V (*Candida* spp.), VI (*Candida stellimalicola*), VII (*Cryptococcus aureus*), VIII (*Cryptococcus carnescens*), XII (*Pichia kudriavzevii*), XV (*Pseudozyma aphidis*), XVII (*S. cerevisiae*) and XVIII (*Sporisorium penniseti*) have not been previously reported in literature.

The yeast species most frequently encountered during isolation (Table 3) were *H. guilliermondii* (17.60%), *C. albicans* (16.00%), *S. cerevisiae* (14.40%) and *A. pullulans* (12.00%). Among the yeast isolates, 72 belonged to species that are commonly reported to be wine-associated (WA) yeasts such as *A. pullulans*, *D. hansenii*, *H. guilliermondii*, *P. kudriavzevii*, *P. terricola*, *M. pulcherrima*, *R. mucilaginosa* and *S. cerevisiae* (Loureiro and Malfeito-Ferreira, 2003; Gonzalez-Pombo *et al*., 2008; Moreira *et al*., 2008; Francesca *et al*., 2010; Ocón *et al*., 2010).

Bird/yeast correlations

The distribution of yeasts among birds is reported in Table 3. The highest number of species and isolates were found for the bird groups most numerous. Thus, garden

Table 1. Bird captured[a] in four Sicilian ringing sites.

Bird family	Bird species	No. of birds	No. of birds carrying yeasts	SFA[b] of birds carrying yeasts		Sampling sites[c] (number of birds)
				0–1	2–7	
Sylviidae	*Sylvia borin* (garden warbler)	90	41	26	15	L (5); Ln (6); MdV (4); U (26)
	Sylvia curruca (lesser whitethroat)	1	0	0	0	
	Hippolais polyglotta (melodious warbler)	1	0	0	0	
	Hippolais icterina (icterine warbler)	24	8	6	2	L (2); U (6)
	Acrocephalus scirpaceus (reed warbler)	12	5	1	4	MdV (4); U (1)
	Acrocephalus arundinaceus (great-reed warbler)	3	0	0	0	
	Sylvia atricapilla (blackcap)	2	1	1	0	Ln (1)
	Acrocephalus schoenobaenus (sedge warbler)	6	3	3	0	L (3)
	Phylloscopus trochilus (willow warbler)	7	4	0	4	Ln (4)
	Phylloscopus collybita (chiffchaff)	1	0	0	0	
	Phylloscopus sibilatrix (wood warbler)	5	1	0	1	U (1)
	Sylvia melanocephala (sardinian warbler)	15	0	0	0	
	Sylvia communis (whitethroat)	8	1	0	1	L (1)
	Sylvia cantillans (subalpine warbler)	40	14	2	12	Ln (10); MdV (4)
	Cettia cetti (cetti's warbler)	2	0	0	0	
Muscicapidae	*Ficedula albicollis* (collared flycatcher)	1	0	0	0	
	Ficedula hypoleuca (pied flycatcher)	6	0	0	0	
	Muscicapa striata (spotted flycatcher)	51	13	10	3	MdV (2); U (11)
Turdidae	*Phoenicurus phoenicurus* (redstart)	12	4	4	0	L (1); Ln (3)
	Turdus philomelos (song thrush)	1	0	0	0	
	Erithacus rubecula (robin)	1	1	1	0	Ln (1)
	Luscinia megarhynchos (nightingale)	4	1	1	0	MdV (1)
	Monticola solitarius (blue rock-thrush)	1	1	0	1	Ln (1)
	Saxicola rubetra (winchat)	23	9	7	2	L (7); U (2)
Hirundinidae	*Hirundo rustica* (swallow)	12	2	2	0	Ln (2)
Passeridae	*Passer montanus* (tree sparrow)	1	0	0	0	
	Passer hispaniolensis (Spanish sparrow)	8	4	3	1	Ln (3); MdV (1)
Oriolidae	*Oriolus oriolus* (golden oriole)	3	0	0	0	
Fringillidae	*Carduelis cannabina* (linnet)	2	0	0	0	
Laniidae	*Lanius senator* (woodchat shrike)	2	0	0	0	
Motacillidae	*Anthus trivialis* (tree pipit)	2	0	0	0	
Paridae	*Parus major* (great tit)	2	1	1	0	MdV (1)
Total		349	114 (32.66%)	68	46	L (19); Ln (31); MdV (18); U (46)

a. The birds were captured by means of a Mist-nets with four shelf-nets (100 m in length) placed in the proximity of the woody areas in each site of ringing and treated following the instructions of the 'Istituto Superiore per la Protezione e la Ricerca Ambientale' (ISPRA). All birds were quickly ringed, identified at species level by phenotypic analysis (Mullarney *et al*., 1999) and soon after ringing the bird cloacae were examined for yeast presence following the methodology of Francesca and colleagues (2010).
b. SFA, subcutaneous fat amount.
c. L, Lampedusa; Ln, Linosa; MdV, Mazara del Vallo; U, Ustica.

warbler and subalpine warbler were the richer sources of fungal biodiversity. However, some WA yeasts, such as *S. cerevisiae*, the most important species in winemaking (Fleet, 2003), were similarly distributed among garden warbler, icterine warbler, redstar, whitethroat, subalpine warbler and winchat, while seven isolates were collected from spotted flycatcher.

Wine-related yeasts

The 58.40% of total yeasts and the majority of WA yeasts (63.89%) were isolated from birds with an SFA value comprised between zero and one (Table 1). Some species as *D. hansenii*, *M. pulcherrima* and *R. mucilaginosa* were obtained only from birds with 0–1 SFA. Except for *H. guilliermondii* (45.45%), the most abundant WA yeast species, such as *A. pullulans* (66.66%) and *S. cerevisiae* (66.67%), were mainly collected from birds with low SFA. The species *P. kudriavzevii* and *P. terricola* are also considered WA.

To our knowledge, this research represents the first report on the isolation and characterization of WA yeasts carried by birds during migration and provides additional elements needing to be analysed when a strain is defined as autochthonous. It is already known that some insects (*Drosophila*, *Apis* and *Vespa*) may carry micro-organisms, yeasts included, on their bodies (Snowdon and Cliver, 1996) and their gut (Ricci *et al*., 2011), but their contribution to the spreading of yeasts is within short distances.

In this study, a new finding deepens the knowledge about the environmental distribution of *S. cerevisiae*. Besides the fact that birds were analysed soon after landing, the sites of Lampedusa is not typically character-

Table 2. Molecular identification of yeasts.

R.P.	5.8S-ITS PCR[a]	Size of restriction fragments[a]			26S PCR[b]	Size of restriction fragments[b]			Species (% identity)[c,d]	Accession number
		CfoI	HaeIII	HinfI		HinfI	MseI	ApaI		
I	600	180 + 165 + 90	430 + 150	230 + 175 + 125	1100	490 + 410 + 190	610 + 360 + 95	n.c.	*Aureobasidium pullulans* (99)	HQ641272
II	550	290 + 260	460 + 90	280 + 270	1100	490 + 400 + 190	610 + 425	n.c.	*Candida albicans* (99)	HQ641284
III	900	380 + 165 + 140	660 + 220	350 + 260 + 50	1050	500 + 220 + 200 + 180	700 + 370	700 + 420	*Candida glabrata* (100)	HQ641276
IV	480	105 + 90 + 75 + 55	450	260 + 210	1150	490 + 240 + 180 + 130 + 110	1000 + 90	700 + 390	*Candida inconspicua* (99)	HQ641283
V	460	200 + 180 + 80	450	220 + 190 + 50	1100	490 + 400 + 100 + 90	390 + 375 + 285 + 75	n.c.	*Candida* spp. (95)	HQ641271
VI	525	200 + 155 + 130	515	290 + 220	1100	500 + 400 + 180	n.c.	n.c.	*Candida stellimalicola* (99)	HQ641277
VII	520	250 + 200 + 70	520	290 + 170	1160	470 + 290 + 210 + 190	400 + 370 + 270	n.c.	*Cryptococcus aureus* (99)	HQ641274
VIII	550	280 + 240	350 + 90 + 65	250 + 250	1100	440 + 280 + 220 + 205	425 + 380 + 260 + 75	n.c.	*Cryptococcus carnescens* (99)	HQ641265
IX	650	355 + 295	495 + 95 + 60	270 + 240 + 140	1100	280 + 200 + 180 + 150	400 + 370 + 270	n.c.	*Cryptococcus magnus* (99)	HQ641280
X	650	300 + 300 + 50	420 + 150 + 90	325 + 325	1100	490 + 410 + 190	610 + 320 + 115 + 75	n.c.	*Debaromyces hansenii* (99)	HQ641266
XI	750	320 + 310 + 105	750	350 + 200 + 180	1000	415 + 395 + 190 + 105	610 + 500 + 440 + 100 + 75	n.c.	*Hanseniapora guilliermondii*[e] (99)	HQ641270
XII	500	115 + 90 + 75 + 55	325 + 90 + 75	270 + 225	1100	500 + 250 + 180	n.c.	700 + 400	*Pichia kudriavzevii* (100)	HQ641275
XIII	450	130 + 100 + 90 + 85 + 45	290 + 125	240 + 105 + 105	1100	500 + 350	800 + 200	n.c.	*Pichia terricola* (99)	HQ641279
XIV	400	205 + 100 + 95	280 + 100	200 + 190	1100	380 + 260 + 240	580 + 270 + 140	600 + 420	*Metschnikowia pulcherrima* (98)	HQ641286
XV	780	220 + 170 + 150 + 130	420 + 320	440 + 340	1100	480 + 250 + 200	400 + 370 + 270	n.c.	*Pseudozyma aphidis* (99)	HQ641278
XVI	640	320 + 240 + 80	425 + 215	340 + 225 + 75	1100	500 + 400 + 200	400 + 300 + 250	n.c.	*Rhodotorula mucilaginosa* (99)	HQ641269
XVII	850	375 + 335 + 140	320 + 240 + 170 + 130	370 + 130 + 110	1100	500 + 220 + 180	n.c.	n.c.	*Saccharomyces cerevisiae* (99)	HQ641267
XVIII	825	370 + 250 + 150	300 + 240 + 160 + 60	400 + 200 + 130	1100	500 + 260 + 200	400 + 380 + 300	n.c.	*Sporisorium penniseti* (97)	HQ641273

a. According to Esteve-Zarzoso and colleagues (1999).
b. According to Baleiras-Couto and colleagues (2005).
c. According to O'Donnel (1993).
d. According to BlastN search of D1/D2 26S rRNA gene sequences in NCBI database.
e. The 5.8S-ITS gene was also digested with DdeI endonuclease confirming the restriction profile reported by Esteve-Zarzoso and colleagues (1999).
All values for the 5.8S-ITS PCR, 26S PCR and restriction fragments are given in bp.
R.P., restriction profile; n.c., not cut.

Table 3. Yeasts carried by migratory birds.

	Bird species																		
	A (2)	B (1)	C (90)	D (2)	E (24)	F (4)	G (12)	H (12)	I (1)	J (6)	K (8)	L (51)	M (40)	N (12)	O (8)	P (7)	Q (23)	R (5)	
Yeast species	•[a]	■	•	■	•	•	•	•	•	•	■	•	•	•	•	•	•	•	Total No.
A. pullulans			8		4	1							1					1	15
C. albicans		1	7	1	1		1	2			4		2				1		20
C. glabrata													2						2
C. inconspicua							2									1			3
Candida spp.			3		2							2							7
C. stellimalicola														2					2
Cr. aureus			3																3
Cr. carnescens																2			2
Cr. magnus												2	1				6		9
D. hansenii			2																2
H. guilliermondii	1	1	4				1		1		4		9			1			22
P. kudriavzevii		1	3										1						5
P. terricola								1					2						3
M. pulcherrima			3																3
Ps. aphidis										3									3
R. mucilaginosa			4																4
S. cerevisiae			3		2		1					7	1		2		2		18
Sp. penniseti								2											2
Total	1	3	40	1	9	1	5	5	1	3	8	11	19	2	2	4	9	1	125

a. The symbol indicates the classification of birds ringed according to their migration strategy: •, trans-saharan migratory bird; ■, partial migratory bird (Svensson, 1992).
All birds ringed were classified according to their migration strategy (trans-saharan or partial) (Svensson, 1992).
Letters: A, blackcap; B, blue rock-thrush; C, garden warbler; D, great tit; E, icterine warbler; F, nightingale; G, redstar; H, reed warbler; I, robin; J, sedge warbler; K, Spanish sparrow; L, spotted flycatcher; M, subalpine warbler; N, swallow; O, whitethroat; P, willow warbler; Q, winchat; R, wood warbler.
The number of individuals analysed per species is reported between brackets.

Table 4. Typing of *Saccharomyces cerevisiae* isolates.

				Patterns				
						Minisatellite analysis[e]		
Isolate code	Isolation source (bird code)	SFA[a]	Ringing site[b]	Interdelta analysis[c]	Microsatellite multiplex PCR[d]	*aga*1	*sed*1	*dan*4
ULSc24	Icterine warbler (br1)	1	L	Int1	Mul1	Mina1	Mins1	Mind1
ULSc26	Icterine warbler (br2)	1	L	Int1	Mul1	Mina1	Mins1	Mind1
ULSc28	Whinchat (br3)	1	L	Int1	Mul1	Mina1	Mins1	Mind1
ULSc29	Whinchat (br3)	2	L	Int2	Mul2	Mina2	Mins2	Mind2
ULSc30	Whitetroat (br4)	2	L	Int1	Mul1	Mina1	Mins1	Mind1
ULSc31	Whitetroat (br5)	2	L	Int1	Mul1	Mina1	Mins1	Mind1
ULSc32	Redstar (br6)	1	L	Int1	Mul1	Mina1	Mins1	Mind1
ULSc37	Garden warbler (br7)	3	L	Int1	Mul1	Mina1	Mins1	Mind1
ULSc139	Garden warbler (br8)	2	U	Int3	Mul3	Mina3	Mins1	Mind1
ULSc140	Garden warbler (br9)	2	U	Int3	Mul3	Mina3	Mins1	Mind1
ULSc144	Spotted flycatcher (br10)	0	U	Int3	Mul3	Mina3	Mins1	Mind1
ULSc145	Spotted flycatcher (br11)	0	U	Int3	Mul3	Mina3	Mins1	Mind1
ULSc146	Spotted flycatcher (br12)	1	U	Int3	Mul3	Mina3	Mins1	Mind1
ULSc148	Spotted flycatcher (br13)	1	U	Int3	Mul3	Mina3	Mins1	Mind1
ULSc186	Spotted flycatcher (br14)	1	U	Int3	Mul3	Mina3	Mins1	Mind1
ULSc326	Spotted flycatcher (br15)	1	MdV	Int4	Mul4	Mina4	Mins3	Mind3
ULSc338	Spotted flycatcher (br16)	1	U	Int5	Mul5	Mina5	Mins4	Mind4
ULSc339	Subalpine warbler (br17)	0	L	Int6	Mul6	Mina6	Mins5	Mind5
Total of different patterns				6	6	6	5	5

a. SFA, subcutaneous fat amount.
b. L, Lampedusa; U, Ustica; MdV, Mazara del Vallo.
c. According to Legras and Karst (2003).
d. According to Vaudano and Garcia-Moruno (2008).
e. According to Marinangeli and colleagues (2004).

ized by wine production, and thus, the contamination of bird cloacae with local *S. cerevisiae* has to be excluded.

Due to their technological importance, the species *S. cerevisiae* was investigated at strain level. The 18 isolates (Table 3) belonging to this species were subjected to molecular typing by applying three techniques (interdelta analysis, minisatellite analysis based on the separate amplification of three genes and microsatellite multiplex PCR of polymorphic microsatellite loci); the corresponding patterns are reported in Table 4. The 18 isolates were allotted into six strains. The strain ULSc326, isolated from Mazara del Vallo site, was characterized by a profile not found for the other *S. cerevisiae* strains. The nine isolates obtained from Lampedusa island were grouped into three strains, while the eight isolates from Ustica island were divided into two strains. The patterns found for *S. cerevisiae* strains of Lampedusa were different from those found for *S. cerevisiae* strains of Ustica. These observations make plausible the statement that the leaving sites of birds landed at Ustica were different from those landed at Lampedusa, and it is also acceptable to state that the birds bringing *S. cerevisiae* with the same pattern profiles were originating from the same area. This affirmation is possible because a single bird (stonechat), captured in Lampedusa, brought two distinct strains.

Non-wine-related yeasts

The 58.40% of non-WA yeasts was obtained from birds with 0–1 SFA. *Candida inconspicua* and *C. glabrata* have been already isolated from birds (Cafarchia *et al*., 2006; Lord *et al*., 2010). *Candida inconspicua* has been detected in the oenological environment as contaminant of grape must (Le Roux *et al*., 1973), while *C. stellimalicola* has been isolated only once during the fermentation of grape must (Ocón *et al*., 2010). Few studies reported *Cryptococcus* spp. as occasional contaminants of grapes (Prakitchaiwattana *et al*., 2004; Li *et al*., 2010), barrel surfaces before wine contact (Renouf *et al*., 2006) and corks (Fleet, 2003). It is clear that these species have no role in winemaking processes for their sporadic isolation. *Candida albicans*, *Pseudozyma aphydis* and *Sporisorium penniseti* had never been reported to be isolated from wine environments.

Persistence tests

From the persistence tests performed in this study on birds, it was calculated an average timing for yeast dissemination by birds that is reasonably around 12 h from ingestion. The *S. cerevisiae* strain GR1, introduced in three robins through feeding, was isolated from bird mouths until 6 h after inoculation, as showed by inter-

delta analysis. In the same period, the isolation of this strain from cloacae was negative. The test became positive for GR1 strain presence in cloacae after 12 h. In the next monitoring steps, the strain inoculated was no more detected, neither from mouths nor from cloacae. The results were identical for all three birds employed in the test. From these results, it can be asserted that the permanence of a given *S. cerevisiae* strain in birds lasts no longer than 12 h. Thus, the dissemination of yeasts by birds is supposed to be possible a few hours after ingestion and up to 12 h. This interval is enough to cover a distance of approximately 300–350 km (Moreau, 1961; 1972) and it is compatible with a no-stop flight. In fact, most of the birds positive for yeast isolation were characterized by a low SFA (comprised between 0 and 1). Furthermore, the persistence tests also demonstrated that *S. cerevisiae* survives the transit through the gastric apparatus of birds. However, in case of yeast strains able to colonize the intestinal tract of birds their release might be gradual and prolonged with the possibility to be disseminated in a wider area than that covered in 12 h.

For some authors, the autochthonous yeasts are linked to vineyard and/or cellar habitat in a given area (Lopes *et al*., 2002; Schuller *et al*., 2005), and for this reason, they are found in consecutive years (Torija *et al*., 2001; Schuller *et al*., 2005). All this statements support the idea of 'terroir', which is defined as an ecosystem in which the vine interacts with the environmental factors (soil and climate) affecting the quality and typicality of the wine produced in a particular location (Terroir Viticoles, 2006). From this perspective, the dissemination of yeasts by migratory birds might contribute to explain the differences observed in vineyards examined in consecutive years (Schuller *et al*., 2005; Valero *et al*., 2005).

Conclusions

Data showed by this study demonstrated yeasts may be disseminated by migrating birds. In particular, wine-related yeasts are vehiculated in active state during bird movements. The last finding highlights the needing of better analysing the concept of 'autochthonous' yeasts in winemaking, since yeasts selected for the oenological aptitudes in a given environment may not be indigenous.

Acknowledgements

The authors are grateful to Professor Bruno Massa, Dr Paolo Lucido, Dr Ciro Sannino and Antonio Alfonzo for their help with analysis and interpretation of data. Dr Stefania D'Angelo and the staff of Riserva Naturale Integrale Lago Preola and Gorghi Tondi (Mazara del Vallo, TP, Italy) are also thanked for their supervision during bird ringing.

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