

An in vitro evaluation of the biocidal effect of oregano and cloves' volatile compounds against microorganisms colonizing an oil painting—A pioneer study

Lucrezia Gatti, Federica Troiano, Violetta Vacchini, Francesca Cappitelli and Annalisa Balloi

Table S1. Inhibition scale degree. Examples of contactless test representing different inhibition halo values: [+] for medium inhibition (approximately one third of the petri dish), [++] for high inhibition (approximately the half of the petri dish) and [+++] for total inhibition.



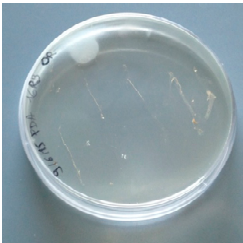
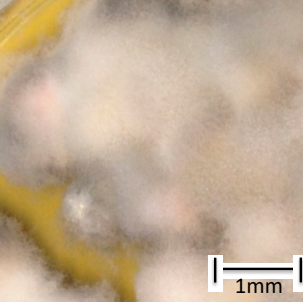
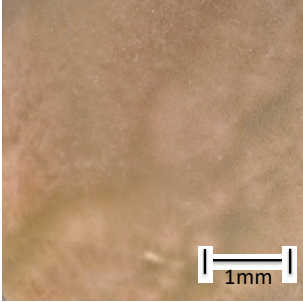
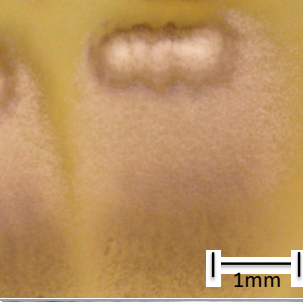
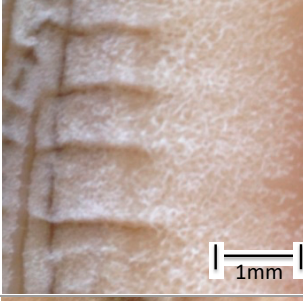
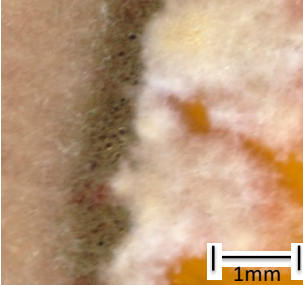
[+]	[++]	[+++]
		

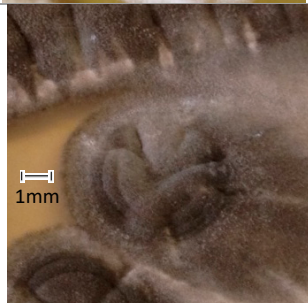
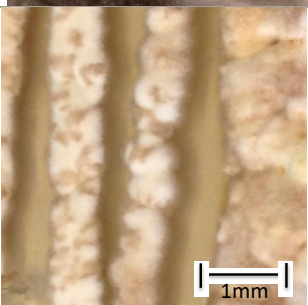

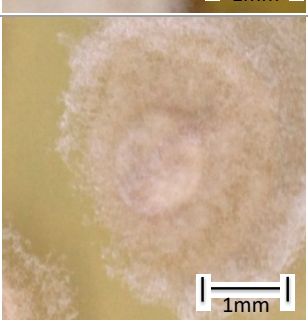
Table S2. Clusterization of bacterial isolates according to their morphology.

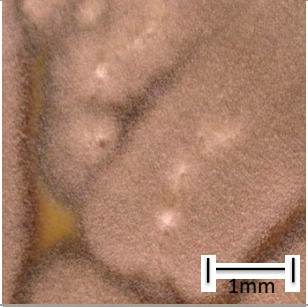
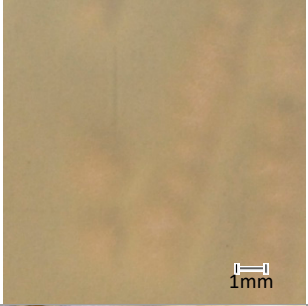
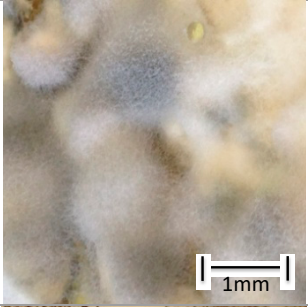
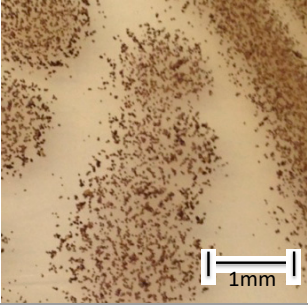
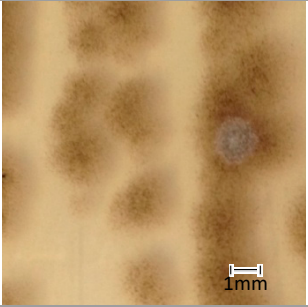
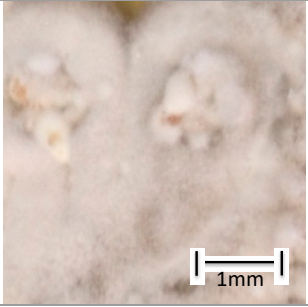
Bacterial Cluster	Morphological Description
Cluster I	white color, wavy edges, irregular shape, flat relief, smooth and translucent surface, mucous texture
Cluster II	white-yellow color, wavy edges, circular shape, raised relief, smooth and translucent surface, mucous consistency
Cluster III	white color, whole edges, circular shape, raised relief, smooth and translucent surface, mucous consistency
Cluster IV	white color, whole edges, circular shape, flat relief, smooth and translucent surface, mucous consistency
Cluster V	white color with brown center, whole edges, circular shape, raised relief, smooth and translucent surface, mucous consistency
Cluster VI	black color, whole edges, circular shape, raised relief, smooth and matt surface, creamy texture
Cluster VII	yellow color, whole edges, irregular shape, flat relief, smooth and translucent surface, mucous consistency
Cluster VIII	white color, whole edges, circular shape, raised relief, smooth and translucent surface, creamy consistency
Cluster IX	white color, whole edges, irregular shape, flat relief, smooth and opaque surface, mucous consistency
Cluster X	white color, whole edges, circular shape, raised relief, smooth and translucent surface, creamy consistency
Cluster XI	white color, whole edges, irregular shape, flat relief, smooth and translucent surface, mucous consistency
Cluster XII	white opaque color, whole edges, irregular shape, flat relief, smooth surface, mucous consistency
Cluster XIII	cream color, whole edges, circular shape, flat relief, smooth and translucent surface, mucous consistency

Table S3. Clusterization of fungi isolates according to their morphology.

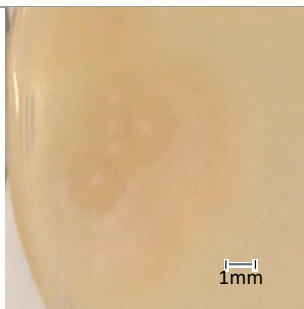
Fungal Cluster	Morphology	Morphological Description
Cluster I		white color, filamentous edge, rhizoidal shape, rough and opaque surface, dusty consistency
Cluster II		white color, filamentous edge, rhizoidal shape, rough and opaque surface, dusty consistency
Cluster III		brown color, filamentous edge, rhizoidal shape, rough and opaque surface, dusty consistency
Cluster IV		white color, filamentous edge, rhizoidal shape, rough and opaque surface, dusty consistency
Cluster V		white-brown color, lobed edge, rhizoidal shape, rough and opaque surface, dusty texture

Cluster VI		yellow color, curled edge, irregular shape, rough and dull surface, dusty texture
Cluster VII		brown color, lobed edge, rhizoidal shape, rough and opaque surface, dusty texture
Cluster VIII		white color and darker center, full edge, circular shape, rough and opaque surface, creamy consistency
Cluster IX		white color, filamentous edge, filamentous shape, rough and opaque surface, dusty texture
Cluster X		brown color, lobed edge, rhizoidal shape, rough and opaque surface, dusty texture
Cluster XI		white color, lobed edge, rhizoidal shape, rough and opaque surface, dusty texture

Cluster XII		brown-black color, filamentous edge, rhizoidal shape, rough and opaque surface, dusty texture
Cluster XIII		white-yellow color, lobed edge, rhizoidal shape, rigid and opaque surface, dusty consistency
Cluster XIV		black color, wavy edge, irregular shape, rough and matte surface, dusty texture
Cluster XV		white-yellow color, curled edge, irregular shape, rough and opaque surface, powdery texture
Cluster XVI		white color, filamentous edge, filamentous shape, rough and opaque surface, dusty texture
Cluster XVII		brown color and white border, lobed border, rhizoidal shape, rough and opaque surface, dusty texture

Cluster XVIII		brown color, opaque, eroded edge, irregular shape, rough and dull surface, dusty texture
Cluster XIX		white color, odulated edge, circular shape, rough and opaque surface, dusty texture
Cluster XX		white and gray color, filamentous edge, filamentous shape, rough and opaque surface, dusty texture
Cluster XXI		brown color, filamentous edge, filamentous shape, rough and opaque surface, dusty texture
Cluster XXII		brown color, filamentous edge, rhizoidal shape, rough and opaque surface, dusty consistency
Cluster XXIII		white color, filamentous edge, filamentous shape, rough and opaque surface, dusty texture

**Cluster
XXIV**



white color, filamentous edge,
filamentous shape, rough and
opaque surface, dusty texture

Table S4. Clustering of bacterial strains isolated from the canvas painting and their phylogenetic identification by 16S rRNA gene sequencing. Different subscript letters indicate the bacterial isolates for the same cluster. NI = not identified strain.

Cluster	Sampling Point	Internal Code	Identities	Closest Taxonomic Relatives	Accession Number	Query Coverage	Similarity
I	2R	Ia	587/607	<i>Bacillus</i> sp.	NZ_CM000745.1	100%	96.71%
I	3R	Ib	1148/1198	<i>Bacillus</i> sp.	MK_480129.1	98%	96%
I	6R	Ic	840/840	<i>Bacillus subtilis</i> subsp. <i>Subtilis</i> str 168 (Kunst et al. 1997)	NC_000964.3	100%	100%
I	21F	Id	1000/1008	<i>Bacillus thuringiensis</i> str. 97-27 (Han et al. 2006)	NC_005957.1	100%	99%
II	7R	Iia	843/908	<i>Xanthomonadaceae</i>	NZ_CP018731.1	99%	93%
III	7R	IIIa	889/926	<i>Streptomyces</i> sp.	NZ_DF968281.1	100%	96%
IV	7R	Iva		NI			
V	9F	Va		NI			
V	10F	Vb		NI			
VI	9F	Via	450/481	<i>Stenotrophomonas</i> sp.	NC_010943.1	75%	94%
VII	11F	VIIa	984/989	<i>Pseudomonas psychrotolerans</i> str. PRS08-11306 (Hauser et al. 2004)	NZ_CP018758.1	100%	99%
VII	13F	VIIb		NI			
VII	16F	VIIc	848/878	<i>Micrococcus luteus</i> NCTC 2665 (Young et al. 2010)	NC_012803.1	99%	97%
VII	22F	VIIId	305/312	<i>Bacillus luteus</i> str. JC167 (Subhash et al. 2014)	NR_133701.1	98%	98%
VII	24F	VIIe	688/774	<i>Caulobacteraceae</i>	NZ_CP022048.2	100%	89%
VIII	12F	VIIIa	936/1037	<i>Xanthomonadaceae</i>	NC_010943.1	97%	91.15%
IX	13F	IXa	911/937	<i>Cellulosimicrobium cellulans</i> str. PSBB019 (Metcalf and Brown 1957)	NZ_CP021383.1	100%	97%
X	14F	Xa	788/848	<i>Paenibacillaceae</i>	NZ_BCNM01000057.1	100%	93%
X	20F	Xb	815/877	<i>Bacillaceae</i>	NC_000964.3	100%	93%
XI	14F	Xia		NI			
XII	16F	XIIa	1051/1121	<i>Paenibacillus</i> sp.	NZ_CP015286.1	93%	94%
XIII	18F	XIIa	986/1006	<i>Bacillus simplex</i> sr. SH-B26 (Priest et al. 1988)	NZ_CP011009.1	99%	98%

Table S5. Clusterization of fungal strains isolated from the canvas painting and their phylogenetic identification by ITS gene sequencing. NI= not identified strain; NA= any author has been reported in NCBI database.

Cluster	Sampling Point	Internal Code	Identifications	Closest Taxonomic Relatives	Accession Number	Query Coverage	Similarity
I	3R	Ia	536/536	<i>Penicillium chrysogenum</i> str. GZU-BCECJXF2-2 (Thom, 1910)	GU565149.1	99%	100%
II	10F	IIa	478/478	<i>Penicillium corylophilum</i> isolate E20335 (Dierckx, 1901)	MK267412.1	100%	100%
II	13F	IIb	514/514	<i>Alternaria</i> sp. str.HXH9 [NA]	MK880492.1	100%	100%
II	5F	IIc		NI			
II	8F	IId		NI			
II	18F	IIE		NI			
III	8F	IIIa	492/492	<i>Penicillium chrysogenum</i> isolate AYMA5 (Thom, 1910)	MK643348.1	100%	100%
III	13F	IIIb	120/143	<i>Trichocomaceae</i>	KM816770.1	37%	83.92%
IV	9F	IVa	516/516	<i>Aspergillus</i> sp. isolate JJGG-73 (Tirab, 1908)	MK644120.1	100%	100%
IV	9F	IVb	506/507	<i>Cephalotheca foveolata</i> isolate UTHSCSA DI 14-21 (Takashi et al. 2006)	KJ573100.1	100%	99.8%
IV	10F	IVc		NI			
IV	12F	IVd	569/571	<i>Penicillium</i> sp. DQ25 [NA]	FJ647576.1	100%	99%
IV	11F	IVe	512/512	<i>Cephalotheca foveolata</i> isolate UTHSCSA DI 14-21 (Takashi et al. 2006)	KJ573100.1	100%	100%
IV	14F	IVf	427/427	<i>Cephalotheca foveolata</i> isolate UTHSCSA DI 14-21 (Takashi et al. 2006)	KJ573100.1	100%	100%
IV	12F	IVg	480/484	<i>Cladosporium halotolerans</i> DTO:308-B3 (Zalar et al. 2007)	MF473102.1	100%	99.17%
IV	14F	IVh	285/306	<i>Sordariaceae</i>	JX081244.1	61%	93.14%
IV	16F	IVi	517/518	<i>Penicillium</i> sp.	MK817616.1	97%	99.81%
IV	16F	IVl	293/296	<i>Penicillium chrysogenum</i> str. F-90 (Thom, 1910)	MF077260.1	100%	98.99%
IV	16F	IVm	520/520	<i>Penicillium chrysogenum</i> str. PA2_4 (Thom, 1910)	KT200273.1	100%	100%
IV	18F	IVn	540/540	<i>Cephalotheca foveolata</i> isolate UTHSCSA DI 14-21 (Takashi et al. 2006)	KJ573100.1	100%	100%
IV	14F	IVo	496/496	<i>Penicillium chrysogenum</i> str. Fes1701 (Thom, 1910)	MK240330.1	100%	100%
IV	16F	IVp	519/520	<i>Penicillium chrysogenum</i> isolate UWR_107 (Thom, 1910)	KY465761.1	100%	99.81%

IV	19F	IVq	516/517	<i>Cephalotheca foveolata</i> isolate UTHSCSA DI 14-21 (Takashi et al. 2006)	KJ573100.1	99%	100%
IV	19F	IVr	390/414	<i>Cephalotheca foveolata</i> isolate UTHSCSA DI 14-21 (Takashi et al. 2006)	KJ573100.1	99%	94.20%
IV	24F	IVs	482/484	<i>Cephalotheca foveolata</i> isolate UTHSCSA DI 14-21 (Takashi et al. 2006)	KJ573100.1	100%	99.59%
V	9F	Va	528/528	<i>Aspergillus</i> sp. isolate LN898693.1 [NA]	MK605980.1	100%	99%
V	25F	Vb	237/238	Uncultured fungus	JN847480.1	66%	99.58%
VI	9F	VIa	538/539	<i>Cephalotheca foveolata</i> isolate UTHSCSA DI 14-21 (Takashi et al. 2006)	KJ573100.1	99.81%	100%
VII	9F	VIIa	520/520	<i>Cladosporium parahalotolerans</i> str. NKM15 (Bensch and Samson, 2018)	MK796044.1	100%	100%
VIII	5F	VIIIa		NI			
IX	10F	IXa	432/444	<i>Cephalotheca foveolata</i> str. UTHSC 08-2766 (Takashi et al. 2006)	HE599376.1	99%	97.30%
IX	10F	IXb	507/513	<i>Cephalotheca foveolata</i> isolate UTHSCSA DI 14-21 (Takashi et al. 2006)	KJ573100.1	100%	99%
IX	10F	IXc	542/545	<i>Cephalotheca foveolata</i> isolate UTHSCSA DI 14-21 (Takashi et al. 2006)	KJ573100.1	100%	99.63%
IX	10F	IXd	412/426	<i>Cephalotheca</i> sp.	KJ573100.1	94%	97%
IX	12F	IXe	543/545	<i>Cephalotheca foveolata</i> isolate UTHSCSA DI 14-21 (Takashi et al. 2006)	KJ573100.1	100%	99%
IX	12F	IXf		NI			
IX	14F	IXg	168/176	Uncultured fungus clone EMF39 V	JQ989325.1	34%	95.35%
X	10F	Xa	534/534	<i>Aspergillus versicolor</i> str. CY11 (Tirab, 1908)	MH712291.1	99%	100%
X	10F	Xb	537/537	<i>Penicillium chrysogenum</i> str. F-53 (Thom, 1910)	MF077255.1	99%	100%
XI	16F	XIa		NI			
XII	12F	XIIa		NI			
XIII	12F	XIIIa	520/520	<i>Penicillium chrysogenum</i> isolate CC (Thom, 1910)	MK881028.1	100%	100%
XIII	17F	XIIIb	465/466	<i>Penicillium</i> sp.	MK841453.1	100%	99.79%
XIII	19F	XIIIc	552/552	<i>Penicillium chrysogenum</i> isolate E20335 (Thom, 1910)	MK267412.1	100%	100%
XIII	19F	XIII d	539/539	<i>Penicillium</i> sp. isolate SFR-12 [NA]	MK817616.1	100%	100%

XIII	19F	XIIIe	412/414	<i>Penicillium chrysogenum</i> str. Fes1701 (Thom, 1910)	MK240330.1	88%	99.52%
XIV	12F	XIVa	405/408	<i>Trichocomaceae</i>	JN859854.1	100%	92.26%
XV	13F	XVa	409/485	<i>Chaetomiaceae</i>	JF817309.1	100%	84.33%
XVI	14F	XVIa	395/395	<i>Penicillium chrysogenum</i> str. Fes 1701 (Thom, 1910)	MK240330.1	100%	100%
XVII	14F	XVIIa	414/417	<i>Cephalotheca foveolata</i> (Takashi et al. 2006)	KJ573100.1	100%	99%
XVII	14F	XVIIb	437/445	<i>Penicillium chrysogenum</i> str. PA2_4 (Thom, 1910)	KT200273.1	99%	98.42%
XVIII	15F	XVIIIa		NI			
XIX	16F	XIXa	388/388	<i>Phaeosphaeriaceae</i> sp. str. CBS 659.73 (Ahmed et al. 2016)	KY090654.1	100%	100%
XX	17F	XXa	512/512	<i>Penicillium</i> sp. Str. K6 (Thom, 1910)	MK719928.1	100%	100%
XX	25F	XXb		NI			
XXI	21F	XXIa	503/503	<i>Cladosporium</i> sp. voucher VB2.1 [NA]	MH655007.1	100%	100%
XXI	17F	XXIb		NI			
XXII	22F	XXIIa	538/538	<i>Aspergillus insuetus</i> str. CBS 107.25 (Thom and Church, 1929)	MH854799.1	100%	100%
XXII	17F	XXIIb		NI			
XXIII	25F	XXIIIa	284/288	<i>Pichia occidentalis</i> (Kurtzman et al. 2008)	KY849376.1	97%%	98.61%
XXIII	19F	XXIIIb		NI			
XXIV	22F	XXIVa		NI			

Figure S1. Clusterization and relative abundance of bacterial (a) and fungal (b) isolates. For each cluster it is indicated, in brackets, the number of the isolated strains (n).

