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Nafees Bacha, Zerif Echarki, Florence Mathieu, Ahmed Lebrihi. Development of a novel quantitative PCR assay as a measurement for the presence of geosmin-producing fungi. *Journal of Applied Microbiology*, 2015, 118 (5), pp.1144-1151. 10.1111/jam.12747 . hal-02134795

HAL Id: hal-02134795

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Bacha, Nafees and Echarhi, Z. and Mathieu, Florence and Lebrihi, Ahmed Development of a novel quantitative PCR assay as a measurement for the presence of geosmin-producing fungi. (2015) *Journal of Applied Microbiology*, 118 (5). 1144-1151. ISSN 1364-5072

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Development of a novel quantitative PCR assay as a measurement for the presence of geosmin-producing fungi

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Keywords

5- *Penicillium expansum*, cytochrome P450 gene, geosmin, *P. restrictum*, quantitative PCR.

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doi:10.1111/jam.12747

Abstract

Aims: To provide an efficient technique for monitoring the off-flavoured fungal compound geosmin.

Methods and Results: Geosmin-associated *gpe1* gene of *Penicillium expansum* displayed $\geq 99\%$ similarity to cytochrome P450 gene of geosmin-producing *P. restrictum*, but $\leq 40\%$ similarities to geosmin biosynthesis, non-cytochromic gene of *Streptomyces avermitilis* and cytochrome P450 genes of non-geosmin-producing *Neotyphodium lolii*, *Phoma betae* and *P. paxilli*. Serial 10-fold dilutions of *P. expansum*'s DNA was subjected to a previously reported qPCR assay (Atoui *et al.* 2007), utilizing *gpe1* specific primer pair 'SNGpe1F/SNGpe1R'. A linear relationship between DNA quantity and Cycle Threshold (C_t), with strong correlative coefficient, was observed. Using the available physico-chemical method, geosmin was quantified in 188 grape samples. *Penicillium spp.*'s DNA was quantified in these samples, utilizing the developed qPCR assay. A strong positive correlation ($R^2 = 0.97$) between *Penicillium*'s DNA and geosmin concentration was observed. Furthermore, $< 50 \text{ ng } \mu\text{l}^{-1}$ *Penicillium*'s DNA corresponds to geosmin level below the permitted intensity limit i.e. 4, for 'Flavour Profile Analysis'.

Conclusions: *Penicillium spp.*, genomic DNA level can provide an efficient way to quantify geosmin.

Significance and Impact of the Study: This particular qPCR technique can be utilized in numerous food industries, for the timely detection and monitoring of geosmin contamination.

Introduction

Geosmin ($C_{12}H_{22}O$) is a bi-cyclic saturated volatile alcohol having the odour of freshly ploughed earth. This compound exists both as (+) and (−) enantiomers. The odour outbreaks are caused by biological production of the naturally occurring (−) enantiomers, which are some 10 times more potent than the (+) molecules (Watson *et al.* 2000). In 1965, geosmin was for the first time isolated from fisheries, various beverages and water supplies (Gerber and Lechevalier 1965). Later, it was found that this compound is equally responsible for the undesirable musty or off-flavour in drinking water, wine, grape juices,

fish and other food stuffs (Gerber 1979; Heil and Lindsay 1988; Darriet *et al.* 2000; La Guerche *et al.* 2005). Geosmin is commonly associated with another volatile secondary metabolite '2-MethylIsoBorneol (2-MIB)' (Buttery and Garibaldi 1976). It has been reported that, in various *Streptomyces species*, geosmin is produced by the conversion of farnesyl diphosphate into germacradienol and then into geosmin (Jiang *et al.* 2007) by an enzyme 'sesquiterpene synthase'.

A wide variety of micro-organisms, including bacteria, fungi, actinomycetes, blue-green algae and some plants like mosses, can synthesize geosmin (Izaguirre *et al.* 1982; Mattheis and Roberts 1992; Spiteller *et al.* 2002; Lu *et al.*

2003; Zaitlin and Watson 2006). Among various species of fungi that produce geosmin, Basidiomycete species i.e. *Cortinarius herculeus*, *Cystoderma amianthinum* and *Cy. carcharias* (Breheret *et al.* 1999), and various species of *Penicillium* (Pisarnitskii and Egorov 1988; Mattheis and Roberts 1992; Larsen and Frisvad 1995) are of prime significance. One of the *Penicillium sp.*, '*Penicillium expansum*', is often associated with rotten grapes in vineyards (La Guerche *et al.* 2004) and is one of the major contributors to geosmin production, especially in various beverages obtained from these sources. In addition to *P. expansum*, the complementary action of another fungus '*Botrytis cinerea*', acts as a second menace in geosmin biosynthesis in grape-beverages (La Guerche *et al.* 2005). *Botrytis cinerea* has been reported to be present in earthy-tasting grapes. In an *in vitro* study, La Guerche *et al.* (2005) demonstrated that when grape-juice is precultured (for 7 days) with *B. cinerea* strains, the biosynthesis of geosmin by *P. expansum* will be augmented. This means that in earthy-tasting grapes, *B. cinerea* act as an initial model organism for the biosynthesis of geosmin by *P. expansum*.

On the one hand, the earthy odour and diversity in geosmin producers are problematic, and on the other hand, volatile nature of geosmin makes it challenging to precisely detect and monitor this compound with previously described methods. These methods include, Methylene chloride extraction and Gas Chromatography (GC) (Johnsen and Kuan 1987); Gas Chromatography–Mass Spectrometry (GC/MS) (Darriet *et al.* 2000) and Stir Bar Sorptive Extraction (SBSE) followed by on-line thermal desorption (TD) capillary (GC/MS) (Benanou *et al.* 2003) etc. More recently, microextraction and GC/MS were used (with a quantification limit of 15.6 ng l⁻¹) for the analysis of geosmin in grape-juice samples inoculated with *P. expansum* and *B. cinerea* (Morales-Valle *et al.* 2010). Another relatively new sampling procedure known as 'Solid-phase microextraction (SPME)' allows facile analysis of volatile molecules with a single step sample extraction and preconcentration (Sundar and Suranjan 2011). However, majority of these physico-chemical techniques developed and utilized for monitoring geosmin require expensive materials, are time-consuming and usually have carry-over effects. For instance, SBSE has long-time sorbent conditioning; liquid–liquid extraction and SPME use large amounts of potentially toxic and often expensive organic solvents and the sample manipulation involved can lead to undesirable contaminations.

Genetic and/or molecular biology based approaches have been developed more recently to overcome the aforementioned problems. Genes that contribute to geosmin production pathways have been identified and their

variability has been studied, for example, *geoA* (Auffret *et al.* 2011) was used to elucidate gene action in geosmin-producing and nonproducing bacterial genera and cyanobacteria (Ludwig *et al.* 2007; Guttman and van Rijn 2008). A real-time polymerase chain reaction (qPCR) protocol has been developed, targeting *geoA* sequences in bacteria *Streptomyces spp.*, that encode a germacradienol synthase involved in geosmin synthesis (Auffret *et al.* 2011). This method was specifically designed to quantify the lowest level of geosmin in *Streptomyces spp.*, in aquaculture systems.

We report the first development of a qPCR-based technique that can precisely monitor, genomic DNA of geosmin-producing fungi in a given sample. The quantified DNA can have a proportional relation to the geosmin concentration in these samples. Hence, this technique can be utilized to monitor geosmin in a quick possible way and can be a good replacement of the previously developed conventional methods. Furthermore, the developed qPCR will help in the establishment of a molecular based 'Flavour Profile Analysis (FPA)' and will ultimately allow a critical limit to be set for tighter regulation and customer satisfaction.

Methodology

Growth of *Penicillium expansum* and *Penicillium restrictum*

A spore suspension of *P. expansum* M2230 and *P. restrictum* were spread on Yeast Extract Saccharose (YES) agar (Yeast extract, 20 g l⁻¹; Sucrose, 150 g l⁻¹ and Agar, 20 g l⁻¹) media plates. Five to 10 ml of 0.01% Tween 80 solution was utilized for the collection of spores after 7 days of incubation at 28°C. After counting, the spores were stored at -20°C in 25% (v/v) glycerol before use. Conidia were inoculated into 250 ml Erlenmeyer flasks containing 100 ml YES broth medium, and incubated at 28°C for 4 days, without shaking. Mycelia was harvested by filtration through a 0.45 µm filter (Millipore, USA), grounded in liquid nitrogen and then stored at -80°C prior to DNA extraction.

Extraction of fungal DNA

The already grounded mycelium of *P. expansum* and *P. restrictum* were used for total genomic DNA extraction, using a previously reported method (Dao *et al.* 2005). In addition, DNA extractions from 118 grape samples, collected from various French vineyards, were also performed. A portion of fresh and frozen grape berries (300 mg) were weighed and incubated with 1.5 ml extraction buffer (1 mol l⁻¹ Tris-HCl

(Sigma–Aldrich, Saint Quentin Fallavier, France) pH 8, 1.4 mol l⁻¹ NaCl (Sigma–Aldrich), 20 mmol l⁻¹ EDTA (Sigma–Aldrich), 3% CTAB (Sigma–Aldrich) and 15 µl β-mercaptoethanol (Sigma–Aldrich), for 90 min at 65°C under constant shaking on an orbital shaker. After incubation, samples were centrifuged at 7000 g for 5 min at 4°C and the supernatant was collected in a 2 ml Eppendorf tube. One volume of chloroform/isoamyl alcohol (24 : 1) (Sigma–Aldrich) was added, samples were mixed and centrifuged at 7000 g for 20 min at 4°C. The upper aqueous phase was transferred into another tube, adding 0.1 volumes of 10% CTAB (Sigma–Aldrich). Again, 1 volume of chloroform/isoamyl alcohol (24 : 1) was added and samples were mixed and centrifuged at 7000 g for 20 min at 4°C. The upper aqueous phase was transferred into another tube, adding 0.1 volume of cold 2-propanol (Sigma–Aldrich). Samples were then incubated for 60 min at –80°C and centrifuged for 20 min at 14000 g. The pellet was dissolved in 300 µl of sterile H₂O and processed according to the EZNA Fungal DNA Miniprep Kit (Biofidal, Vaulx en Velin, France) protocol. DNA clean-up through a Hi-bond[®] spin column was performed, and finally, DNA was eluted in 100 µl of de-ionized H₂O.

Primer design and specificity of the PCR reaction

The protein blast service of the ‘National Center for Biotechnology Information (NCBI)’ (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) has been utilized to identify genes having closer identity with the target geosmin-associated cytochrome P450 genes ‘*gpe1*’ of *P. expansum* M2230 (Genbank accession number: AER70129.1). The online multiple alignment service (<http://multalin.toulouse.inra.fr/multalin/>) was utilized to identify two regions which were highly conserved between *gpe1* and cytochrome P450 gene of geosmin-producing *P. restrictum* (accession number: KC427062.1) (Fig. 1). The identified regions were nonconserved with geosmin nonproducing *Neotyphodium lolii* (accession number: AAW88512.1), *Phoma betae* (accession number: BAD29968.1), *P. paxilli* (accession number: AAK11528.1) and *Streptomyces avermitilis* (accession number: BAC69874.1). A forward and a reverse primer i.e. SNgpe1F: cggatgtgtcaatgacctc and SNgpe1R: tgttattccttg-gagtctc, respectively, were designed at the selected conserved regions, with the help of PRIMER EXPRESS software (Applied Biosystems, Foster City, CA). Primer synthesis was performed by Distribio, France. To test the specificity of the primer pair, the isolated DNA of *P. expansum* was subjected to PCR with the *Taq* recombinant polymerase (Invitrogen, Cergy Pontoise, France). Amplification was

carried out in a 50 µl reaction mixture containing: 5 µl of *Taq* polymerase buffer 10×, 1.5 µl of 50 mmol l⁻¹ MgCl₂, 1 µl of 10 mmol l⁻¹ dNTP (Promega, Charbonnières, France), 1 µmol of each primer, 1.5 U of *Taq* and 50 ng of genomic DNA. The volume was made up to 50 µl with deionized H₂O. Reaction conditions were: 94°C for 4 min, (94°C for 40 s, 58°C for 40 s and 72°C for 40 s) × 35 cycles followed by incubation at 72°C for 10 min. The amplified products were examined by agarose gel electrophoresis. The same experiment was performed for *P. restrictum* as a confirmatory test.

The β-tubulin gene was amplified as a positive control. The primers used in this amplification were: TubF: ctcgagcgtatgaactgtctac; TubR: aaacctggaggcagctgc. The same PCR conditions used for *gpe1* gene amplification were utilized for β-tubulin gene amplification.

Real-time PCR reactions

The ABI PRISMR 7700 instrument (Applied Biosystems) was used for quantitative real-time PCR (qPCR) amplification and detection. Duplicates of 25 µl reaction mixture in MicroAmp optical 96-well reaction plates were sealed with optical adhesive covers (Applied Biosystems). Each reaction well contained 2.5 µl of template DNA, 12.5 µl of 2× Quantitect SYBR[®] Green I Mix (Qiagen, Courtaboeuf, France), and 12.5 pmol each of SNgpe1F and SNgpe1R primers. Real-time qPCR was conducted with the following cycling conditions: 50°C for 2 min, 95°C for 15 min, followed by 40 cycles of 94°C for 15 s, 58°C for 30 s and 72°C for 30 s.

To generate the standard curve, a 10-fold dilution of *P. expansum* DNA (concentration determined previously), were subjected to real-time PCR. The standard curve is a plot of the threshold cycle (C_t) vs log DNA concentration. DNA extracted from grape samples were then subjected to qPCR. The quantity of *Penicillium spp.* DNA was calculated according to the generated standard curve. A control sample without a DNA template was included in all experimental runs.

Geosmin quantification

All of the 118 grape samples were cut into small pieces with a sterile surgical blade and each individual sample was placed in separate tubes. To each tube, 10 ml of 20% ethanol was added. After vortexing, the tubes were incubated for 1 h at room temperature and at 200 rev min⁻¹. The filtered samples were sent to Exact Laboratory at Macon (France) for quantification of geosmin, using gas chromatography–mass spectrometry (GC–MS), with a stated quantification limit of 10 ng l⁻¹.

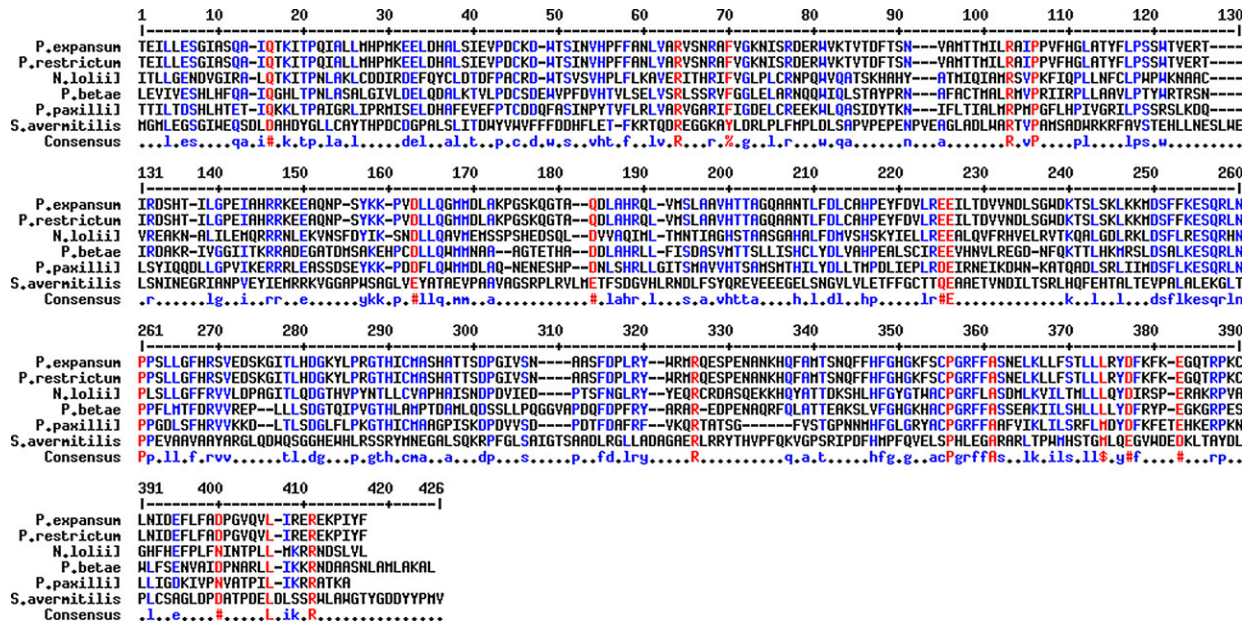


Figure 1 Multiple sequence alignment of the predicted amino acid sequence: The top two rows represent the amino acid sequence of cytochrome P450 from geosmin-producing *P. expansum* (accession number: AER70129.1) and *P. restrictum* (accession number: KC427062.1), respectively, which are compared with non-geosmin-producing i.e. *Neotyphodium lolii* (accession number: AAW88512.1), *Phoma betae* (accession number: BAD29968.1), *Penicillium paxilli* (accession number: AAK11528.1) and another non-cytochromic gene responsible for geosmin production in *Streptomyces avermitilis* (accession number: BAC69874.1).

Results

PCR Primers Selection and Specificity

A geosmin-associated cytochrome P450 gene ‘*gpe1*’ previously identified and characterized in *P. expansum* (Siddique *et al.* 2012) was the target of the present study. The predicted amino acid sequence of the 1-14 kb *gpe1* gene displayed significantly a high degree of similarity ($\geq 99\%$) to the cytochrome P450 gene identified in another geosmin-producing *Penicillium* sp. i.e. *P. restrictum* (Fig. 1). On the other hand, *gpe1* displayed low level similarities ($\leq 40\%$) to other non-geosmin-producing fungi including *Neotyphodium lolii*, *Phoma betae* and *P. paxilli*. It has also been observed that a non-cytochromic gene ‘*geoA*’ of *Streptomyces avermitilis*, important for the biosynthesis of geosmin has significant low level similarities to *gpe1*.

The online multiple alignment study revealed several highly conserved regions between *gpe1* and cytochrome P450 type gene of geosmin-producing *P. restrictum* (Fig. 1). Most of these regions were nonconserved with same type gene of *N. lolii*, *P. betae*, *P. paxilli* and *geoA* of *S. avermitilis*. From these, two regions ‘T D V V N D L’ and ‘E D S K G I T’ were selected to design a forward primer ‘SNGpe1F’ and reverse primer ‘SNGpe1R’ respectively. These primers were designed to amplifying a 149 bp fragment of the *gpe1* gene. A single PCR was

performed to check the specificity of these primers. The primer pair successfully amplified the target fragment of cytochrome P450 genes in both *P. expansum* and *P. restrictum* (Fig. 2). In this PCR, a 340 bp fragment of the universal β -tubulin gene was amplified as a positive control in both the above mentioned fungi (Fig. 2).

From the above PCR amplification, we conclude that the designed primers can specifically amplify cytochrome P450 genes in most geosmin-producing fungi.

qPCR Development for quantification of fungal geosmin

Serial 10-fold dilutions of *P. expansum* DNA were subjected to real-time PCR according to the procedure developed by Atoui *et al.* 2007. They were conjugated with SYBR[®] Green 1 dye, utilizing the developed *gpe1* specific primer pair SNGpe1F/SNGpe1R. In this particular process the quantification depends on measuring the intensity of a fluorescent signal, which is proportional to the amount of DNA generated during the PCR amplification. SDS software of the ‘Applied Biosystems’ real-time PCR machine was used to calculate the threshold cycle (C_t) value that corresponds to the PCR cycle number at which fluorescence was detected.

A standard curve was obtained by plotting C_t value vs logarithm of concentration at each DNA dilution. The linear correlation coefficient of the standard curve was

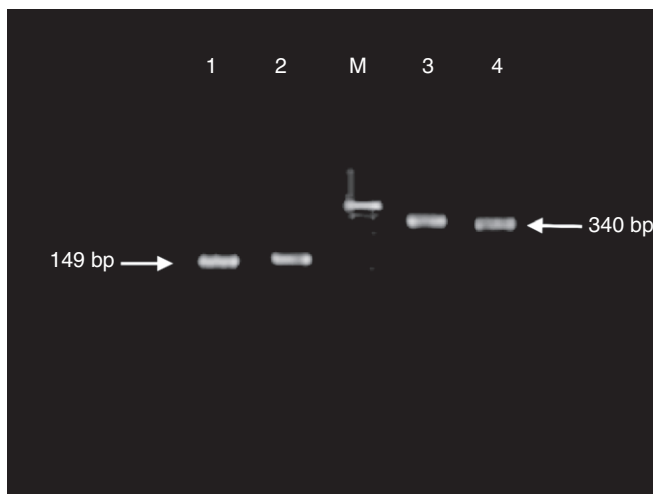


Figure 2 Genomic PCR of Cytochrome P450: A PCR product (149 bp) was amplified from *P. expansum* and *P. restrictum* using SNgpe1F/SNgpe1R primers. β -tubulin (fragment size 340 bp) was included as an internal control using primers TubF and TubR. Both products were resolved on 1.5% of agarose by gel electrophoresis. Lane 1 represents *P. restrictum*; Lane 2, *P. expansum*; Lane M represent 100 bp marker while Lane 3 & 4 represent positive control (β -tubulin) in both *P. expansum* and *P. restrictum* respectively.

$R^2 = 0.95$ (Fig. 3). The standard curve generated here could be used for DNA quantification of geosmin-producing *Penicillium spp.*, from any beverage/food source.

Quantification of geosmin and geosmin-producing fungal DNA through qPCR

A total of 118 grape samples were provided by farmers from various vineyards that were expected to have geosmin contamination. These samples were analysed for the occurrence of various geosmin-producing fungi through our developed qPCR system. DNA was extracted from the provided samples and subjected to qPCR with the system described above. The amount of geosmin associated with cytochrome P450 gene in an unknown sample was obtained by interpreting its C_t value against the

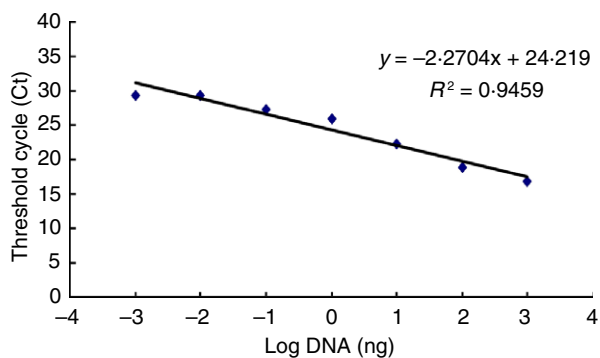


Figure 3 Correlation between Log₁₀ DNA amount (ng) vs the real-time PCR cycle threshold (C_t)qRTPCR: Standard curve showing the Log₁₀ DNA amount (ng) vs the real-time PCR cycle threshold (C_t) using SNgpe1F/SNgpe1R primers for 10-fold dilutions of *Penicillium expansum* pure genomic DNA. The assay shows a linear relationship between the DNA amount and C_t with a strong correlation coefficient ($R^2 = 0.95$).

standard curve, expressed in ng of DNA μl^{-1} of sample. The samples were also analysed for geosmin content using gas chromatography–mass spectrometry (GC–MS) (quantification limit 10 ng l^{-1}).

The geosmin-producing fungal DNA (ng DNA μl^{-1} of sample) quantified through our developed system was then plotted against geosmin content (ng l^{-1}) for all samples. The resultant regression curve showed a strong, positive correlation ($R^2 = 0.97$) between fungal DNA and geosmin concentrations. The geosmin free samples had a low level of DNA, as expected (Fig. 4). These findings suggest that, assessment of geosmin-producing fungal DNA, through our developed PCR system can be used for a quick indirect detection and monitoring of geosmin contamination in any type of sample.

Discussion

The organic compound ‘Geosmin’ is a problem for fisheries, vineyards, and other beverage/water suppliers due to its unpleasant aroma and ‘muddy’ flavour (Gerber and Lechevalier 1965; Buttery and Garibaldi 1976). To date, the ‘Environmental Protection Agency (EPA)’ has not defined a maximum permissible level for geosmin concentration and the related compound, 2-Methylisoborneol in drinking water/beverages (You 2012). This could possibly be due to its volatile nature (which makes this compound a challenge for most food suppliers and food processing industries to timely detect, monitor and decontaminate from contaminated samples) (Darriet *et al.* 2001), and the currently available physico-chemical detection and quantification techniques. Most of these techniques are associated with several disadvantages. They can be expensive, time-consuming, suffer from carry-over effects, potentially toxic and can lead to undesirable contaminations (La Guerche *et al.* 2004;

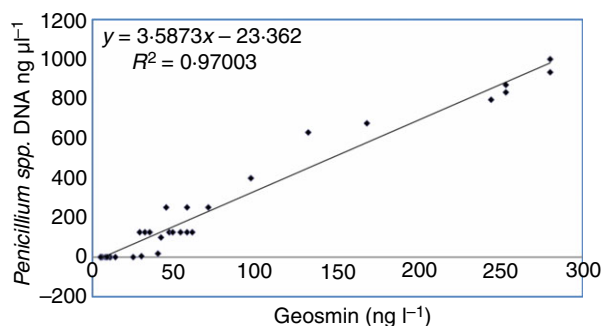


Figure 4 Correlation studies between the amount of *Penicillium expansum* DNA and geosmin concentrations in 118 grape samples. DNA was quantified using real-time PCR with primers SNgpe1F/SNgpe1R and SYBR Green as the fluorescent dye.

Morales-Valle *et al.* 2011). In the past fungi, the principal producer of geosmin in various food commodities remained ignored in toxicological and microbiological research. Herein, the two geosmin-producing *Penicillium spp.*, i.e. *P. expansum* and *P. restrictum*, have been targeted for the development of an efficient geosmin monitoring method. This method could possibly replace the available disadvantageous physico-chemical techniques and would help to define a maximum permitted concentration of geosmin, for quality control and customer satisfaction.

Several nonfungal organisms have been targeted for studying geosmin. It has been reported that geosmin is produced by the conversion of farnesyl diphosphate to germacradienol then geosmin (Jiang *et al.* 2007) by a sesquiterpene synthase encoded by homologous genes found in *Streptomyces coelicolor* A3(2) (*cyc2*), *S. avermitilis* (*geoA*) or *S. peucetius* (*Spterp13*). Other studies negate this statement and suggested that 'terpene synthase-encoding genes are rather involved in the biosynthesis of other similar compounds, but not geosmin' (Cane and Watt 2003; Cane *et al.* 2006; Ludwig *et al.* 2007; Ghimire *et al.* 2008; Jiang *et al.* 2008). A putative terpene synthase gene has been found in the genome of *Myxococcus xanthus* (accession number YP_634376). Another gene identified in the genome of several *Streptomyces* strains, has been found to encode the functional monoterpene cyclase also known as 2-MIB synthase, which is involved in the biosynthesis of 2-MIB (2MIB has similar aroma to geosmin and is strongly associated with the presence of geosmin) (Buttery and Garibaldi 1976; Komatsu *et al.* 2008; Wang and Cane 2008). It has also been suggested that the synthesis of MIB is similar in cyanobacteria and actinomycetes, but cyanobacteria showed a unique lineage possibly explained by multiple rearrangements of the genes in this operon (Giglio *et al.* 2010; Wang *et al.*

2011). However, such genes were not found in the genome of other MIB producers such as myxobacteria. Previous research from our group identified a cytochrome P450 gene '*gpe1*' in fungi i.e. *P. expansum*. The inactivation of this particular gene through insertion of *hygromycin B phosphotransferase (hph)* cassette of resistance from *Escherichia coli* revealed that *gpe1* is involved in the biosynthesis of geosmin (Siddique *et al.* 2012). Therefore, *gpe1* was further targeted with the objective to develop a specific PCR-based assay to quantify geosmin-associated *Penicillium spp.*, DNA, and ultimately correlate it to geosmin concentration.

Alignment of the predicted amino acid sequence of *gpe1* with the cytochrome P450 gene of geosmin producer, *P. restrictum*, the *geoA* gene of geosmin biosynthesis in *S. avermitilis* and other P450 genes of some non-geosmin producer, revealed that *gpe1* has a very high degree of similarity to the cytochrome P450 gene of *P. restrictum*, with lower levels of similarity to the *geoA* gene of geosmin biosynthesis in *S. avermitilis* and other P450 type genes found in geosmin nonproducers (Fig. 1). In the past, PCR-based assays were successfully utilized to detect and quantify various fungal and bacterial species and their corresponding important metabolites. Atoui *et al.* (2007) utilized PCR to detect *Aspergillus carbonarius* in various grape samples. They further developed a qPCR technique to quantify *A. carbonarius* DNA and ultimately ochratoxin A in these samples (Atoui *et al.* 2007). A similar technique was developed by Marc-Auffret *et al.* (2011) for the quantification of geosmin-producing *Streptomyces spp.*, and hence geosmin. The technique of Marc-Auffret *et al.* (2011) is specific for monitoring geosmin produced by *Streptomyces spp.*, and not any other source such as fungi. Siddique *et al.* (2012), utilized a simple PCR approach, to amplify a fragment of cytochrome P450 gene and successfully differentiate geosmin-producing and nonproducing fungi. To develop a qPCR assay, the alignment data (Fig. 1) was utilized to design specific primers for the amplification and quantification of the *gpe1* gene. The primer pair presented similar properties to that of Siddique *et al.* (2012), and specifically amplified the *gpe1* gene in both the target geosmin-producing *Penicillium* species i.e. *P. expansum* and *P. restrictum* (Fig. 2). This primer pair was further utilized to quantify geosmin-producing fungal DNA (specifically *Penicillium spp.*, DNA) extracted from the available grape samples, through qPCR. The quantified DNA was ultimately correlated with the concentration of geosmin in these samples. This was achieved by plotting the DNA quantity against geosmin contents (directly measured by GC-MS) of 118 grape samples. We observed a strong, positive correlation between the presence of the fungus and the quantity of geosmin detected.

A technique standardized in 1995 and known as FPA has been utilized for identifying sample taste(s) and odour(s) (the technique is described in 'standard methods' <http://standardmethods.org/store/ProductView.cfm?ProductID=437>). In the USA, six laboratory and plant personnel were trained to perform the rating test for geosmin. They set an action level of 15 ng l⁻¹ geosmin, which is equivalent to a FPA intensity of 4 (Dietrich 2004). They mentioned that above this limit customer complaints will be likely to increase markedly. Analysing the correlation curve, which we have obtained from comparing genomic DNA concentration and geosmin quantity of 118 grape samples (Fig 4), we observe that a DNA content lower than 50 ng μl⁻¹ presumably correspond to geosmin quantity of <15 ng l⁻¹. This means that <50 ng μl⁻¹ of DNA could assure good security and corresponds to FPA intensity of <4. Our developed qPCR assay can efficiently quantify the amount of geosmin-producing fungal genomic DNA, which can be an indicator of geosmin in a given sample.

Based on the above outcome, we suggest that the amount of genomic DNA of geosmin producers correlates with the amount of geosmin. Hence, by monitoring the genomic DNA through our developed qPCR, the quantity of geosmin in a given sample can be estimated.

Acknowledgements

The authors are grateful to Dr Hannah Maple (IEU Metabolomics Manager, School of Social and Community Medicine, University of Bristol) and Dr Zafar (Department of Agriculture Chemistry, The University of Agricultural Peshawar, Pakistan) for their help in improving the linguistic quality of the manuscript.

Conflict of interest

No conflict of interest declared.

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