Letters in Applied Microbiology
November 2013, Volume 57, Issue 5, pages 385–392
http://dx.doi.org/10.1111/lam.12143

Cytotoxicity and mycotoxin production of shellfish-derived *Penicillium* spp., a risk for shellfish consumers

M. Geiger[1,2], Y. Guitton[1], M. Vansteelandt[1], I. Kerzaon[1,3], E. Blanchet[1], T. Robiou du Pont[1], J.C. Frisvad[4], P. Hess[2], Y.F. Pouchus[1,*], O. Grovel[1]

[1] MMS, University of Nantes, Nantes, France
[2] Phycotoxins Laboratory, IFREMER, Nantes, France
[3] UMR 5557 CNRS-UCBL, University of Lyon 1, Lyon, France
[4] Center for Microbial Biotechnology, Technical University of Denmark, Lyngby, Denmark

*: Corresponding author : Yves-François Pouchus, email address : yves-francois.pouchus@univ-nantes.fr

Abstract :

In order to assess the putative toxigenic risk associated with the presence of fungal strains in shellfish-farming areas, *Penicillium* strains were isolated from bivalve molluscs and from the surrounding environment, and the influence of the sample origin on the cytotoxicity of the extracts was evaluated. Extracts obtained from shellfish-derived Penicillia exhibited higher cytotoxicity than the others. Ten of these strains were grown on various media including a medium based on mussel extract (*Mytilus edulis*), mussel flesh-based medium (MES), to study the influence of the mussel flesh on the production of cytotoxic compounds. The MES host-derived medium was created substituting the yeast extract of YES medium by an aqueous extract of mussel tissues, with other constituent identical to YES medium. When shellfish-derived strains of fungi were grown on MES medium, extracts were found to be more cytotoxic than on the YES medium for some of the strains. HPLC-UV/DAD-MS/MS dereplication of extracts from *Penicillium marinum* and *P. restrictum* strains grown on MES medium showed the enhancement of the production of some cytotoxic compounds. The mycotoxin patulin was detected in some *P. antarcticum* extracts, and its presence seemed to be related to their cytotoxicity. Thus, the enhancement of the toxicity of extracts obtained from shellfish-derived *Penicillium* strains grown on a host-derived medium, and the production of metabolites such as patulin suggests that a survey of mycotoxins in edible shellfish should be considered.

Significance and Impact of the Study

Penicillium strains isolated from bivalve molluscs produce extracts exhibiting a higher cytotoxicity than extracts from *Penicillium* strains isolated from the surrounding marine environment. The use of a mussel-based medium for cultures of some shellfish-derived strains enhances the cytotoxicity of extracts when compared with classical media. The production of cytotoxic compounds and of the mycotoxin patulin on such a host-derived medium highlights a potential health risk for shellfish consumers.

Keywords : cytotoxicity ; Dereplication ; marine fungi ; mass spectrometry ; *Penicillium* ; Shellfish

1. Introduction

In marine environments such as hypersaline waters, sediments, beaches sands or shellfish farming areas, *Penicillium* is one of the predominant genera (Khudyakova et al., 2000, Sallenave-Namont et al., 2000, Mancini et al., 2005, Butinar et al., 2011). Among the wide diversity of *Penicillium* species, numerous marine-derived *Penicillium* strains have been described for the production of toxic metabolites (Bugni and Ireland, 2004, Blunt et al., 2009, Ebel, 2010, Blunt et al., 2011) and some are known to produce mycotoxins such as ochratoxin A, penitrem A, roquefortine C or patulin (Frisvad et al., 2004, Paterson et al., 2004). Marine-derived fungi form a group of potential contaminants for marine bivalves such as mussels (Zvereva and Vysotskaya, 2005, Zielinski et al., 2009). In previous studies, it has been shown that *Penicillium* strains sampled from shellfish farming areas produced roquefortine C and patulin (Vansteelandt et al., 2012) and that some toxic fungal metabolites like gliotoxin or peptaibols produced by marine-derived fungi can be detected in marine sediments and can be accumulated in filter-feeding molluscs under laboratory conditions (Sallenave et al., 1999, Grovel et al., 2003). *In situ* contamination of bivalves by fungal compounds could be one of the origins of the unexplained toxicities of seafood observed along the French coast since the beginning of the 1990's (Belin et al., 2009).

In this study, the cytotoxicity of shellfish-derived *Penicillium* strains in comparison with environment-derived ones was investigated. Subsequently, a host-derived medium was used to evaluate the influence of mussel flesh on the production of secondary metabolites and mycotoxins by shellfish-derived Penicillia.

2. Results and discussion

2.1. Influence of strain origin on cytotoxicity of culture extracts

Cytotoxicity of culture extracts from 24 *Penicillium* strains isolated from shellfish farming areas and grown on MEA medium was evaluated on KB cells. Activities obtained for isolates from shellfish were compared to those from their immediate environment (Figure 1). The number of inactive extracts from the two groups was similar. However, amongst toxic extracts, high cytotoxicities were found to be predominant in the extracts obtained from shellfish-derived isolates, with 45% of the extracts exhibiting an IC_{50} < 10 µg mL^{-1}, whereas only 10% of extracts from environment-derived strains exhibited such high cytotoxicity. As samples of shellfish, sediment and seawater were gathered in the same place at the same moment, it seems that the origin of the samples has an influence on the cytotoxicity profiles of strains. Some *Penicillium* strains isolated from shellfish have been previously reported to be more toxic on an *Artemia salina* bioassay than others isolated from the marine environment (Sallenave-Namont et al., 2000, Matallah-Boutiba et al., 2012). This influence of the sample origin on the activity has also been reported for extracts of fungi isolated from different species of sponges (Holler et al., 2000, Thirunavukkarasu et al., 2011) or type of sediment (Khudyakova et al., 2000). Thus, the observation of a highest number of cytotoxic extracts from shellfish-derived isolates could suggest the operation of some mechanism of selection from either of the organisms, leading to a kind of association.

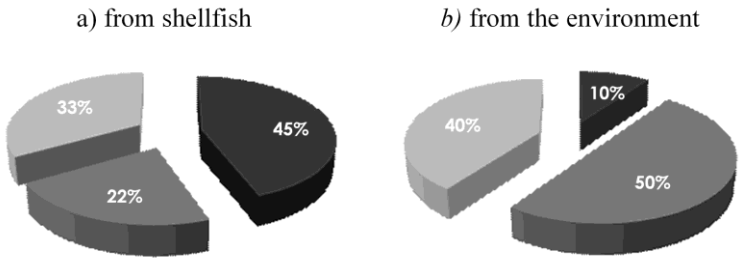

Figure 1. Comparison of crude extracts cytotoxicity for a) shellfish-derived (n=14) and b) environment-derived (n=10) *Penicillium* spp. strains cultured on MEA medium. Dark grey for IC_{50} < 10 µg ml^{-1}; medium grey for 10 < IC_{50} < 30 µg ml^{-1}; light grey for IC_{50} > 30 mg ml^{-1}

Associations between fungi and marine macro-organisms have been mainly studied through the investigation of the biodiversity or chemodiversity of sponge-derived fungi. Sponges are often associated to micro-organisms which have a profound impact on host biology (Wilkinson, 1983). Some sponge-derived fungi may be opportunistic and contribute to localized lesions and diseases (Li and Wang, 2009). These fungi may also contribute to host defense via the production of biologically active metabolites (Holler et al., 2000, Han et al., 2009, Thirunavukkarasu et al., 2011). However, limited data are available on the ecological function of fungal communities living within these invertebrates. Associations of fungi with bivalves have rarely been studied: some marine fungi have been reported to exhibit a pathogenic character towards different species of molluscs (mussels, oysters, abalones) by infecting the shell or the flesh (Alderman and Jones, 1971, Grindley et al., 1998, Van Dover et al., 2007), but none of the other kind of associations such as commensalism or mutualism have been described. It could be envisaged that the different types of relationship described between sponges and fungi could be transposed to bivalve molluscs.

2.2. Influence of shellfish constituents on the cytotoxicity of shellfish-derived Penicillia

Ten fungal strains isolated from bivalves were grown on a mussel flesh-based medium (MES) obtained by substituting the yeast extract of the usual YES medium by a mussel flesh aqueous extract, in order to assess the influence of mussel constituents on the production of cytotoxic metabolites. Table 1 summarizes the cytotoxicity of culture extracts obtained for the MES medium as well as five other common media.

Table 1. Cytotoxicity of extracts of *Penicillium* spp. strains isolated from bivalves and grown on the MES medium and the five classical media (IC_{50}, µg ml^{-1}).

		Culture medium					
strain	species	MES	YES	CYA	DCA	MEA	PDA
MMS50	*P. venetum*	> 30.0	> 30.0	12.5	> 30.0	> 30.0	25.0
MMS163	*P. antarcticum*	5.0	16.9	> 30.0	> 30.0	8.8	> 30.0
MMS231	*P. polonicum*	21.6	> 30.0	6.3	24.1	> 30.0	20.9
MMS266	*P. marinum*	15.7	> 30.0	> 30.0	20.5	15.0	26.6
MMS270	*P. bialowiezense*	> 30.0	> 30.0	25.0	> 30.0	> 30.0	> 30.0
MMS330	*P. ubiquetum*	26.9	11.1	19.1	16.8	13.9	18.7
MMS393	*P*. sp.	> 30.0	> 30.0	> 30.0	> 30.0	> 30.0	> 30.0
MMS399	*P. ligerum*	14.4	25.7	16.5	21.4	> 30.0	> 30.0
MMS404	*P. brevicompactum*	> 30.0	> 30.0	> 30.0	> 30.0	> 30.0	> 30.0
MMS417	*P. restrictum*	4.9	> 30.0	6.3	> 30.0	12.5	> 30.0

All ten strains selected exhibited a similar growth on MES and YES media, showing that mussel constituents are also favorable to the development of fungi. In this way, fermentation studies have shown that mussel processing wastewaters can be depurated by fungi and yeasts, and such mussel-based media have proven to be suitable for the industrial production of amylase by several fungal species (Gonzalez et al., 1992, Torrado et al., 2013). Among the 60 extracts, 33 exhibited no activity, 22 a medium cytotoxicity and 5 were highly cytotoxic. IC_{50} obtained for MES and CYA media appeared to have the similar distribution*, i.e*. four IC_{50} were higher than 30 $\mu g.ml^{-1}$, four were between 10 and 30 $\mu g.ml^{-1}$ and two were lower than 10 $\mu g.ml^{-1}$, implying that these two media seemed to be equally favorable to induce the production of active metabolites by fungi although their composition were highly different. The CYA medium has been reported to be one of the most strongly inducing production of the widest range of secondary metabolites (Frisvad et al., 2004). This study showed that the MES medium seems to induce in a similar manner the production of a large number of extrolites some of them being cytotoxic. On the contrary, the YES medium was the less favorable for the production of cytotoxic compounds, with only three extracts exhibiting an activity. For five strains (*P. antarcticum*, *P. polonicum*, *P. marinum*, *P. restrictum* and *P.* sp MMS351), the MES extracts were more cytotoxic than the corresponding YES extracts showing that shellfish-derived fungi grown on a host-derived medium could express a specific metabolome and could enhance the production of cytotoxic compounds. This influence of host-derived media on the specific expression of metabolites has been previously demonstrated with the stimulation of the production of corymbiferan lactones by necrotrophic fungi (Overy et al., 2006).

2.3. Cytotoxic metabolites responsible for cytotoxicities of MES extracts

In order to investigate the compounds responsible for the enhanced activities on MES media, metabolic profiles of strains were analysed using HPLC-UV/DAD-HRMS/MS, and the composition of extracts obtained on the MES and YES media were compared. The dereplication of extracts was performed on the basis of the accurate mass, UV spectrum and MS/MS spectrum of the main chromatographic peaks. The results obtained for three strains presenting either a high cytotoxicity on MES medium or a high differential activity between the MES and YES extracts are shown. Table 2 lists peaks which could be annotated.

MES and YES extracts of MMS266 (*P. marinum*) exhibited different chromatographic profiles (Figure 2A, 2B). Surprisingly, none of the cytotoxic indole alkaloids communesins or chaetoglobosins, some characteristic metabolites of this species, were found to be produced on these two media. The most abundant peaks observed in the YES extract were not observed in the MES extract, whereas several peaks were specific of the latter. Four of them (peaks A, B, C, E) were identified as penostatin derivatives, a group of compounds initially described from marine *Penicillium* strains and known to exhibit significant cytotoxicities on various cell lines (Takahashi et al., 1996, Iwamoto et al., 1999). The fifth identified compound, also specifically produced on MES medium, was fusoxysporone, a viscidane-type diterpene first isolated from a *Fusarium oxysporum* (Abraham and Hanssen, 1992). No biological activities have been described for this compound. Thus, further investigations on toxic effects of both penostatins and fusoxysporone would be required to assess their potential involvement in the cytotoxicity of MES extracts and their putative human health effects if they were produced *in situ* in mussels.

For the MMS417 strain (*P. restrictum*), YES and MES extracts exhibited chromatographic profiles of greater similarity (Figure 2C, 2D). The main difference was the relative abundance of peak C which represented 4.8 % of the total peak areas on YES and 14.1 % on MES. This compound could be annotated as pestalotin, a metabolite previously isolated from a *Penicillium* sp. strain, or less likely as pestalrone B, a *Pestalotiopsis karstenii* molecule

(Kimura et al., 1986, Luo et al., 2012). All the other annotated compounds were also pyran-2-one derivatives (Table 2). As for peak C, peak B was thought to be hydroxypestalotin rather than pestalrone B, as it has once been reported in a *Penicillium* sp. strain (Kirihata et al., 1992). Pestalotin derivatives have been described to exhibit only weak cytotoxicity against the human tumor cell line U-251 (Luo et al., 2012). Thus, the high activity of the MES extract could also be attributed to one of the minor peaks that remained unidentified.

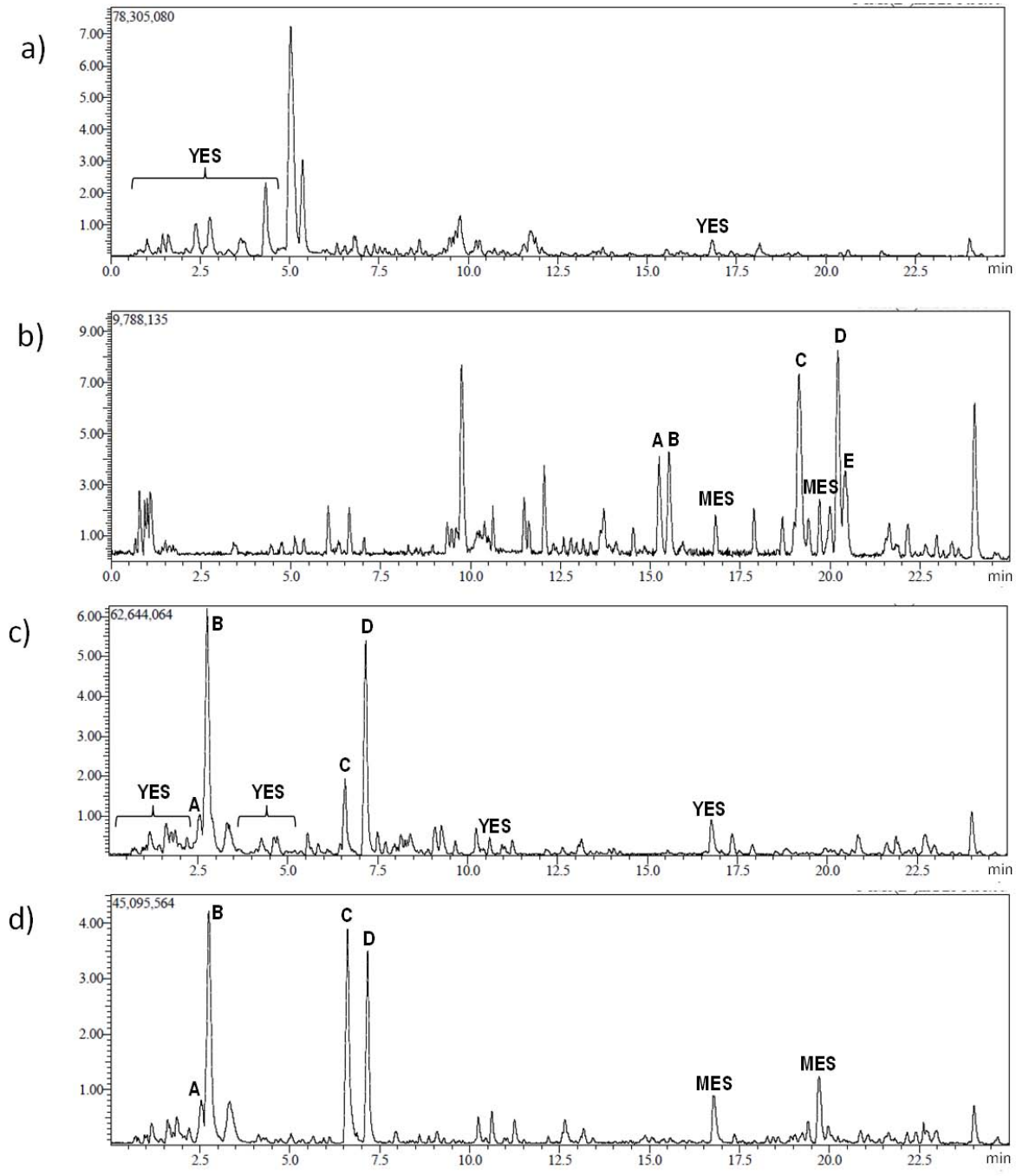

Figure 2. ESI positive mode base peak chromatograms (BPC) of crude extracts of a) MMS266 on YES medium, b) MMS266 on MES medium, c) MMS417 on YES medium and d) MMS417 on MES medium. Peaks annotated YES=YES medium component and MES=MES component.

Table 2. Annotated peaks observed on YES and MES chromatograms for MMS266 and MMS417.

Peak	Experimental *m/z* $[M+H]^+$	Molecular formula	Theoretical *m/z* $[M+H]^+$	ppm diff	Identification parameters			Annotation
					MS	MS/MS	UV	
Strain MMS266								
A, B	345.2418	$C_{22}H_{32}O_3$	345.2424	1.74	X	X	X	Penostatin derivatives
C	327.23	$C_{22}H_{30}O_2$	327.2319	5.81	X	X	X	Penostatin derivatives
D	287.2356	$C_{20}H_{30}O$	287.2369	4.53	X	X	X	Fusoxysporone
E	327.2283	$C_{22}H_{30}O_2$	327.2319	8.86	X	X	nf*	Penostatin derivatives
Strain MMS417								
A	229.1064	$C_{11}H_{16}O_5$	229.1071	3.06	X	X	X	LLP880-gamma
B	231.1214	$C_{11}H_{18}O_5$	231.1227	5.62	X	X	ni**	Hydroxypestalotin or Pestalrone A
C	215.1262	$C_{11}H_{18}O_4$	215.1278	7.44	X	X	nf	Pestalotin or Pestalrone B
D	213.1112	$C_{11}H_{16}O_4$	213.1121	4.22	X	ni	ni	5,6-dihydro-4-methoxy-6-(1-oxopentyl)-2H-pyran-2-one

*nf: data not fully congruent with literature; **ni: no information on MS/MS or UV spectra in literature

Extracts of the strain MMS163 (*P. antarcticum*) were highly cytotoxic when it was grown on MES and MEA media, whereas no activity was observed for those obtained from cultures on CYA, PDA and DCA media. As marine isolates of this species are known to produce patulin (Vansteelandt et al., 2012), a mycotoxin cytotoxic on various cell lines (Iwamoto et al., 1999, Heussner et al., 2006), the six extracts were dereplicated with a focused attention to patulin detection (Table 3).

Table 3. Annotated peaks observed on extracts of MMS163.

Medium	IC_{50} ($\mu g\ ml^{-1}$)	Annotated compounds				
		patulin	chrysogine	cladosporin	5'-hydroxy-asperentin	terrestric acid
MES	5.0	X	-	X	X	-
YES	16.9	X	-	X	X	X
MEA	8.8	X	-	X	X	-
CYA	> 100.0	-	X	X	X	X
DCA	> 100.0	-	X	X	X	-
PDA	> 100.0	-	X	X	-	-

Cladosporin and 5'-hydroxy-asperentin were identified in six and five extracts, respectively. Cladosporin is a secondary metabolite initially isolated from *Cladosporium cladosporioides* (Scott and Van, 1971). This compound has been shown to exhibit antifungal, antimicrobial and antimalarial activities (Scott and Van, 1971, Anke et al., 1978, Hoepfner et al., 2012). To our knowledge, bioactivities of the cladosporin derivative 5'-hydroxy-asperentin have not yet been reported. Patulin was detected in the three cytotoxic extracts and not in the others, contrary to the alkaloid chrysogine which was only present in the non-active ones. This indicates that the cytotoxicity of the extracts is strongly related to the presence of patulin, and that there is a biosynthetical shift in the routes leading to these two compounds. Several

investigations have shown that the biosynthesis of patulin is highly regulated and dependent of abiotic factors such as medium components. Glucose is a favorable carbon source, whereas nitrogenous compounds inhibit its production (Grootwassink and Gaucher, 1980, Rollins and Gaucher, 1994), and manganese is required for the expression of the isoepoxydon dehydrogenase gene implicated in the synthesis of patulin intermediates (Scott et al., 1986, Puel et al., 2010). In the present study, no correlation could be observed between any medium component and patulin or chrysogine production. Due to its cytotoxic, mutagenic, genotoxic, immunotoxic, neurotoxic and teratogenic effects (Andersen et al., 2004), patulin is considered as a major mycotoxin and its presence in food is regulated (FAO, 2004). Thus, the stimulation of patulin production by mussel flesh constituents could lead to its accumulation in edible shellfish and then induce a putative health risk for their consumers.

In summary, this study shows that the most toxigenic *Penicillium* strains were found in association with natural samples corresponding to bivalve molluscs as opposed to sediment and seawater. As advised by Overy et al. (2006), who proposed to grow necrotrophic fungi on host-derived media to enhance the production of specific metabolites, the use of a mussel-based medium for the cultivation of shellfish-derived strains led to the observation of more cytotoxic compounds for some strains. Thus, the production of such toxic compounds may be generally enhanced by shellfish constituents. Further investigations must focus in priority on the isolation and identification of these compounds to assess the potential risk for human shellfish consumers. Moreover, the identification of the fungal toxin patulin in a *Penicillium* strain isolated from shellfish strengthens the interest of monitoring *Penicillium* mycotoxins in shellfish-farming areas.

3. Material and methods

3.1. Fungal strains

Twenty-four *Penicillium* strains were randomly selected from the marine-derived fungal strain collection of the laboratory. These strains had been isolated from cultured shellfish (cockles and mussels) and their immediate environment (sediment and seawater) gathered along the western coast of France. Strains were identified using their phenotypic characteristics, by metabolite profiling or by sequencing the internal transcribed spacers (ITS-1 and -2) and beta-tubulin regions of their genomic DNA. Sequences were compared to CBS and Genbank databanks. Three strains remained unidentified as they did not grow during the identification procedure. The list of all strains is given in supporting information (table S1). For the study of shellfish-derived Penicillia, 10 strains, isolated from shellfish and all belonging to different species, were kept from the previous selection.

3.2. Culture and extraction

Culture media: strains were grown on 6 different culture media all prepared with 15 g L^{-1} of agar in sterilized natural seawater (salinity of 32.8 psu) containing additionally: 5 g Czapek extract, 5 g yeast extract and 30 g saccharose for Czapek Yeast extract Agar medium (CYA); 40 g glucose and 10 g casein enzymatic digest for Dextrose Casein Agar medium (DCA); 5 mg $CuSO_4$, 10 mg $ZnSO_4$, 1 g peptone, 20 g malt extract and 20 g glucose for Malt Extract Agar medium (MEA, medium used for the cytotoxicity screening of the 24 strains); 5 mg $CuSO_4$, 10 mg $ZnSO_4$, 4 g potato extract and 20 g glucose for Potato Dextrose Agar medium (PDA); 5 mg $CuSO_4$, 10 mg $ZnSO_4$, 0.5 g $MgSO_4$, 20 g yeast extract and 150 g saccharose for Yeast Extract Sucrose medium (YES). Mussel Extract Saccharose (MES) medium was realised by incorporating 20g of a freshly prepared mussel extract instead of the 20 g of

yeast extract of the YES medium. Mussel extract was obtained by collecting total flesh of blue mussels (*Mytilus edulis*) from Normandy, France. The flesh was crushed and centrifuged at 6000 g, 5°C during 30 min. Supernatant was successively passed through filters of decreasing porosity (50, 5 and 1 µm; Sartorius, Göttigen, Germany) and finally lyophilized. The extract obtained was ground in order to obtain a homogeneous powder. Bioassay performed on non-inoculated MES medium extracts showed no cytotoxicity of the mussel flesh constituents.

Fungal cultures and extract preparation: each culture was carried out in triplicates in Erlenmeyer flasks containing 50 mL of solid medium; cultures were incubated at 27°C for 12 days under natural light. Fungal mycelium was extracted together with the agar layer, in order to obtain both internal and excreted compounds. The cultures were extracted twice with 100 mL of dichloromethane/ethyl-acetate 1:1 (v/v). Mixtures were then sonicated during 30 min and macerated overnight. After filtration on Büchner funnel, the organic phase was dehydrated on Na_2SO_4. Spores were removed by filtration on regenerated cellulose 0.45 µm filters (Sartorius, Göttingen, Germany). Organic phases were evaporated to dryness leading to crude extracts.

3.3. Cytotoxicity assay

Cytotoxicity was evaluated on KB cells, a human epidermoid carcinoma cell line, with a 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) coloration after 72 h of incubation according to the protocol of Ruiz et al. (2007). Results were expressed as the concentration that inhibited 50% of cell growth (IC_{50}). Experiments were carried out in duplicate. Culture medium containing MeOH 5% was used as negative control and penicillic acid was used as positive control (IC_{50} = 18.8 µg mL^{-1}). Cytotoxicity was considered as high if IC_{50} was under 10 µg mL^{-1} and low if IC_{50} was between 10 and 30 µg mL^{-1}. Extracts with IC_{50} higher than 30 µg mL^{-1} were considered as inactive.

HPLC-DAD/ESI-IT-TOF-MS analyses

HPLC-DAD/ESI-IT-TOF-MS^n analyses were performed with a Shimadzu LCMS-IT-TOF instrument composed of two LC-20ADxr pumps, a SIL-20ACxr autosampler, a CTO-20AC column oven, an SPD-M20A PDA detector and a CBM-20A system controller, coupled to a mass spectrometer with an ESI ion source, and an hybrid Ion Trap-Time-Of-Flight mass analyser (Shimadzu, Kyoto, Japan). HPLC and EI-IT-MS analysis conditions are given as supporting informations. Dereplication of extracts was performed according to the method described by Vansteelandt et al. (2012). The data obtained were analysed using the fungal metabolite database of Nielsen and Smedsgaard (2003) and literature.

Acknowledgements

We acknowledge the *Region des Pays de la Loire* for funding of this study through the COLNACOQ and CHIMIMAR programs, and Yolaine Joubert and Solène Brochard for her help in identification of fungal strains, cultures and extractions. Authors have no conflict of interest to declare.

References

Abraham, W.R. and Hanssen, H.P. (1992) Fusoxysporone - a new type of diterpene from *Fusarium oxysporum*. *Tetrahedron* **48**:10559-10562.

Alderman, D.J. and Jones, E.B.G. (1971) Physiological requirements of two marine phycomycetes, *Althornia crouchii* and *Ostracoblabe implexa*. *T Brit Mycol Soc* **57**:213-225.

Andersen, B., Smedsgaard, J. and Frisvad, J.C. (2004) *Penicillium expansum*: consistent production of patulin, chaetoglobosins, and other secondary metabolites in culture and their natural occurrence in fruit products. *J Agr Food Chem* **52**:2421-2428.

Anke, H., Zaehner, H. and Koenig, W.A. (1978) Metabolic products of microorganisms. 170. On the antibiotic activity of cladosporin. *Arch Microbiol* **116**:253-257.

Belin, C., Soudant, D. and Amzil, Z. (2009) Surveillance des toxines lipophiles dans les coquillages. Analyses statistique et comparaison des résultats obtenus par deux méthodes d'analyse : les bio-essais sur souris et les analyses chimiques par CL-SM/SM. Rapport exécuté dans le cadre de la Convention Etudes DGAL / Ifremer, correspondant à la Subvention pour charges de service public, *Programme 206*, 94 pp, Octobre 2009.

Blunt, J.W., Copp, B.R., Hu, W.P., Munro, M.H.G., Northcote, P.T. and Prinsep, M.R (2009) Marine natural products. *Nat Prod Rep* **26**:170-244.

Blunt, J.W., Copp, B.R., Munro, M.H.G., Northcote, P.T. and Prinsep, M.R. (2011) Marine natural products. *Nat Prod Rep* **28**:196-268.

Bugni, T.S. and Ireland, C.M. (2004) Marine-derived fungi: a chemically and biologically diverse group of microorganisms. *Nat Prod Rep* **21**:143-163.

Butinar, L., Frisvad, J.C. and Gunde-Cimerman, N. (2011) Hypersaline waters - a potential source of foodborne toxigenic aspergilli and penicillia. *FEMS Microbiol Ecol* **77**:186-199.

Ebel, R. (2010) Terpenes from marine-derived fungi. *Mar Drugs* **8**:2340-2368.

FAO (2004) Worldwide Regulation for Mycotoxins in Food and Feed in 2003. In: FAO Food and Nutrition Paper vol. 81, p 180 pp. Rome.

Frisvad, J.C., Smedsgaard, J., Larsen, T.O. and Samson, R.A. (2004) Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. *Stud Mycol* **49**:201-241.

Gonzalez, M.P., Siso, M.I.G., Murado, M.A., Pastrana, L., Montemayor, M.I. and Miron, J. (1992) Depuration and valuation of mussel-processing wastes. Characterization of amylolytic postincubates from different species grown on an effluent. *Bioresource Technol* **42**:133-140.

Grindley, R.M., Keogh, J.A. and Friedman, C.S. (1998) Shell lesions in New Zealand *Haliotis* spp. (Mollusca, Gasteropoda). *J Shellfish Res* **17**:805-811.

Grootwassink, J.W. and Gaucher, G.M. (1980) De novo biosynthesis of secondary metabolism enzymes in homogeneous cultures of *Penicillium urticae*. *J Bacteriol* **141**:443-455.

Grovel, O., Pouchus, Y.F. and Verbist, J.F. (2003) Accumulation of gliotoxin, a cytotoxic mycotoxin from *Aspergillus fumigatus*, in blue mussel (*Mytilus edulis*). *Toxicon* **42**:297-300.

Han, Y., Yang, B., Zhang, F., Miao, X. and Li, Z. (2009) Characterization of antifungal chitinase from marine *Streptomyces* sp. DA11 associated with South China sea sponge *Craniella Australiensis*. *Mar Biotechnol* **11**:132-140.

Heussner, A.H., Dietrich, D.R. and O'Brien, E. (2006) In vitro investigation of individual and combined cytotoxic effects of ochratoxin A and other selected mycotoxins on renal cells. *Toxicol in Vitro* **20**:332-341.

Hoepfner, D., McNamara, C.W., Lim, C.S., Studer, C., Riedl, R., Aust, T., McCormack, S. L., Plouffe, D. M., Meister, S., Schuierer, S., Plikat U., Hartmann, N., Staedtler, F., Cotesta, S., Schmitt, E. K., Petersen, F., Supek, F., Glynne R. J., Tallarico, J.A., Porter, J.A., Fishman M.C., Bodenreider C., Diagana, T. T., Movva N.R. and Winzeler

E.A. (2012) Selective and specific inhibition of the *Plasmodium falciparum* Lysyl-tRNA synthetase by the fungal secondary metabolite cladosporin. *Cell Host & Microbe* **11**:654-663.

Holler, U., Wright, A.D., Matthee, G.F., Konig, G.M., Draeger, S., Aust, H.J., and Schulz, B. (2000) Fungi from marine sponges: Diversity, biological activity and secondary metabolites. *Mycol Res* **104**:1354-1365.

Iwamoto, C., Minoura, K., Oka, T., Ohta, T., Hagishita, S. and Numata, A. (1999) Absolute stereostructures of novel cytotoxic metabolites, penostatins A–E, from a *Penicillium* species separated from an *Enteromorpha alga*. *Tetrahedron* **55**:14353-14368.

Khudyakova, Y.V., Pivkin, M.V., Kuznetsova, T.A. and Svetashev, V.I. (2000) Fungi in sediments of the sea of Japan and their biologically active metabolites. *Microbiol-Moscow* **69**:608-611.

Kimura, Y., Hamasaki, T. and Nakajima, H. (1986) Stereochemistry and biological activities of LL-P880γ, a pestalotin analog, produced by *Penicillium citreo-viride*. *Agr Biol Chem Tokyo* **50**:1649-1650.

Kirihata M, Ohe M, Ichimoto I, Ueda H (1992) Stereoselective synthesis of unnatural stereoisomers of LL-P880fi and LL-P880y, pestalotin analogues from Penicillium sp. *Biosci Biotech Biochem* **56**:1825-1828.

Li, Q. and Wang, G. (2009) Diversity of fungal isolates from three Hawaiian marine sponges. *Microbiol Res* **164**:233-241.

Luo, D.Q., Zhang, L., Shi, B.Z. and Song, X.M. (2012) Two new oxysporone derivatives from the fermentation broth of the endophytic plant fungus *Pestalotiopsis karstenii* isolated from stems of *Camellia sasanqua*. *Molecules* **17**:8554-8560.

Mancini, L., D'Angelo, A.M., Pierdominici, E., Ferrari, C., Anselmo, A., Venturi, L., Fazzo, L., Formichetti, P., Iaconelli, M. and Pennelli, B. (2005) Microbiological quality of Italian beach sands. *Microchem J* **79**:257-261.

Matallah-Boutiba, A., Ruiz, N., Sallenave-Namont, C., Grovel, O., Amiard, J.C., Pouchus, Y.F. and Boutiba, Z. (2012) Screening for toxigenic marine-derived fungi in Algerian mussels and their immediate environment. *Aquaculture* **342-343**:75-79.

Nielsen, K.J. and Smedsgaard, J. (2003) Fungal metabolite screening: database of 474 mycotoxins and fungal metabolites for dereplication by standardised liquid chromatography–UV–mass spectrometry methodology. *J Chromatogr A* **1002**:111-136.

Overy, D.P., Smedsgaard, J., Frisvad, J.C., Phipps, R.K. and Thrane, U. (2006) Host-derived media used as a predictor for low abundant, in planta metabolite production from necrotrophic fungi. *J Appl Microbiol* **101**:1292-1300.

Paterson, R.R.M., Venâncio, A. and Lima, N. (2004) Solutions to *Penicillium* taxonomy crucial to mycotoxin research and health. *Res Microbiol* **155**:507-513.

Puel, O., Galtier, P. and Oswald, I.P. (2010) Biosynthesis and toxicological effects of patulin. *Toxins* **2**:613-631.

Rollins, M.J. and Gaucher, G.M. (1994) Ammonium repression of antibiotic and intracellular proteinase production in *Penicillium urticae*. *Appl Microbiol Biot* **41**:447-455.

Ruiz, N., Wielgosz-Collin, G., Poirier, L., Grovel, O., Petit, K.E., Mohamed-Benkada, M., Robiou du Pont, T., Bissett, J., Vérité, P., Barnathan, G. and Pouchus, Y.F. (2007) New Trichobrachins, 11-residue peptaibols from a marine strain of *Trichoderma longibrachiatum*. *Peptides* **28**:1351-1358.

Sallenave-Namont, C., Pouchus, Y.F., Robiou du Pont, T., Lassus, P. and Verbist, J.F. (2000) Toxigenic saprophytic fungi in marine shellfish farming areas. *Mycopathologia* **149**:21-25.

Sallenave, C., Pouchus, Y.F., Bardouil, M., Lassus, P., Roquebert, M.F. and Verbist, J.F. (1999) Bioaccumulation of mycotoxins by shellfish: Contamination of mussels by metabolites of a *Trichoderma koningii* strain isolated in the marine environment. *Toxicon* **37**:77-83.

Scott, P.M. and Van, W.W. (1971) Cladosporin, a new antifungal metabolite from *Cladosporium cladosporioides*. *J Antibiot* **24**:747-755.

Scott, R.E., Jones, A., Lam, K.S. and Gaucher, G.M. (1986) Manganese and antibiotic biosynthesis. I. A specific manganese requirement for patulin production in *Penicillium urticae*. *Can J Microbiol* **32**:259-267.

Takahashi, C., Numata, A., Yamada, T., Minoura, K., Enomoto, S., Konishi, K., Nakai, M., Matsuda, C. and Nomoto, K. (1996) Penostatins, novel cytotoxic metabolites from a *Penicillium* species separated from a green alga. *Tetrahedron Lett* **37**:655-658.

Thirunavukkarasu, N., Suryanarayanan, T., Girivasan, K., Venkatachalam, A., Geetha, V., Ravishankar, J. and Doble, M. (2011) Fungal symbionts of marine sponges from Rameswaram, southern India: species composition and bioactive metabolites. *Fungal Diversity* **55**:37-46.

Torrado, A., Vázquez, J.A., Prieto, M.Á., Fuciños, P., Montemayor, M.I., Pastrana, L., González, M.P. and Murado, M.Á. (2013) Amylase production by *Aspergillus oryzae* in a solid-state bioreactor with fed-batch operation using mussel processing wastewaters as feeding medium. *J Chem Technol Biot* **88**:226-236.

Van Dover, C.L., Ward, M.E., Scott, J.L., Underdown, J., Anderson, B., Gustafson, C., Whalen, M. and Carnegie, R.B. (2007) A fungal epizootic in mussels at a deep-sea hydrothermal vent. *Mar Ecol* **28**:54-62.

Vansteelandt, M., Kerzaon, I., Blanchet, E., Fossi Tankoua, O., Robiou Du Pont, T., Joubert, Y., Monteau, F., Le Bizec, B., Frisvad, J.C., Pouchus, Y.F. and Grovel, O. (2012) Patulin and secondary metabolite production by marine-derived *Penicillium* strains. *Fungal Biology* **116**:954-961.

Wilkinson, C.R. (1983) Net primary productivity in coral reef sponges. *Science* **219**:410-412.

Zielinski, O., Busch, J.A., Cembella, A.D., Daly, K.D., Engelbrektsson, J., Hannides, A.K. and Schmidt, H. (2009) Detecting marine hazardous substances and organisms: sensors for pollutants, toxins, and pathogens. *Ocean Science* **5**:329-349.

Zvereva, L.V. and Vysotskaya, M.A. (2005) Filamentous fungi associated with bivalve mollusks from polluted biotopes of Ussuriiskii Bay, sea of Japan. *Ecol Microorg* **31**:382-385.

Supporting informations

Table S1. List of the studied strains with their origins and their mode of identification

Strain	Species	Sampling characteristics			Identification		
		Sample type	localization	Geographical type	molecular biology	phenotypic characters	metabolic profiling
MMS 5	*chrysogenum*	cockles	Le Croisic	laguna	X	X	-
MMS 14	*antarcticum*	cockles	Le Croisic	laguna	X	X	X
MMS 15	*antarcticum*	cockles	Le Croisic	laguna	X	X	X
MMS 29	*citreonigrum*	sediment	Le Croisic	laguna	-	X	-
MMS 42	*expansum*	sediment	Le Croisic	laguna	X	-	X
MMS 50	*venetum*	mussels	Loire estuary	rocky coast	-	X	X
MMS 163	*antarcticum*	mussels	Loire estuary	rocky coast	X	-	X
MMS 194	*canescens*	seawater	La Baule	sandy bay	-	X	X
MMS 231	*polonicum*	mussels	La Baule	sandy bay	X	-	-
MMS 266	*marinum*	mussels	La Baule	sandy bay	-	X	X
MMS 270	*bialowiezense*	cockles	La Baule	sandy bay	-	X	X
MMS 330	*ubiquetum*	mussels	Loire estuary	rocky coast	-	X	X
MMS 351	*ligerum**	seawater	Loire estuary	rocky coast	X	-	X
MMS 388	*ligerum*	mussels	Loire estuary	rocky coast	X	-	X
MMS 393	sp.**	mussels	Loire estuary	rocky coast	-	-	-
MMS 399	*ligerum*	mussels	Loire estuary	rocky coast	X	-	X
MMS 404	*brevicompactum*	cockles	Le Croisic	laguna	-	X	X
MMS 417	*restrictum*	cockles	Le Croisic	laguna	X	-	-
MMS 460	*canescens*	sediment	Le Croisic	laguna	-	X	X
MMS 556	*atramentosum*	sediment	Bourgneuf Bay	sandy bay	-	X	X
MMS 747	*ligerum*	sediment	Bourgneuf Bay	sandy bay	X	-	X
MMS 906	radicum	sediment	Loire estuary	rocky coast	X	-	-
MMS 967	sp.	sediment	Loire estuary	rocky coast	-	-	-
MMS 976	sp.	sediment	Le Croisic	laguna	-	-	-

* *P. ligerum* = new species sill undescribed; **sp. = species not identified

HPLC and ESI-IT-MS conditions

HPLC was performed with a Kinetex C18 column (100 mm × 2.1 mm i.d.; 2.6 µm particle size) from Phenomenex (Torrance, California, USA). A binary gradient of water + 0.1% formic acid (FA) *vs* acetonitrile + 0.1% FA was used. The injection volume was 5 µL, the flow rate was 0.3 mL min^{-1}, the column oven temperature was 40 °C, and UV spectra (DAD) were recorded between 190 and 800 nm.

The conditions of ESI-IT-TOF-MS^n analysis were: (1) flow rate: 0.3 mL min^{-1}; (2) detection mode: positive ion; (3) mass range: MS, m/z 100–1000; MS^2, m/z 50–1000; (4) heat block and curved desolvation line temperature: 230 °C; nebulizing nitrogen gas flow: 1.5 L min^{-1}; Interface voltage: (+), 4.5 kV; detector voltage of the TOF analyze: 1.61 kV; for MS the ion accumulation time: 30 ms; (5) MS^2 fragmentation were performed by a data-dependent program; the ion accumulation time: 40 ms relative collision-induced dissociation energy: 100% with 50% Collision gas; ultra-high-purity argon was used as the collision gas in the collision-induced dissociation (CID) (6) All data were recorded and analysed using Shimadzu software: LCMS solution Version 3.60, Formula Predictor Version 1.2, and Accurate Mass Calculator (Shimadzu, Kyoto, Japan); (7) a NaTFA solution (2.5 mM) was used to calibrate the mass range from 50 to 1000 Da.