Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

REVIEW ARTICLE

Penicillium roqueforti: a multifunctional cell factory of high value-added molecules

R. Mioso[1], F.J. Toledo Marante[2] and I. Herrera Bravo de Laguna[3]

1 Department of Biotechnology, Federal University of Paraíba, João Pessoa, Paraíba, Brazil
2 Department of Chemistry, University of Las Palmas de Gran Canaria, Gran Canaria, Spain
3 Department of Biology, University of Las Palmas de Gran Canaria, Gran Canaria, Spain

Keywords
biotechnology, cell-factory, enzymes, metabolites, *Penicillium roqueforti*.

Correspondence
Irma Herrera Bravo de Laguna, Departamento de Biología, Universidad de Las Palmas de Gran Canaria, Campus Universitario de Tafira, Edificio de Ciencias Básicas, Gran Canaria - 35017, Spain.
E-mail: irmagallotia@hotmail.com

2014/1264: received 20 June 2014, revised 18 October 2014 and accepted 20 November 2014

doi:10.1111/jam.12706

Summary

This is a comprehensive review, with 114 references, of the chemical diversity found in the fungus *Penicillium roqueforti*. Secondary metabolites of an alkaloidal nature are described, for example, ergot alkaloids such as festuclavine, isofumigaclavines A and B, and diketopiperazine alkaloids such as roquefortines A–D, which are derived from imidazole. Other metabolites are marcfortines A–C, PR-toxin, eremofortines A–E, mycophenolic and penicillic acids, and some γ-lactones. Also, recent developments related to the structural characteristics of botryodiplodin and andrastin are studied—the latter has anticancer properties. Finally, we discuss the enzymes of *P. roqueforti*, which can participate in the biotechnological production of high value-added molecules, as well as the use of secondary metabolite profiles for taxonomic purposes.

Introduction

Fungi produce, store and release chemicals that affect other organisms and determine the existence of chemical interactions, which give the producer adaptive advantages. These organic compounds come from secondary metabolic pathways which are closely related to the routes that give rise to the primary metabolites, that is carbohydrates, lipids, proteins and nucleic acids (Turner 1971).

The use of new technologies and optimization processes has allowed us to characterize these metabolites, which have theoretical and practical implications of great importance to current biotechnological industrial development and represent a source of compounds that could be applied to different fields (e.g. nutraceutical, cosmetic and pharmaceutical).

Thus, the saprophytic fungus *Penicillium roqueforti* Thom is ideally placed, because it is widely involved in the fundamental degradation processes found in nature and it produces a variety of enzymes and secondary metabolites based on various types of substrates (e.g. carbon sources). It is no surprise that there is a significant volume of information associated with this organism.

Penicillium roqueforti has been used in biotechnology for the dairy industry, and its enzymatic system has been well-characterized biochemically. While it is only described as a terrestrial fungus, different studies have shown that *P. roqueforti* is able to grow in saline solutions and that spore germination is inhibited only at NaCl concentrations >100 g l^{-1} (Kubeczka 1968; Godinho and Fox 1981). Indeed, a marine-derived strain with halotolerant characteristics has been isolated and has grown successfully in the KMV-modified broth prepared with sea water at 35 g l^{-1} salinity (Mioso *et al.* 2014, in press).

This fungus also has many other favourable fermentation characteristics, such as its tolerance for growing at low pH values and the ability to use both pentoses and hexoses as substrates, which make it a natural candidate for industrial biotechnological production. Also, a variety of genetic techniques are being developed to improve the productivity of these organisms. Thus, the consolidation of these and other technologies related to transgenic production will generate a technology platform that could be used in the sustainable manufacture of high value-added products at low cost (Punt *et al.* 2002; Ward 2012).

Chemical diversity of the fungus

Enzymes

Enzymes are molecules of a proteinic nature that are able to facilitate and accelerate chemical reactions. Their use in the biotechnology industry, through catalysing biotransformation processes, presents a number of advantages. Due to their high specificity towards the substrate, they do not contribute to undesirable secondary reactions. They also exhibit high catalytic activity, and regioselective and stereoselective reactions occur at low concentrations and in less aggressive experimental conditions (Sibilla and Domínguez de María 2013; Asgher *et al.* 2014).

The current interest that has been generated within the scientific community in relation to these biological catalysts is due to their variety and distribution in a wide range of fungal and bacterial micro-organisms. Their great biotechnological potential is, therefore, applicable in different fields of research, including the pharmaceutical and chemical industries (Colen 2006). It is worth noting, because it relates to *P. roqueforti*, that their proteolytic and lipolytic enzymes make them suitable for biotechnological applications (Gobbetti *et al.* 1998), such as removing scales from fish, the production of high-quality lipids from food waste products and the preparation of dairy drinks (Hassanien *et al.* 1986; Woloszyn *et al.* 1988; Szoltysek 1999). Also, *P. roqueforti* is used in preserving meat products through fermentation processes (Jingxin *et al.* 2013) and in the production of pectinolytic enzymes from agricultural by-products such as pumpkin oil cake (Peričin *et al.* 2007) and coffee husks (Fernandes 2014).

Lipolytic enzymes

Penicillium roqueforti is a filamentous fungus used during the process for making blue cheese. Some strains play an important role in the maturation of these cheeses, which occurs due to the consumption of lactic acid through beta-oxidation and the strong acid and alkaline lipolytic action of the extracellular enzymes (Martínez-Rodríguez *et al.* 2014). The result of this process is the formation of two methyl ketones, 2-heptanone and 2-nonanone, which are considered to be responsible for the odour and flavour of the blue cheese (Cao *et al.* 2014).

The enzymatic transformation of lower quality industrial raw materials, such as fats and oils, can also be improved with the use of lipases (Lobyreva and Marchenkova 1980). A current example is in the biocatalytic production of biodiesel from animal fat residues (Rivera *et al.* 2009).

Many of these lipases of industrial interest are produced by the submerged culture system of selected strains (Eitenmiller *et al.* 1970). The lipases of fungal origin are preferred to the ones of animal origin, because the former are thermally stabler and do not denature either at high temperatures or with changes in pH (Dheeman *et al.* 2010). Some lipases are regioselective, reacting with triglyceride at the sn-1 or sn-3 position. The lipases obtained from *P. roqueforti* exhibit specificity, even in respect to short-chain fatty acids, or in other words, they are acyl-specific short-chain fatty acids.

Penicillium roqueforti produces several types of active lipolytic proteins which differ in molecular weight, amino acid/carbohydrate composition, substrate specificity and preference for incubation conditions such as temperature and pH (Lobyreva and Marchenkova 1980; Mase *et al.* 1995). These lipases are used to modify the physical and chemical properties of fats and industrial oils through the catalysis of the interesterification reactions (Lamberet and Menassa 1983). Lipases can be immobilized in synthetic polymers, to control the bioconversion. Thus, one can obtain valuable products from the fats and oils of industrial wastes (Temesvari and Biacs 1996).

Proteolytic enzymes

The enzymatic conversion of industrial raw materials also occurs through the use of proteases. In biochemical terms, *P. roqueforti* presents a complex proteolytic system consisting of two extracellular endopeptidases (acid protease and metalloprotease) and exopeptidases (acid carboxypeptidase and alkaline aminoprotease), which are commonly used in the dairy industry (Modler *et al.* 1974; Gripon 1977; Gripon *et al.* 1977; Le Bars and Gripon 1981; Rosenthal *et al.* 1996; Igoshi *et al.* 2007).

In terms of bioprocesses, the maturing of Roquefort cheese is carried out through solid-state fermentation of strains of *P. roqueforti*, in which the substrate is the cheese itself. Thus, the intense proteolytic action of the enzymes of *P. roqueforti* causes the release of peptides such as amino acids, which have both high and low molecular weights (Fuquay *et al.* 2011).

Penicillium roqueforti secretes an aspartyl protease, ASPA, which generates the main extracellular activity (Gente *et al.* 2001). Its extracellular endopeptidases are responsible for degrading the alpha- and beta-caseins. The acid protease attacks the beta-casein, which results in peptides being released, even in unfavourable pHs (Zevaco *et al.* 1973; Modler *et al.* 1974; Gripon *et al.* 1977). Similarly, the metalloprotease degrades the beta-casein. These processes result in high concentrations of amino acids in the cheese (Gripon *et al.* 1980).

Decarboxylase and deaminase enzymes

It is known that tryptamine, tyramine and histamine—which are nonvolatile amines produced by decarboxylation

of the amino acids tryptophan, tyrosine and histidine, respectively—are present in Roquefort cheese. This is due to the presence of decarboxylase enzymes (Hwang *et al.* 1976; De Boer and Kuik 1987; Gripon 1993). Also, ammonia is present from the deamination of the tryptophan, tyrosine and histidine, which in turn indicates the presence of deaminases (Rabie 1989).

Primary metabolites and macroconstituents

In relation to the chemical composition of *P. roqueforti*, it is worth mentioning the pioneering works concerning its fatty acid profile and its lipid metabolism, including those by Lawrence (1965), and the studies on its ability to form 2-heptanone and other methyl ketones by the oxidative fission of fatty acids (Gehrig and Knight 1958; Jackson and Hussong 1958). According to Dartey and Kinsella (1973), the fungus could use this mechanism as a form of auto-detoxication. Simultaneously, volatile aldehydes and secondary alcohols were detected in the cheese manufactured with this fungus (Dolezalek and Hoza 1969).

In a chromatographic study of the total lipidic fraction of *P. roqueforti*, Kaufmann *et al.* (1966) described the major presence of the palmitic, oleic and linoleic acids esterified in the form of phospholipids and/or triacylglycerides, as well as free steroids with the structure of ergosterol. Subsequently, based on the growth phases (Lawrence 1967; Salvadori and Salvadori 1967; Fan and Kinsella 1976), new data on the biogenesis of the unsaturated fatty acids were provided, and, more recently, the fatty acid profile has been reconfirmed (Lomascolo *et al.* 1994; Mioso *et al.* 2014).

Shimp and Kinsella (1977a) determined the macronutrient content of the mycelium of *P. roqueforti* and showed that, under optimal growing conditions, it is possible to find high levels of carbohydrates, proteins and lipids. They also explained that the less polar lipids consist predominantly of triacylglycerides, diglycerides and free fatty acids, while the most polar ones are composed of phospholipids and glycolipids; the palmitic, stearic, oleic and linoleic acids are always the most esterifying fatty acids.

Secondary metabolites and high value-added products

In biological studies, it has been determined that most of the secondary metabolites defend the producer organism against predators, competitors and pathogens (Levin 1976; Pawlik 1993; McClintock and Baker 2001). As a component of ecosystems, fungi also produce secondary metabolites, which play an important role in the defence mechanisms by acting as allomones. To remove these undesirable organisms, the filamentous fungi, in particular, accumulate and manufacture antimicrobial and antifouling factors (Toledo Marante *et al.* 2004; Hermosa *et al.* 2014).

It is not surprising, therefore, that among the various kinds of fungi, ascomycetes are recognized as producers of new secondary metabolites with unusual carbon structures. Consequently, it is clear that these apparently defenceless organisms were endowed by evolution with adaptive allomones that bring benefits, and by extrapolation, it is assumed that they could be genuine biofactories of natural drug sources (Toledo Marante *et al.* 2004). A historic example is the case of penicillin and the eradication of tuberculosis (Fleming 1929).

Under certain culture conditions, *P. roqueforti* also shows a pronounced ability to biosynthesize secondary metabolites (Engel and Teuber 1983), some of which have antiparasitic, bacteriostatic (Kopp-Holtwiesche and Rehm 1990; Ruddock 1992; Aninat *et al.* 2001) and anticancer properties (Nielsen *et al.* 2005; Overy *et al.* 2005).

However, these filamentous fungi are able to produce specific toxic metabolites, which initially restricted their use in food (Kolousek and Michalik 1954; Kanota 1969; Scott *et al.* 1977). In this regard, the structures of some alkaloids were isolated and clarified, for example, roquefortine A (**1**), which is also referred to as isofumigaclavine A; roquefortine B (**2**), which is also known as isofumigaclavine B; festuclavine (**3**); and roquefortine C (**4**). All of these alkaloids would come to be known as neurotoxins (Ohmomo *et al.* 1975; Scott *et al.* 1976; Polonsky *et al.* 1977).

The alkaloid roquefortine D (**5**) was subsequently isolated—it was suggested that its biogenesis passes through mevalonic acid, tryptophan and histidine (Ohmomo *et al.* 1978). More recently, some evidence supporting this hypothesis has been provided (Bhat *et al.* 1993). Moreover, Bellinck (1975) studied the chemistry of the pigments of *P. roqueforti* and showed that these compounds are polyphenolic in nature (Fig. 1).

The applicability of fungus in food was even more limited when Wei *et al.* (1973, 1975) purified another mycotoxin from a strain of *P. roqueforti*, which had been isolated from toxic foods. This substance was lethal when administered orally to rats, and its structure was clarified by chemical and spectroscopic methods such as PR-toxin (**6**). These same authors also proved that this mycotoxin has a strong inhibitory effect on the biosynthesis of DNA, RNA and proteins.

Moreau *et al.* (1976) soon discovered three new metabolites—the eremofortines A (**7**), B (**8**) and D (**10**)—biosynthetically related to PR-toxin. They suggested that the latter one is the biogenetic precursor of the 9-OH or 6-OH-furo-eremophilanic sesquiterpenes that have already been found in various organisms (Fig. 2).

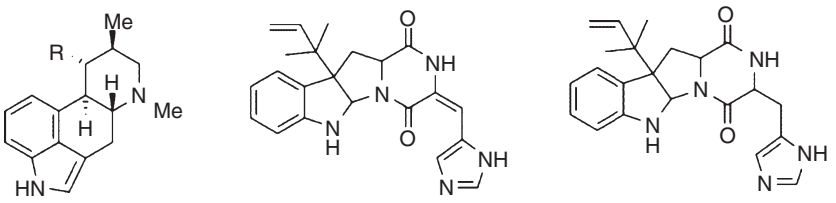

Figure 1 Roquefortine A (isofumigaclavine A) (**1**), roquefortine B (isofumigaclavine B) (**2**), festuclavine (**3**) and roquefortines C (**4**) and D (**5**).

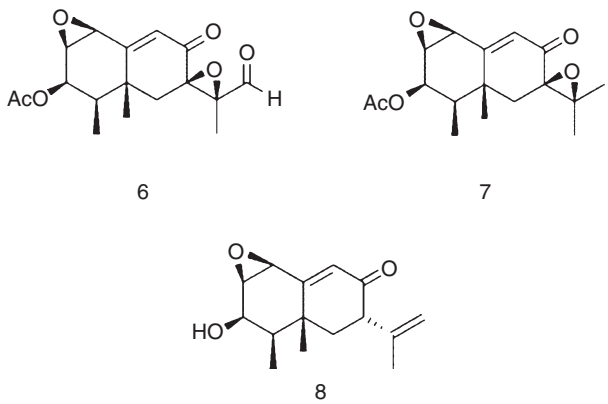

Figure 2 PR-toxin (PRT, **6**), and eremofortines A (**7**) and B (**8**).

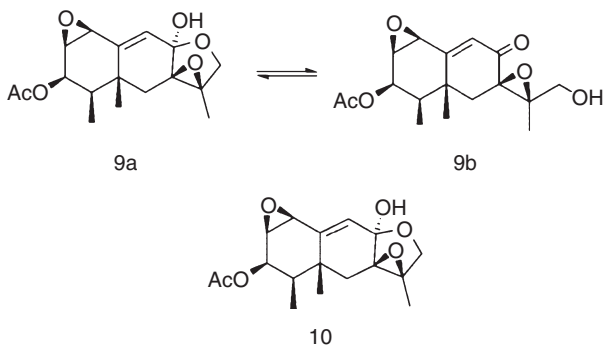

Figure 3 Eremofortines C (**9a/9b**) and D (**10**).

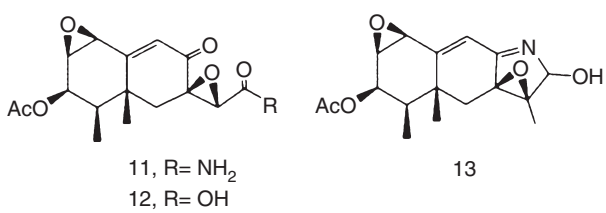

Figure 4 Eremofortine E (PR-amide, **11**), PR-acid (**12**) and PR-imine (**13**).

Moreau *et al.* (1977) subsequently showed the two structural shapes of the eremofortine C (**9a/9b**), and Arnoux *et al.* (1977) completed the structural description of eremofortine D (**10**) (Fig. 3).

Moreau *et al.* (1980b) linked eremofortines A, B and C with the appearance of PR-toxin in the toxic strains and suggested that sesquiterpene eremofortine C is its direct biosynthetic precursor.

The structures of eremofortines A, B, C, D and E were completed from a stereochemical viewpoint, by correlation with the absolute configuration of PR-toxin. Also, the structure of eremofortine E or PR-amide (**11**) was elucidated by X-ray diffraction (Moreau *et al.* 1980a). Wei *et al.* (1989) then involved these metabolites in the biosynthetic and degradation pathway of PR-toxin (Fig. 4).

Finally, the chemical and biological activity of these eremophilanic sesquiterpenes was revised. The factors related to the culture conditions that affect the production of the PR-toxin were described, as well as its degradation in the self-producing *P. roqueforti* (Scott 1984; Chang *et al.* 1991). The metabolites released and accumulated in the medium were eremofortine E (PR-amide, **11**), PR-acid (**12**) and PR-imine (**13**) (Chang *et al.* 1993, 1996).

The advances in the description of the secondary metabolism of *P. roqueforti* must also highlight: the description of the absolute configuration of the isofumigaclavine A (**1**) (Arnoux *et al.* 1978); the structural explanation of marcfortines A (**14**), B (**15**) and C (**16**) (Polonsky *et al.* 1980; Prangé *et al.* 1981); and the description of the crystal structure of the aristolochene synthase (AS) enzyme (Caruthers *et al.* 2000). The AS is the first terpenoid cyclase enzyme of fungal origin, and its structure shows the active centres that presumably are involved in the cyclization of farnesyl pyrophosphate (**26**), leading to aristolochene hydrocarbon (**17**) which is the biogenetic precursor of all sesquiterpenes and is structurally related to the PR-toxin described above. Note that both compound (**26**) and (**17**) have the carbon structure of the eremophilane (**18**) (Figs 5 and 6).

Additionally, Olivigni and Bullerman (1978) detected penicillic acid (**19**) and patulin (**20**) in an atypical strain of *P. roqueforti*. Lafont and Debeaupuis (1980) described mycophenolic acid (**21**), and Brueckner and Reinecke (1988) identified aminoisobutyric acid by GC-MS (Fig. 7).

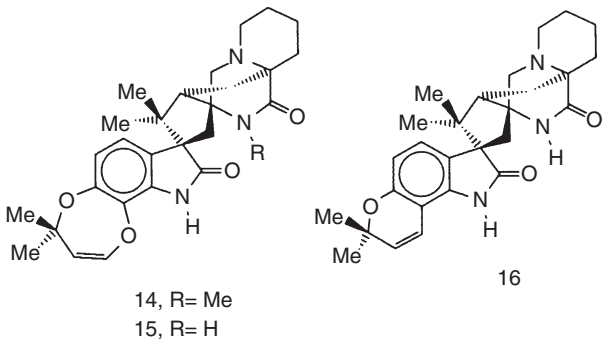

Figure 5 Marcfortines A (**14**), B (**15**) and C (**16**).

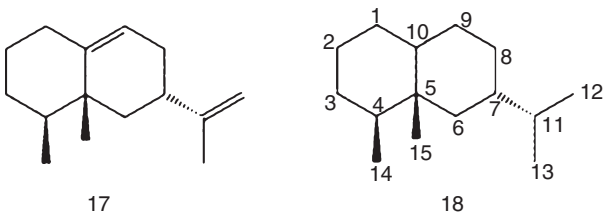

Figure 6 (+)-Aristolochene (**17**) and the eremophilane carbon skeleton (**18**).

Chalier and Crouzet (1992) indicated that γ-lactones are responsible for the smell of the blue cheese. They described 4-dodecanolide (γ-C12, **22**), (Z)-6-dodecen-4-olide {(6Z)-γ-C12: 1, **23**} and the minority 4-hexanolide (γ-C6), as well as the stereochemistry at carbon 4 {S to **22** and also S to **23**} that *P. roqueforti* makes from the oleic and linoleic acids.

Finally, botryodiplodin (**24**), which had previously been found in *Botryodiplodia theobromae*, was isolated from a nonproducing PR-toxin strain of *P. roqueforti*, (Moreau *et al.* 1982). The authors determined its absolute configuration by X-ray diffraction and reported its mutagenic activity (Fig. 8).

Andrastins (**25**), which are components of the fungus *P. roqueforti* that gives the classic blue colour to the Roquefort cheese (Fernández-Bodega *et al.* 2009), have been described previously by Danish (Nielsen *et al.* 2005; Overy *et al.* 2005) and Japanese (Shiomi *et al.* 1996; Uchida *et al.* 1996a,b) researchers. Andrastins A–D are potent inhibitors of the farnesyl transferase which is an important enzyme involved in cholesterol biosynthesis (Omura *et al.* 1996; Shiomi *et al.* 1996; Uchida *et al.* 1996a,b). Andrastin A exhibits potent antitumor activity by acting on the oncogenic *Ras* protein and also in the regulation of cell proliferation and cell differentiation (Uchida *et al.* 1996b; Rho *et al.* 1998) (Fig. 9).

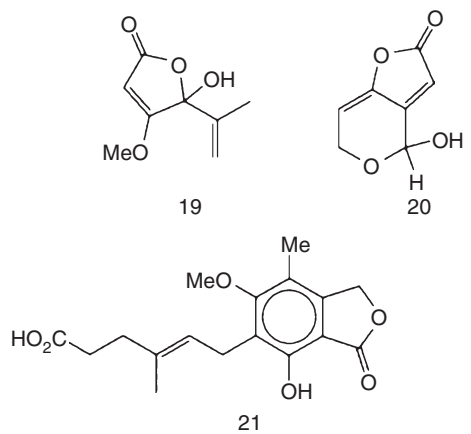

Figure 7 Penicillic acid (**19**), patulin (**20**) and mycophenolic acid (**21**).

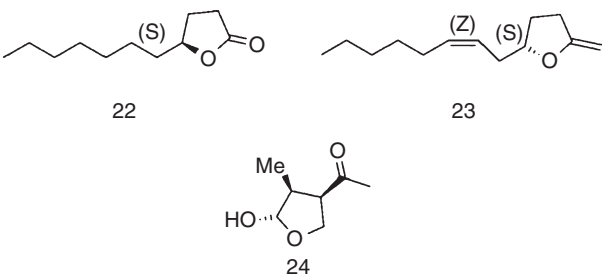

Figure 8 4-dodecanolide (**22**), (Z)-6-dodecen-4-olide (**23**) and botryodiplodin (**24**).

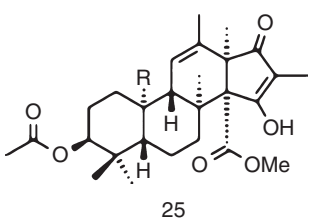

Figure 9 Andrastin (**25**).

Chemotaxonomy

Traditionally, the taxonomy of the *P. roqueforti* group has been based on morphological criteria, its diffusion capacity in standardized culture media and biochemical analysis of the profiles—the variety *P. roqueforti* var. *roqueforti* is used in the manufacture of cheese, and *P. roqueforti* var. *carneum* is the producer of patulin (**20**) (Pitt 1979; Frisvad 1981; Engel and Teuber 1983; Blomquist *et al.* 1992; Lomascolo *et al.* 1994). However, the modern tools of molecular genetics combined with biochemical profiles have highlighted the need to reclassify the *P. roqueforti* group, dividing it into three species:

P. roqueforti, *Penicillium carneum* and *Penicillium paneum* (Boysen *et al.* 1996, 2000).

Currently, the use of solid-phase microextraction techniques followed by capillary chromatography analysis with mass detection (GC-MS) allows the detection of volatile sesquiterpenes which are intermediates of various metabolic pathways in *P. roqueforti* (Demyttenaere *et al.* 2002; Jelen 2002). This confirms the hypothesis that the AS enzyme is responsible for the cyclization of the farnesyl pyrophosphate (**26**) to aristolochene (**17**), which is considered to be the biogenetic precursor of PR-toxin.

That is why Calvert *et al.* (2002), Demyttenaere *et al.* (2002) and Jelen (2002) proposed the AS enzyme with germacrene A (**27**), valencene (**28**), β-elemene (**29**), β-gurjunene (**30**), α-chamigrene (**31**), α-panasinsene (**32**), β-patchoulene (**33**), α-selinene (**34**), di-epi-α-cedrene (**35**), β-himachalene (**36**) and β-bisabolene (**37**), as the profile of volatile components, to allow chemotaxonomical differentiation of the producer strains of PR-toxin from those that cannot biosynthesize it (Figs 10 and 11). The authors noted that these chemical markers, detectable by GC-MS, were absent in the nontoxic strains of *P. roqueforti* that were analysed.

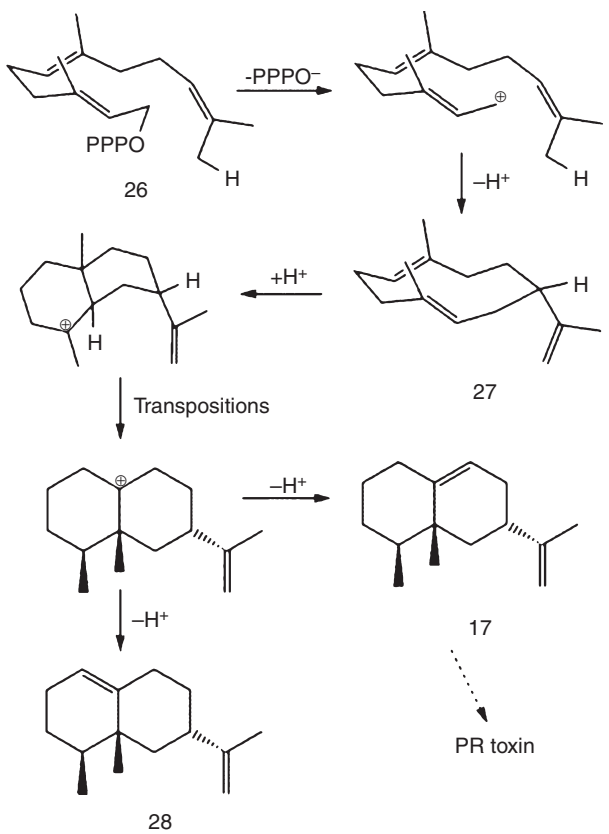

Figure 10 Biogenesis of volatile sesquiterpenes involved in the pathway of PR-toxin (**6**) in toxic strains of *Penicillium roqueforti*.

The identification of the secondary metabolites can help in the taxonomic differentiation, although the results should be taken with caution (Scott *et al.* 1977; Shimp and Kinsella 1977b; Maheva *et al.* 1984; Hassanien *et al.* 1986; Gock *et al.* 2003). That is why Jelen (2002) studied the influence of the culture conditions (temperature and water content in the broth), and he concluded that, although they have an influence on the number of sesquiterpenes produced (quantitative analysis), they do not have an influence on the profile (qualitative analysis) which is unique and characteristic for the toxic strains. The reason for this specificity has been justified by structural studies of the AS enzyme (Caruthers *et al.* 2000; Calvert *et al.* 2002; Deligeorgopoulou and Allemann 2003), providing evidence that the farnesyl pyrophosphate accommodates itself to the active AS site, having the necessary quasi-cyclic conformation (**26**) that makes possible the nucleophilic attack of C1 by the double bond at C10, stereospecifically producing (S)-germacrene A (**27**), which is an intermediary involved in the path of the PR-toxin

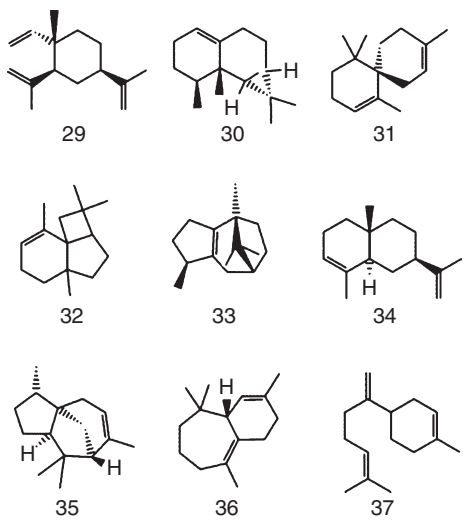

Figure 11 Volatile tracers in the toxic strains of *Penicillium roqueforti*.

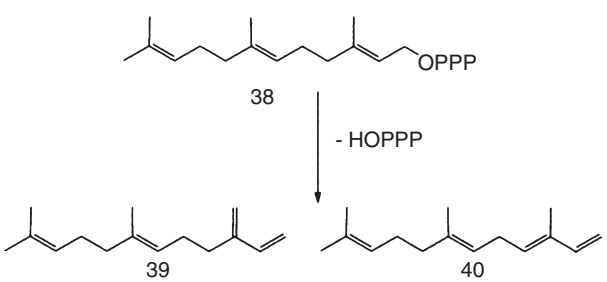

Figure 12 Biogenesis of some volatile tracers found in the nontoxic strains of *Penicillium roqueforti*.

(see Fig. 10), whereas the AS mutation at a single point (residue 92) makes it an enzyme that diverts the biogenetic route to the production of straight-chain terpenoids, such as (E)-β-farnesene (**39**) and (E,E)-α-farnesene (**40**), thereby allowing accommodation in the active site of the farnesyl pyrophosphate with its quasi-linear conformation (**38**)—see Fig. 12.

Concluding remarks and perspectives

Penicillium roqueforti is a filamentous fungus which is very often found in food. Although its origin is unclear, references and legends associated with this species date back over 2000 years. It can be considered to be 'domesticated' because of its relation to the development of artisanal cheeses. Nowadays, there are various strains known to us which are used in the food industry for the production of dairy products. About a century ago, the biotechnological research of the chemical diversity of the metabolites found in this fungus began to be improved worldwide. Thus, the comprehensive review of the chemistry of this fungus has allowed us to do a retrospective study of the secondary metabolites (e.g. festuclavine, isofumigaclavines and roquefortines) which are alkaloidal in nature. Other metabolites are marcfortines, PR-toxin, eremofortines, mycophenolic acid, penicillic acid and γ-lactones. Similarly, recent developments related to the structural characteristics of botryodiplodin and andrastin are addressed—the latter has anticancer properties. In conclusion, we discussed the use of secondary metabolite profiles for taxonomic purposes.

In the coming decades, it is expected that this fungus will be grown, even in marine conditions, to produce enzymes as products with high added value. Additionally, its applications in food will be increased, including the production of nonproducing strains of mycotoxins for foods.

Acknowledgments

The authors would like to thank the National Council of Technological and Scientific Development (CNPq, Brazil) for the postdoctoral fellowship granted to R.M., as well as the Sergipe State Foundation for Research and Innovation (FAPITEC/SE) for the financial support to the Regional Scientific Development Project (DCR, no. 150804/2011-3), without which this review could not have been completed. Grateful acknowledgement is made of the financial support towards the SI-697 project (ULPAPD-08/01-5) by the Canary Government (Agencia Canaria de Investigación, Innovación y Sociedad de la Información, ACIISI) and ICIC (Instituto Canario de Investigación del Cáncer).

Conflict of Interest

There is no conflict of interest to declare.

References

Aninat, C., Hayashi, Y., André, F. and Delaforge, M. (2001) Molecular requirements for inhibition of cytochrome p450 activities by roquefortine. *Chem Res Toxicol* **14**, 1259–1265.

Arnoux, B., Pascard, C. and Moreau, S. (1977) Eremofortin D, a valencane-class sesquiterpene. *Acta Crystallogr B Struct Crystal Cryst Chem* **33**, 2930–2932.

Arnoux, B., Merrien, M.A., Pascard, C., Polonsky, J. and Scott, P.M. (1978) Crystal structure and absolute configuration of isofumigaclavine A, a metabolite of *Penicillium roqueforti*. *J Chem Res* **6**, 210–211.

Asgher, M., Shahid, M., Kamal, S. and Iqbal, H.M.N. (2014) Recent trends in immobilization and industrial applications of ligninolytic enzymes: review. *J Mol Catal B Enzym* **101**, 56–66.

Bellinck, C. (1975) Chemical study of *Penicillium* and *Trichoderma* pigments. *Ann Microbiol* **126**, 131–142.

Bhat, B., Harrison, D.M. and Lamont, H.M. (1993) The biosynthesis of the mould metabolites Roquefortine and Aszonalenin from L-[2,4,5,6,7-2H5] Tryptophan. *Tetrahedron* **49**, 10663–10668.

Blomquist, G., Andersson, B., Andersson, K. and Brondz, I. (1992) Analysis of fatty acids. A new method for characterization of molds. *J Microbiol Methods* **16**, 59–68.

Boysen, M., Skouboe, P., Frisvad, J. and Rossen, L. (1996) Reclassification of the *Penicillium roqueforti* group into three species on the basis of molecular genetic and biochemical profiles. *Microbiology* **142**, 541–549.

Boysen, M.E., Jacobsson, K.G. and Schnürer, J. (2000) Molecular identification of species from the *Penicillium roqueforti* group associated with spoiled animal feed. *Appl Environ Microbiol* **66**, 1523–1526.

Brueckner, H. and Reinecke, C. (1988) GC-MS detection of Aib-containing polypeptide mycotoxins (antibiotics) in *Penicillium roqueforti* and other filamentous fungi. *J High Resolut Chromatogr Commun* **11**, 735–738.

Calvert, M.J., Ashton, P.R. and Allemann, R.K. (2002) Germacrene A is a product of the Aristolochene synthase-mediated conversion of Farnesyl pyrophosphate to Aristolochene. *J Am Chem Soc* **124**, 11636–11641.

Cao, M., Fonseca, L.M., Schoenfuss, T.C. and Rankin, S.A. (2014) Homogenization and lipase treatment of milk and resulting methyl ketone generation in blue cheese. *J Agric Food Chem* **62**, 5726–5733.

Caruthers, J.M., Kang, I., Rynkiewicz, M.J., Cane, D.E. and Christianson, D.W. (2000) Crystal structure determination of aristolochene synthase from the blue cheese mold, *Penicillium roqueforti*. *J Biol Chem* **275**, 25533–25539.

Chalier, P. and Crouzet, J. (1992) Production of lactones by *Penicillium roqueforti*. *Biotechnol Lett* **14**, 275–280.

Chang, S.-C., Wei, Y.-H., Wei, D.-L., Chen, Y.-Y. and Jong, S.-C. (1991) *Appl Environ Microbiol* **57**, 2581–2585.

Chang, S.-C., Lu, K.-L. and Yeh, S.-F. (1993) Secondary metabolites resulting from degradation of PR toxin by *Penicillium roqueforti*. *Appl Environ Microbiol* **59**, 981–986.

Chang, S.-C., Yeh, S.F., Li, S.-Y., Lei, W.-Y. and Chen, M.-Y. (1996) A novel secondary metabolite relative to the degradation of PR toxin by *Penicillium roqueforti*. *Curr Microbiol* **32**, 141–146.

Colen, G. (2006) Isolamento e seleção de fungos filamentosos produtores de lipases. PhD Thesis, Federal University of Minas Gerais, Brazil, 206 p.

Dartey, C.K. and Kinsella, J.E. (1973) Metabolism of carbon-14-uniformly-labeled lauric acid to methyl ketones by the spores of *Penicillium roqueforti*. *J Agric Food Chem* **21**, 933–936.

De Boer, E. and Kuik, D. (1987) A survey of the microbiological quality of blue-veined cheeses. *Neth Milk Dairy J* **41**, 227–237.

Deligeorgopoulou, A. and Allemann, R.K. (2003) Evidence for differential folding of farnesyl pyrophosphate in the active site of aristolochene synthase: a single-point mutation converts aristolochene synthase into an (E)-ß-farnesene synthase. *Biochemistry* **42**, 7741–7747.

Demyttenaere, J.C.R., Adams, A., van Belleghem, K., de Kimpe, N., Konig, W.A. and Tkachev, A.V. (2002) De novo production of (+)-aristolochene by sporulated surface cultures of *Penicillium roqueforti*. *Phytochemistry* **59**, 597–602.

Dheeman, D.S., Frias, J.M. and Henehan, G.T.M. (2010) Influence of cultivation conditions on the production of a thermostable extracellular lipase from *Amycolatopsis mediterranei* DSM 43304. *J Ind Microbiol Biotechnol* **37**, 1–17.

Dolezalek, J. and Hoza, I. (1969) Effect of *Penicillium roqueforti* on the formation of aldehydes and methyl ketones. *Prumysl Potravin* **20**, 171–175.

Eitenmiller, R.R., Vakil, J.R. and Shahani, K.M. (1970) Production and properties of *Penicillium roqueforti* lipase. *J Food Sci* **35**, 130–133.

Engel, G. and Teuber, M. (1983) Differentiation of *Penicillium roqueforti* strains by thin-layer chromatography of metabolites. *Milchwissenschaft* **38**, 513–516.

Fan, T.Y. and Kinsella, J.E. (1976) Changes in biochemical components during the germination of spores of *Penicillium roqueforti*. *J Sci Food Agric* **27**, 745–752.

Fernandes, A.P. (2014) Utilization of waste from dry and wet processing of coffee to obtain pectinases. PhD Thesis, Federal University of Lavras, Brazil, 135 p.

Fernández-Bodega, E., Mauriz, E., Gómez, A. and Martín, J.F. (2009) Proteolytic activity, mycotoxins and andrastin A in *Penicillium roqueforti* strains isolated from Cabrales, Valdeón and Bejes-Tresviso local varieties of blue-veined cheeses. *Int J Food Microbiol* **136**, 18–25.

Fleming, A. (1929) On the antibacterial action of cultures of a *Penicillium*, with special reference to their use in the isolation of *B. influenza*. *Br J Exp Pathol* **10**, 226–236.

Frisvad, J.C. (1981) Physiological criteria and mycotoxin production as aids in identification of common asymmetric penicillia. *Appl Environ Microbiol* **41**, 568–579.

Fuquay, J.W., Fox, P.F. and McSweeney, P.L.H. (2011) *Encyclopedia of Dairy Sciences*, 2nd edn. San Diego, CA: Academic Press, 960 p.

Gehrig, R.F. and Knight, S.G. (1958) Formation of ketones from fatty acids by spores of *Penicillium roqueforti*. *Nature* **182**, 1237.

Gente, S., Billon-Grand, G., Poussereau, N. and Fevre, M. (2001) Ambient alkaline pH prevents maturation but not synthesis of ASPA, the aspartyl protease from *Penicillium roqueforti*. *Curr Gen* **38**, 323–328.

Gobbetti, M., Burzigotti, R., Smacchi, E., Corsetti, A. and De Angelis, M. (1998) Microbiology and biochemistry of Gorgonzola cheese during ripening. *Int Dairy J* **7**, 519–529.

Gock, M.A., Hocking, A.D., Pitt, J.I. and Poulos, P.G. (2003) Influence of temperature, water activity and pH on growth of some xerophilic fungi. *Int J Food Microbiol* **81**, 11–19.

Godinho, M. and Fox, P.F. (1981) Effect of sodium chloride on the germination and growth of *Penicillium roqueforti*. *Milchwissenschaft* **36**, 205–208.

Gripon, J.-C. (1977) The proteolytic system of *Penicillium roqueforti*. Purification and properties of an alkaline aminopeptidase. *Biochimie* **59**, 679–686.

Gripon, J.C. (1993) Moulded-ripened cheeses. In *Cheese: Chemistry, Physics and Microbiology* ed. Fox, P.F. pp. 193–255. London: Chapman and Hall.

Gripon, J.-C., Desmazeaud, M.J., Le Bars, D. and Bergere, J.L. (1977) Role of proteolytic enzymes of *Streptococcus lactis*, *Penicillium roqueforti* and *Penicillium caseicolum* during cheese ripening. *J Dairy Sci* **60**, 1532–1538.

Gripon, J.-C., Auberger, B. and Lenoir, J. (1980) Metalloproteases from *Penicillium caseicolum* and *P. roqueforti*: comparison of specificity and chemical characterization. *Int J Biochem* **12**, 451–455.

Hassanien, F.R., Ragab, M. and El-Makhzangy, A. (1986) Studies on the possibility of producing fats from food wastes by using microorganisms. Factors affecting fat production from different fungi. *Fette, Seifen, Anstrichmittel* **88**, 33–38.

Hermosa, R., Cardoza, R.E., Rubio, M.B., Gutiérrez, S. and Monte, E. (2014) Secondary metabolism and antimicrobial metabolites of *Trichoderma*. In *Biotechnology and Biology of Trichoderma* eds. Gupta, V.G., Schmoll, M., Herrera-Estrella, A., Upadhyay, R.S., Druzhinina, I. and Tuohy, M., pp 125–138. Amsterdam: Elsevier.

Hwang, D.H., Lee, Y.J. and Kinsella, J.E. (1976) ß-Ketoacyl decarboxylase activity in spores and mycelium of *Penicillium roqueforti*. *Int J Biochem* **7**, 165–171.

Igoshi, K., Hara, H. and Kobayashi, H. (2007) Two kinds of extracellular protease from wheat bran medium cultured by *Penicillium roqueforti*. *Milk Sci* **56**, 1–7.

Jackson, H.W. and Hussong, R.V. (1958) Secondary alcohols in blue cheese and their relation to methyl ketones. *J Dairy Sci* **41**, 920–924.

Jelen, H.H. (2002) Volatile sesquiterpene hydrocarbons characteristic for *Penicillium roqueforti* strains producing PR toxin. *J Agric Food Chem* **50**, 6569–6574.

Jingxin, S., Ming, H., Guanghong, Z., Xinglian, X., Meiling, Y. and Shuling, W. (2013) *CN pat. 103005474*.

Kanota, K. (1969) Toxic metabolites of *Penicillium roqueforti*. *Eisei Shikensho Hokoku* **87**, 31–35.

Kaufmann, H.P., Ahmad, A.K.S. and Radwan, S.S. (1966) Component lipids of *Penicillium roqueforti* and *Penicillium caseicolum*. *Fette, Seifen, Anstrichmittel* **68**, 498–503.

Kolousek, J. and Michalik, S. (1954) Nitrogenous constituents and metabolites of *Penicillium roqueforti*. *Sbornik Ceskoslov Akad Zemedel Ved* **27**, 281–286.

Kopp-Holtwiesche, B. and Rehm, H.J. (1990) Antimicrobial action of roquefortine. *J Environ Pathol Toxicol Oncol* **10**, 41–44.

Kubeczka, K.H. (1968) Comparative studies on the biogenesis of volatile secondary products. II Molds. *Archiv Mikrobid* **60**, 139–159.

Lafont, P. and Debeaupuis, J.P. (1980) Photodensitometric determination of PR-toxin, metabolite of *Penicillium roqueforti*. *J Chromatogr* **198**, 481–488.

Lamberet, G. and Menassa, A. (1983) Purification and properties of an acid lipase from *Penicillium roqueforti*. *J Dairy Res* **50**, 459–468.

Lawrence, R.C. (1965) Use of 2,4-dinitrophenylhydrazine for the estimation of micro amounts of carbonyls. *Nature* **205**, 1313–1314.

Lawrence, R.C. (1967) Metabolism of triglycerides by spores of *Penicillium roqueforti*. *J Gen Microbiol* **46**, 65–76.

Le Bars, D. and Gripon, J.C. (1981) Role of *Penicillium roqueforti* proteinases during blue cheese ripening. *J Dairy Res* **48**, 479–487.

Levin, D.A. (1976) The chemical defenses of plants to pathogens and herbivores. *Annu Rev Ecol Syst* **7**, 121–159.

Lobyreva, L.B. and Marchenkova, A.I. (1980) Isolation and characteristics of *Penicillium roqueforti* lipases. *Mikrobiologiia* **49**, 924–930.

Lomascolo, A., Dubreucq, E., Perrier, V., Galzy, P. and Grimaud, J. (1994) Mycelial lipid composition of three *Penicillium* strains. *J Dairy Sci* **77**, 2160–2167.

Maheva, E., Djelveh, G., Larroche, C. and Gros, J.B. (1984) Sporulation of *Penicillium roqueforti* in solid substrate fermentation. *Biotechnol Lett* **6**, 97–102.

Martínez-Rodríguez, Y., Acosta-Muñiz, C., Olivas, G.I., Guerrero-Beltrán, J., Rodrigo-Aliaga, D., Mujica-Paz, H., Welti-Chanes, J. and Sepulveda, D.R. (2014) Effect of high hydrostatic pressure on mycelial development, spore viability and enzyme activity of *Penicillium roqueforti*. *Int J Food Microbiol* **168–169**, 42–46.

Mase, T., Matsumiya, Y. and Matsuura, A. (1995) Purification and characterization of *Penicillium roqueforti* IAM 7268 lipase. *Biosci Biotechnol Biochem* **59**, 329–330.

McClintock, J.B. and Baker, B.J. (2001) *Marine Chemical Ecology*, p 609. Boca Raton: CRC Press.

Mioso, R., Toledo Marante, F.J., de Bravo Laguna, I.H., González, J.E.G. and Rodríguez, J.J.S. (2014) Biomolecules produced in liquid-state fermentation by a marine-derived fungus, *Penicillium roqueforti*. *Quim Nova* **37**, 260–267.

Mioso, R., Toledo Marante, F.J. and Bravo de Laguna, I.H. (in press) Chemical constituents of the fermentation broth of the marine-derived fungus *Penicillium roqueforti*. *Rev Iberoam Micol*, doi:10.1016/j.riam.2014.01.004.

Modler, H.W., Brunner, J.R. and Stine, C.M. (1974) Extracellular protease of *Penicillium roqueforti*. Production and characteristics of crude enzyme preparation. *J Dairy Sci* **57**, 523–527.

Moreau, S., Gaudemer, A., Lablache-Combier, A. and Biguet, J. (1976) Metabolites de *Penicillium roqueforti*: PR toxine et metabolites associes. *Tetrahedron Lett* **11**, 833–834.

Moreau, S., Moule, Y. and Bousquet, J.F. (1977) Relationships between the chemical structure and the biological properties of some eremophilane compounds related to PR toxin. *Ann Nutr Aliment* **31**, 881–884.

Moreau, S., Biguet, J., Lablache-Combier, A., Baert, F., Foulon, M. and Delfose, C. (1980a) Structures et stereochimie des sesquiterpenes de *Penicillium roqueforti*: PR toxin et eremofortines A, B, C, D, E. *Tetrahedron* **36**, 2989–2997.

Moreau, S., Lablache-Combier, A. and Biguet, J. (1980b) Production of eremofortins A, B, and C relative to formation of PR toxin by *Penicillium roqueforti*. *Appl Environ Microbiol* **39**, 770–776.

Moreau, S., Lablache-Combier, A., Biguet, J., Foulon, C. and Delfosse, M. (1982) Botryodiplodin, a mycotoxin synthesized by a strain of *Penicillium roqueforti*. *J Org Chem* **47**, 2358–2359.

Nielsen, K.F., Dalsgaard, P.W., Smedsgaard, J. and Larsen, T.O. (2005) Andrastins A-D, *Penicillium roqueforti* metabolites consistently produced in blue-mold-ripened cheese. *J Agric Food Chem* **53**, 2908–2913.

Ohmomo, S., Sato, T., Utagawa, T. and Abe, M. (1975) Production of alkaloids and related substances by fungi. Isolation of festuclavine and three new indole alkaloids, roquefortine A, B, and C from the cultures of *Penicillium roqueforti*. *Nippon Nogei Kagaku Kaishi* **49**, 615–623.

Ohmomo, S., Oguma, K., Ohashi, T. and Abe, M. (1978) Isolation of a new indole alkaloid, roquefortine D, from the cultures of *Penicillium roqueforti*. *Agric Biol Chem* **42**, 2387–2389.

Olivigni, F.J. and Bullerman, L.B. (1978) Production of penicillic acid and patulin by an atypical *Penicillium roqueforti* isolate. *Appl Environ Microbiol* **35**, 435–438.

Omura, S., Inokoshi, J., Uchida, R., Shiomi, K., Masuma, R., Kawakubo, T., Tanaka, H., Iwai, Y. *et al.* (1996) Andrastins A-C, new protein farnesyltransferase inhibitors produced by *Penicillium* sp. FO-3929. Producing strain, fermentation, isolation, and biological activities. *J Antibiot* **49**, 414–417.

Overy, D.P., Larsen, T.O., Dalsgaard, P.W., Frydenvang, K., Phipps, R., Munro, M.H. and Christophersen, C. (2005) Andrastin A and barceloneic acid metabolites, protein farnesyl transferase inhibitors from *Penicillium albocoremium*: chemotaxonomic significance and pathological implications. *Mycol Res* **109**, 1243–1249.

Pawlik, J.R. (1993) Marine invertebrate chemical defenses. *Chem Rev* **93**, 1911–1922.

Peričin, D.M., Mađarev, S.Z., Radulović, L.M. and Škrinjar, M.M. (2007) Bioprocessing for value added products from oil cakes. *Acta Period Technol* **38**, 35–40.

Pitt, J.I. (1979) *The Genus Penicillium*, p 346. London: Academic Press.

Polonsky, J., Merrien, M.A. and Scott, P.M. (1977) Roquefortine and isofumigaclavine A, alkaloids from *Penicillium roqueforti*. *Ann Nutr Aliment* **31**, 693–698.

Polonsky, J., Merrien, M.A., Prangé, T., Pascard, C. and Moreau, S. (1980) Isolation and structure (x-ray analysis) of marcfortine A, a new alkaloid from *Penicillium roqueforti*. *J Chem Soc, Chem Commun* **13**, 601–602.

Prangé, T., Billion, M.A., Vuilhorgne, M., Pascard, C., Polonsky, J. and Moreau, S. (1981) Structures of marcfortine B and C (x-ray analysis), alkaloids from *Penicillium roqueforti*. *Tetrahedron Lett* **22**, 1977–1980.

Punt, P.J., van Biezen, N., Conesa, A., Albers, A., Mangnus, J. and van den Hondel, C.A. (2002) Filamentous fungi as cell factories for heterologous protein production. *Trends Biotechnol* **20**, 200–206.

Rabie, A.M. (1989) Acceleration of blue cheese ripening by cheese slurry and extracellular enzymes of *Penicillium roqueforti*. *Lait* **69**, 305–314.

Rho, M.C., Toyoshima, M., Hayasshi, M., Uchida, R., Shiomi, K., Komiyama, J. and Omura, S. (1998) Enhancement of drug accumulation by andrastin A produced by *Penicillium* sp. FO-3929 in vincristine-resistant KB cells. *J Antibiot* **51**, 68–72.

Rivera, I., Villanueva, G. and Sandoval, G. (2009) Biodiesel production from animal grease wastes by enzymatic catalysis. *Grasas Aceites* **60**, 468–474.

Rosenthal, I., Bernstein, S. and Rosen, B. (1996) Alkaline phosphatase activity in *Penicillium roqueforti* and blue-veined cheeses. *J Dairy Sci* **79**, 16–19.

Ruddock, J. (1992) *Brit. UK pat. GB2247016*.

Salvadori, P. and Salvadori, B.B. (1967) Attack on some higher fatty acids by different strains of *Penicillium roqueforti*. *Lait* **47**, 605–611.

Scott, P.M. (1984) PR Toxin. In *Mycotoxins - Production, Isolation, Separation and Purification* ed. Betina, V. pp. 469–474. Amsterdam: Elsevier Science Publishers.

Scott, P.M., Merrien, M.A. and Polonsky, J. (1976) Roquefortine and isofumigaclavine A, metabolites from *Penicillium roqueforti*. *Experientia* **32**, 140–142.

Scott, P.M., Kennedy, B.P.C., Harwig, J. and Blanchfield, B.J. (1977) Study of conditions for production of roquefortine and other metabolites of *Penicillium roqueforti*. *Appl Environ Microbiol* **33**, 249–253.

Shimp, J.L. and Kinsella, J.E. (1977a) Lipids of *Penicillium roqueforti*. Influence of culture temperature and age on unsaturated fatty acids. *J Agric Food Chem* **25**, 793–799.

Shimp, J.L. and Kinsella, J.E. (1977b) Composition of the mycelium of *Penicillium roqueforti*. *J Food Sci* **42**, 681–684.

Shiomi, K., Uchida, R., Inokoshi, J., Tanaka, H., Iwai, Y. and Omura, S. (1996) Andrastins A-C, new protein farnesyltransferase inhibitors, produced by *Penicillium* sp. FO-3929. *Tetrahedron Lett* **37**, 1265–1268.

Sibilla, F. and Domínguez de María, P. (2013) Integrating white biotechnology in lignocellulosic biomass transformations: from enzyme-catalysis to metabolic engineering. In *The Role of Catalysis for the Sustainable Production of Bio-Fuels and Bio-Chemicals* eds Triantafyllidis, K., Lappas, A. and Stöcker, M. pp. 445–466. Amsterdam: Elsevier.

Szoltysek, K. (1999) *PL pat. 176037*.

Temesvari, J. and Biacs, P.A. (1996) Immobilization of lipase and its investigation. *Acta Aliment* **25**, 277–290.

Toledo Marante, F.J., García Castellano, A., León Oyola, F. and Bermejo Barrera, J. (2004) Ecología química en hongos y líquenes. *Rev Acad Colomb Ci Exact* **109**, 509–528.

Turner, W.B. (1971) *Fungal Metabolites*, p 446. New York: Academic Press.

Uchida, R., Shiomi, K., Inokoshi, J., Sunazuka, T., Tanaka, H., Iwai, Y. and Omura, S. (1996a) Andrastins A-C, new protein farnesyltransferase inhibitors produced by *Penicillium* sp. FO-3929. II Structure elucidation and biosynthesis. *J Antibiot* **49**, 418–424.

Uchida, R., Shiomi, K., Inokoshi, J., Tanaka, H., Iwai, Y. and Omura, S. (1996b) Andrastin D, novel protein farnesyltransferase inhibitor produced by *Penicillium* sp. FO3929. *J Antibiotics* **49**, 1278–1280.

Ward, O. (2012) Production of recombinant proteins by filamentous fungi. *Biotechnol Adv* **30**, 1119–1139.

Wei, R.D., Still, P.E., Smalley, E.B., Schnoes, H.K. and Strong, F.M. (1973) Isolation and partial characterization of a mycotoxin from *Penicillium roqueforti*. *Appl Microbiol* **25**, 111–114.

Wei, R.D., Schnoes, H.K., Hart, P.A. and Strong, F.M. (1975) Structure of PR toxin, a mycotoxin from *Penicillium roqueforti*. *Tetrahedron* **31**, 109–114.

Wei, Y.H., Chang, S.C., Li, S.Y. and Lu, C.Y. (1989) Secondary metabolites and enzymes involved in the biosynthesis and degradation of PR toxin. *Acad Sin Inst Bot Monogr Ser* **9**, 371–390.

Woloszyn, J., Szoltysek, K. and Ziobrowski, J. (1988) Use of an enzymic preparation of *Penicillium roqueforti* mycelium for descaling of fish-roach. *Pol Przemysl Spozywczy* **42**, 297–298.

Zevaco, C., Hermier, J. and Gripon, J.C. (1973) Le système protéolytique de *Penicillium roqueforti*. II - Purification et propriétés de la protéase acide. *Biochimie* **55**, 1353–1360.