Detection of *Saccharopolyspora rectivirgula* by Quantitative Real-Time PCR

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The thermophilic actinomycete species *Saccharopolyspora rectivirgula* has been associated with the exogen allergic alveolitis (EAA). EAA is caused by the inhalation of high amounts of airborne spores that can be found for example in environments of agricultural production, compost facilities, mushroom cultivation rooms, or rooms with technical air moistening. Because of the medical relevance of *S. rectivirgula*, a reliable detection system is needed. Therefore, a quantitative real-time polymerase chain reaction (qPCR) primer system was designed, targeting the 16S rRNA gene of the type strain *S. rectivirgula* DSM 43747T and six other *S. rectivirgula* reference strains. Our investigation showed that *S. rectivirgula* presumably own four operons of the 16S rRNA gene, which has to be considered for estimation of cell equivalents. Furthermore, the DNA recovery efficiency from these strains was tested in combination with bioaerosol or material sample as well as the influence of non-target DNA to the recovery rate. Results showed a recovery DNA efficiency of 7–55%. The recovery rate of DNA in a mixture with non-target DNA resulted in ~87%. In summary, a high amplification efficiency using real-time PCR was found, for which estimated concentrations revealed cell numbers of 2.7 × 10^5 cells m^-2 in bioaerosol and 2.8 × 10^6 cells g^-1 fw^-1 in material samples from a duck house. The specificity of the new developed quantification system was shown by generation of two clone libraries from bioaerosol samples, from a duck house, and from a composting plant. Totally, the results clearly show the specificity and practicability of the established qPCR assay for detection of *S. rectivirgula*.

Keywords: bioaerosols; microbial exposure; real-time PCR; *Saccharopolyspora rectivirgula*; 16S rRNA gene

INTRODUCTION

*Saccharopolyspora rectivirgula* (Krassilnikov and Agre, 1964; Korn-Wendisch et al., 1989; Basonym: *Micropolyspora faeni*, Cross et al., 1968 and *Faenia rectivirgula* Kurup and Agre, 1983) is an aerobic, thermophilic, Gram-positive, and filamentous bacterial strains belonging to the phylum Actinobacteria. *Saccharopolyspora rectivirgula* produces short chains of spores both on substrates and on aerial mycelia. The substrate mycelium ranged between 0.5 and 0.8 µm, the aerial mycelium between 0.8 and 1.2 µm, and spores vary between 0.7 and 1.5 µm in diameter (Kurup and Agre, 1983).

*Saccharopolyspora rectivirgula* was first isolated from soil and/or moldy hay in parallel (Lacey, 1990) and is well described as one causative agent of exogenous allergic alveolitis (EAA, a type of hypersensitive pneumonitis, Corbaz et al., 1963; Krassilnikov and Agre, 1964). EAA is an inflammation of the alveoli caused by hypersensitivity to inhaled organic dusts or in detail, by the inhalation of high amounts of different allergens, here of airborne spores of *S. rectivirgula*. The development of an EAA is dependent on predisposition of individuals as well as the nature, intensity, and
duration of exposure. In Eastern Canada, e.g., *S. rectivirgula* was described to be most frequently responsible for ‘farmer’s lung disease’, the classic form of EAA (Cornier et al., 1985). However, high concentrations of *S. rectivirgula* spores were also found in compost facilities, mushroom cultivation, or rooms with technical air moistening (Pepys et al., 1963; Lacey and Crook, 1988; Kutzner and Kempf, 1996; Dannenberg and Driesel, 1999; Duchaine et al., 1999). Because of the clinical relevance, a reliable detection system for *S. rectivirgula* is needed. Current detection methods for *S. rectivirgula* e.g. at working places are often based on cultivation-based approaches. However, identification of *S. rectivirgula* is difficult, especially when environmental samples are analyzed (Duchaine et al., 1999). In addition, culture-based methods are time consuming and low in specificity and non-viable or dead bacterial cells, which can also cause allergic reactions, remain undetected. Hence, molecular approaches can be a useful alternative. Therefore, the aim of this study was the development of a quantitative real-time PCR (qPCR) assay for the specific detection of *S. rectivirgula* esp. in bioaerosols. Furthermore, influences on DNA extraction efficiencies should be analyzed to advert possibly underestimation of cell counts using molecular approach. Additionally, because quantification methods targeting the 16S rRNA gene possibly leading to an overestimation of analyzed cell counts, the amount of 16S rRNA gene copies in *S. rectivirgula* should be investigated.

**MATERIALS AND METHODS**

**Bacterial strains and environmental samples**

Testing the species-specific qPCR assay, we investigated seven *S. rectivirgula* strains obtained from the DSMZ (DSM 43747T, DSM 43113, DSM 43114, DSM 43371, DSM 43755, DSM 43169 and DSM 43163). Twelve other *Saccharopolyspora* strains (DSM 44350T, DSM 45019T, DSM 45119T, DSM 40517T, DSM 44575T, DSM 45244T, DSM 43463T, DSM 44795T, DSM 43856T, DSM 44771T, DSM 44324T and DSM 44065T) also obtained from the DSMZ were used for optimization of qPCR protocol. All strains were either grown on the medium M65 (http://www.dsmz.de) or tryptone soy agar.

Mature compost material was obtained from two composting plants in Germany (anonymous) and straw material was obtained from one duck house in Germany (anonymous). Bioaerosol samples from different composting plants were collected by IPA (Institute for Prevention and Occupational Medicine of the German Social Accident Insurance) using a personal sampling device as described earlier by Fallschissel et al. (2009). Bioaerosol samples from a duck house were taken by a stationary filtration system as described by Martin et al. (2009). Impacted cells were detached and homogenized from the employed polycarbonate filters (0.8 μm pore size, 37 mm in diameter, Whatman, Germany) into 10 ml NaCl 10.9% (w/v) using a stomacher (Stomacher 80 lab systems; Seward, London, UK) for 60 s and stored until usage at −20°C.

**Extraction of DNA from bacterial strains and environmental samples**

Genomic DNA from bacterial strains was extracted after disruption of cells by a 30-s bead-beating step (Precellys 24, Peqlab, Erlangen) with 1 g of 0.1 mm Zirconia beads (Carl Roth GmbH+Co, Karlsruhe) at maximum speed, with the GenElute™ Plant Genomic DNA Kit (Sigma) following the instructions of the manufacturer.

From the environmental samples, total DNA were extracted directly from 0.05 to 0.5 g material or from cells of 10 ml bioaerosol samples, which were concentrated by centrifugation (17 000 g) in a 2-ml reaction tube. The cell pellet was used for direct DNA extraction using the FastDNA®Spin Kit for soil (MP, Biomedicals) following the manufacturer’s instructions. A negative control for DNA extraction, containing only the solutions of the extraction kit, was carried out to examine the purity of the solution of the extraction kit. The extracted DNA was used for further qPCR and cloning analyses.

**Primer design**

The nucleotide sequences of primer Sac-86f and Sac-183R, specific for 16S rRNA sequence fragments from *S. rectivirgula* species, were designed using the freeware programme Primrose 2.17 (Ashelford et al., 2002), including the download of the current actual RDP database (http://rdp.cme.msu.edu/) as well as sequence information’s from all *S. rectivirgula* strains, mentioned above. The developed primers Sac-86f: 9′-TGTTGGTGTTAGATGT-3′ and Sac-183R: 9′-ACCATGCGGCAAAGTGCCT-3′ induce the amplification of a 16S rRNA gene fragment of ~100 bp. By submitting the nucleotide sequence to the PROBE MATCH algorithm of RDP (http://rdp.cme.msu.edu/index.jsp), the primer system initially was tested in silico for its specificity.

**Quantitative real-time polymerase chain reaction**

For preparation of quantification standards, fluorometric-quantified 16S rRNA PCR products (using
universal 16S rRNA primers, 27F/1492R, Lane, 1991), obtained from genomic S. rectivirgula (DSM 43747T) DNA, were employed. For each concentration, the cycle threshold (C_T) value was plotted against the log value of corresponding target number. The calibration curve was generated by the iQ™5 software. Consequently, initial target copy numbers in the environmental samples were calculated as described by Martin et al. (2010). After optimization, the resulting qPCR conditions were initial denaturation at 98°C for 4 min, denaturation at 98°C for 1 min, annealing at 59.6°C for 10 s, and extension at 72°C for 10 s. To proof the occurrence of primer dimers a step of 81°C for 10 s was added. The amplification was carried out at 50 cycles. The PCR was performed in a final volume of 20 μl, using the QuantiTect® SYBR® Green PCR Mix (Qiagen, Germany), with a primer concentration of 200 nM each primer in the iQ™5 Cycler (Biorad, Munich, Germany). For negative control, only SYBR® Green PCR Mix, primer solution and molecular grade water were analyzed. All samples, standards, and controls were analysed in triplicates.

16S rRNA operons: cloning analyses and southern hybridization of bacterial strains

To get detailed information about possibly differences in nucleotide sequences resulting from possibly multiple operons, 16S rRNA gene clone libraries were generated from all seven S. rectivirgula strains. Cloning analyses of the strains and subsequent sequencing of plasmid inserts were done by Agowa (Berlin, Germany) using the M13F primer (Invitrogen Corp., CA, USA).

Furthermore, the 16S rRNA operon copy number was estimated via southern hybridization according to the protocol from rrnDB database (http://ribosome.mmg.msu.edu/rrndb/about.php) and the digoxigenin (DIG)-High Prime Random Labeling and Detection Starter Kit II protocol (Roche, Molecular Biochemicals). First, we isolated genomic DNA from the S. rectivirgula type strain and digested the DNA with different restriction endonucleases (PstI, PvuII, SacI, XmiI, RsrII, Sall, Hind III, Fermentas). In the second step, the digested genomic DNA was separated via agarose gel electrophoresis. Subsequently, we transferred and immobilized the gel-separated genomic DNA to a (+)-charged nylon membrane (Roche, Molecular Biochemicals) and hybridized the membrane-bound genomic DNA with a DIG-labeled 16S rRNA gene probe. After specifically binding of the probe, the immunological detection takes place with an alkaline phosphate-conjugated antibody specific to the DIG moiety on the DNA probe. The hybridized DNA bands were detected with an alkaline phosphatase-activated chemiluminescent substrate.

**DNA extraction efficiency and recovery rate using qPCR**

The DNA extraction efficiency from S. rectivirgula as well as ‘S. rectivirgulas’ DNA recovery efficiency were tested by spiking experiments. Beside the isolation of DNA from pure culture, S. rectivirgula cells were added to bioaerosol and material samples and furthermore, S. rectivirgulas DNA was added to DNA that was isolated from environmental samples. In the first assay, equal amounts of S. rectivirgula cultures [0.008 g fresh water (f.w.)] from different ages (3d, 7d, and 14d) were employed per DNA extraction assay, either as pure culture or spiked to bioaerosol samples out of a duck house or material samples (litter) from the same duck house each in triplicates. Although it is difficult due to the formation of filaments by this species for a rough estimation of applied cell numbers, we presume the fresh weight of Escherichia coli (9.5 × 10⁻¹³ g f.w⁻¹) according to Madigan et al. (2001). Cell equivalents deployed in the assay were determined by calculation of 0.008 g divided by 9.5 × 10⁻¹³ g cells⁻¹, achieve an estimated amount of 8.42 × 10⁹ cell equivalents. DNA extractions were done using the FastDNA®Spin Kit for soil (MP, Biomedicals) following the manufacturer’s instructions. Amount of DNA was quantified fluorometrically (Qubit; Invitrogen). The amounts of S. rectivirgula cell equivalents were measured by real-time PCR approach (see above).

Values for S. rectivirgula originary present in the bioaerosol (2.7 × 10⁶ cell equivalents) and material sample (2.8 × 10⁶ cell equivalents) were considered by subtraction from values measured in the mixture with pure culture.

In the second approach, we investigated a potential inhibition of PCRs by non-target DNA. For this purpose, 1 μl of pure culture DNA (1 ng μl⁻¹, three stages of age) was mixed with 1 μl of bioaerosol DNA (0.5 ng μl⁻¹) each in triplicates. By real-time PCR assay from these mixtures, the 16S rRNA gene copy number of spiked S. rectivirgula DNA and the corresponding potential cell number were determined.

Cloning analyses of environmental samples and sequencing

Prior to quantitative analyses, the specificity of the developed qPCR system in environmental samples was investigated. Therefore, two positive PCR
products obtained from bioaerosol samples of (i) a duck house and (ii) a composting plant were analyzed by generation of two independent clone libraries (as described in Schäfer et al. 2010) and sequence analyses of plasmid inserts of 48 randomly chosen clones from each library. Cloning and sequencing analyses were done by Fraunhofer Institute (Aachen, Germany) using the M13F or M13R primer (Invitrogen Corp.).

Phylogenetic analyses

Similarity searches of all sequences out of all clone libraries against the NCBI database were carried out using BLAST search (http://www.ncbi.nlm.nih.gov/).

Multiple sequence alignment with type strains of the detected genera as well as genetic distance calculations (distance options according to the Kimura-2 model) of the data were also performed using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 4.

RESULTS

16S rRNA operons in S. rectivirgula

Sequence analyses of all S. rectivirgula strains and clone inserts revealed differences both between and within the strains of S. rectivirgula, which may be explained by different 16S rRNA operons. About a fourth of all investigated 16S rRNA insert sequences of each strain could not be assigned to any known genus.

Highest sequence similarity using BLAST® search was detected to one uncultured bacterium found in a compost pile. These sequences however form one distinct cluster with high internal sequence similarity (>99.4%), which shows a clear indication for an unknown 16S rRNA operon in S. rectivirgula.

Southern hybridization revealed the presents of 3–5 bands per lane depending on used enzymes. In four lanes, which mean the digestion by PstI, PvuII, SacI, RsrlI, four distinct bands were visible. Residual lanes showed ambiguous pattern with three and five bands [XmiI (5), Sall (3), Hind III (3)]. To estimate the equivalent cell number of S. rectivirgula, we primarily consider four 16S rRNA operons per S. rectivirgula genome. Based on 16S rRNA cloning analyses, however, we assume one operon of the 16S rRNA gene, which is not amplified by the new developed primer system. Therefore, finally, we calculated three operons per S. rectivirgula genome for qPCR analysis and calculation of cell equivalent units.

Quantitative real-time polymerase chain reaction

The analyses of the 16S rRNA fragment originating from the unexpected 16S rRNA operon showed that this fragment was not amplified (data not shown). Otherwise, a linear correlation ($r^2 = 0.99$) of Ct-values and corresponding target numbers was observed for concentrations between $10^3$ and $10^8$ targets $\mu l^{-1}$. The detection of S. rectivirgula, on the basis of <10^3 targets (35 cycles), was not possible because linear correlation failed. Whereas 16S rRNA genes from non-S. rectivirgula strains in general were not amplified, a weak unspecific gene amplification of Saccharopolyspora cebuensis could not be eliminated. Due to a very low amplification efficiency of S. cebuensis 16S rRNA gene, however, equal initial concentrations (1 ng $\mu l^{-1}$) of S. rectivirgula (DSM 43747T) and S. cebuensis resulted in clear different quantification of 6.4 $\times$ 10^6 versus 4 $\times$ 10^5 cells $\mu l^{-1}$, respectively (data not shown). Additionally, until now, S. cebuensis was only isolated from a Philippine sponge (Pimentel-Elardo et al., 2008) and seems to be not relevant in occupational environments with high exposure to airborne bacteria. The adequacy for the intended use of this PCR approach was indicated by melting curve analysis of PCR products and gel electrophoresis (bands showed the correct molecular size ~100 bp) of the amplicon (data not shown). Furthermore, the results revealed high amplification efficiency (~98%) of the 16S rRNA genes of all available strains of S. rectivirgula using the new designed primer system.

DNA extraction efficiency and recovery rate

Firstly, the results showed DNA extraction efficiency from pure cultures between ~7% in 7-day old cultures, 19.5% in 3 days, and 55% in 14-day old cultures (Table 1, Column 4). The recovery rate from spiking experiments depends on age of the spiked cultures. Generally, the recovery rate was successful and varied between 60 and 100% in spiking experiments, respectively (Table 1, Columns 5 and 6). A worse recovery efficiency of 20% in material sample was found in spiking experiments with cells of a 14-day old culture (Table 1, Column 5). The investigated potential inhibition of PCRs by non-target DNA revealed a recovery between 70 and 100% (Fig. 1).

Specificity of developed primer system

Cloning analyses of PCR products gained with the new Primer system, from bioaerosol samples, of a duck house and a composting plant revealed that all obtained sequences ($n = 96$, each clone library 48) were most closely related (>99%) to 16S rRNA
gene sequences from *S. rectivirgula*, verifying the adequacy of this PCR approach for the intended use.

**Application in environmental samples**

For testing the established qPCR protocol, we investigated bioaerosol and material samples from a composting plant and agricultural environment. Here, application of the method showed concentrations of *S. rectivirgula* between $2.7 \times 10^6$ and $1.0 \times 10^7$ estimated cell counts of *S. rectivirgula* in bioaerosols and between $2.0 \times 10^5$ and $4.5 \times 10^9$ cell counts in material samples. Extended cell numbers of *S. rectivirgula* of $4.5 \times 10^9$ cells g$^{-1}$ fw$^{-1}$ were detected in mature compost and up to $1.0 \times 10^7$ cells m$^{-3}$ in bioaerosol samples from composting plant (Table 2). Estimated cell numbers in straw material of a duck house amount $1.0 \times 10^7$ cells g$^{-1}$ fw$^{-1}$. Bioaerosol samples out of the duck houses showed estimated cell numbers between 2.7 and $9.2 \times 10^5$ cells m$^{-3}$.

**DISCUSSION**

Current detection methods for *S. rectivirgula* based on its cultivation. Therefore, it is hardly to compare the few existing investigations with investigations of the present study. However, our results tend to result in clear higher *S. rectivirgula* concentrations. Lacey and Crook (1988), for example, detected *S. rectivirgula* together with *Thermoactinomyces* spp. in a concentration of $1.5 \times 10^5$ cfu m$^{-3}$ air in mushroom farms. And Ranalli *et al.* (1999) detected up to $5.2 \times 10^3$ cfu m$^{-3}$ air thermophilic *Actinomycetes* in diary barns, where they also detected similar amounts of thermophilic *Actinomycetes* in hay samples ($3.3 \times 10^3$ g$^{-1}$). In contrast within the present study, the estimated amount of *S. rectivirgula* cells using qPCR assay was $1.0 \times 10^7$ cells per g$^{-1}$ straw material and $2.8 \times 10^6$ cells per m$^{-3}$ in bioaerosol samples from duck houses (Table 2). In general, this observation is in agreement with detected differences between culture-based and real-time

<table>
<thead>
<tr>
<th>Culture age (d)</th>
<th>Amount of culture in DNA extraction approach</th>
<th>Estimated equivalent cell number</th>
<th>Cell number detected by qPCR from pure culture (recovery %) ($n = 3$)</th>
<th>Cell number in straw material samples (recovery %, related to the pure culture recovery)</th>
<th>Cell number in bioaerosol samples (recovery %, related to the pure culture recovery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.008 g</td>
<td>$8.42 \times 10^9$</td>
<td>$1.60 \times 10^9 \pm 2.42 \times 10^8$ (19.4%)</td>
<td>$1.24 \times 10^8 \pm 2.07 \times 10^8$ (77.6%)</td>
<td>$1.47 \times 10^8 \pm 4.65 \times 10^8$ (91.8%)</td>
</tr>
<tr>
<td>7</td>
<td>0.008 g</td>
<td>$8.42 \times 10^9$</td>
<td>$5.67 \times 10^8 \pm 7.4 \times 10^7$ (6.7%)</td>
<td>$5.94 \times 10^8 \pm 1.14 \times 10^8$ (100%)</td>
<td>$5.61 \times 10^8 \pm 8.29 \times 10^7$ (99.0%)</td>
</tr>
<tr>
<td>14</td>
<td>0.008 g</td>
<td>$8.42 \times 10^9$</td>
<td>$4.65 \times 10^9 \pm 3.19 \times 10^8$ (55.3%)</td>
<td>$9.28 \times 10^8 \pm 1.55 \times 10^8$ (19.9%)</td>
<td>$2.80 \times 10^9 \pm 3.61 \times 10^7$ (60.2%)</td>
</tr>
</tbody>
</table>

**Fig. 1.** Recovery of target DNA (1 ng µl$^{-1}$) from pure cultures (PC) with an age of 3d, 7d, and 14d in a mixture with DNA extracted from bioaerosols (BES). Values are means of $n = 9 \pm$ SD.
PCR-based quantifications (Fallschissel et al., 2009). Reasons resulting in underestimation of the concentration using culture-based methods are manifold, e.g. microbes, which are viable but not culturable. These bacteria are viable but their metabolic activity is very low and therefore reproduction failed (Staley and Konopka, 1985; Heidelberg et al., 1997; Zinder and Salyers, 2001; Oliver, 2005). Furthermore, desiccation and/or sampling procedure could result in loss of cultivability. Especially, an air sampling by personal carried devices during a whole working day, which is the basic requirement for a precise exposure measurement, is not feasible in cultivation-based approaches because many bacteria are not resistant to sampling stress and desiccation (Marthi et al., 1990; Potts 1994; Durand et al., 2002). Here, qPCR permit the analysis of bioaerosol samples that were collected over a period of several hours.

But even for these DNA-based methods, bias has been shown (Chandler, 1998; Polz and Cavanaugh, 1998; Acinas et al., 2005) and methods generally should be used with caution. Therefore, our investigations should mention the limitations according to the DNA extraction efficiency and/or recovery. In particular, the loss of genomic DNA (up to 93%, Table 1, Column 4) in our study at the extraction procedure was comparable to those found earlier (Mumy and Findlay, 2004; Einen et al., 2008; Fallschissel et al., 2009). In this context, an impact on DNA extraction efficiency was detected according to the age of the culture (Table 1). Here, may be (time of) sporation and fragmentation of the culture influences DNA extraction efficiency because of the difficulty of DNA extraction from spores. These findings basically show the difficulty in exact quantification of bacteria in environmental samples. However, in comparison to culture-based analyses of thermophilic Actinomycetes, qPCR show a clear improvement because a species-specific cultivation and a morphological differentiation to other thermophilic Bacillus spp. and Geobacillus spp. is hardly possible (Albrecht and Kämpfer, 2006).

In consideration of the estimated loss of DNA by the isolation method, the real concentrations of S. rectivirgula in investigated samples seem to be 2- to 10-fold higher. A potential inhibition of PCR by co-extracted inhibitory substances or non-target DNA seems negligible because the recovery rate varied between 70 and 100% (Fig. 1).

Basically, a cell number can be deduced from PCR-based gene quantification. For this, the knowledge about number of target sequences for primer hybridization is most relevant. The primer system was used in the present study targeting the 16S rRNA gene sequence, which possibly occur multiple (Acinas et al., 2004). Prior to quantification in

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total cell count</th>
<th>Estimated cell counts ( S. \text{ rectivirgula (g fw)} ) or (m(^3))</th>
<th>Share compared to the total cell count in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material: straw</td>
<td>n.d.</td>
<td>(1.0 \times 10^7)</td>
<td>n.d.</td>
</tr>
<tr>
<td>ES-6.1-1</td>
<td>n.d.</td>
<td>(2.8 \times 10^6)</td>
<td>n.d.</td>
</tr>
<tr>
<td>ES-6.4-1</td>
<td>n.d.</td>
<td>(1.3 \times 10^6)</td>
<td>n.d.</td>
</tr>
<tr>
<td>ES-6.7-1</td>
<td>n.d.</td>
<td>(2.0 \times 10^5)</td>
<td>n.d.</td>
</tr>
<tr>
<td>Compost Du</td>
<td>n.d.</td>
<td>(4.5 \times 10^6)</td>
<td>n.d.</td>
</tr>
<tr>
<td>Compost Dra</td>
<td>n.d.</td>
<td>(7.0 \times 10^4)</td>
<td>n.d.</td>
</tr>
<tr>
<td>Bioaerosol BES 071010-8/17</td>
<td>n.d.</td>
<td>(4.00 \times 10^7)</td>
<td>2.3</td>
</tr>
<tr>
<td>BES 6.1-1-3</td>
<td>(3.13 \times 10^7)</td>
<td>(2.7 \times 10^5)</td>
<td>0.85</td>
</tr>
<tr>
<td>BC 090806-24-Witz</td>
<td>(1.60 \times 10^8)</td>
<td>(5.06 \times 10^5)</td>
<td>0.3</td>
</tr>
<tr>
<td>BC 090930-31-Gand</td>
<td>(1.10 \times 10^8)</td>
<td>(2.35 \times 10^6)</td>
<td>2.1</td>
</tr>
<tr>
<td>BC 090916-16-Bohm</td>
<td>(3.30 \times 10^7)</td>
<td>(1.01 \times 10^7)</td>
<td>30.7</td>
</tr>
<tr>
<td>BC 090930-30-Gand</td>
<td>(1.20 \times 10^7)</td>
<td>(1.29 \times 10^6)</td>
<td>10.8</td>
</tr>
<tr>
<td>BC 090923-26-Nieh</td>
<td>(2.30 \times 10^6)</td>
<td>(1.05 \times 10^6)</td>
<td>45.6</td>
</tr>
<tr>
<td>BC 090707-29 Hof</td>
<td>(6.00 \times 10^6)</td>
<td>(4.26 \times 10^5)</td>
<td>7.0</td>
</tr>
<tr>
<td>BC 090923-27 Nieh</td>
<td>(8.50 \times 10^6)</td>
<td>(5.78 \times 10^5)</td>
<td>6.7</td>
</tr>
<tr>
<td>BC 090909-39 Lemgo</td>
<td>(3.90 \times 10^6)</td>
<td>(5.86 \times 10^5)</td>
<td>15.3</td>
</tr>
</tbody>
</table>

n.d., not detected; ES, straw from different duck houses including faeces; BES, bioaerosol from a duck house; BC, bioaerosol from a compost plant; total cell count was measured by DAPI (4′,6-diamidino-2-phenylindol) staining according to Martin et al. (2010), estimated cell counts were detected by qPCR, values of qPCR are means of triplicates.
environmental samples, the 16S rRNA gene copy number was examined by southern hybridization. Depending on employed restriction enzymes, the pattern revealed operon numbers between three and five (mostly four bands). Pattern with three bands may result from crude separation of DNA within the agarose gel. Because cloning analyses supporting the hypotheses of four operons (about a fourth of all investigated 16S rRNA insert sequences of all strains could not be assigned to the original sequence) within the present study, calculations of cell counts were made in presumption that *S. rectivirgula* exhibit four 16S rRNA operons. Finally, we calculated with three operons per *S. rectivirgula* genome in qPCR analysis and calculation of cell equivalent units because amplification of the untypical sequence failed. If 16S rRNA of *S. rectivirgula* is analyzed without a previous cloning analyses, this unexpected sequence was detectable as background sequence in chromatograms of 16S rRNA gene sequence analysis of all investigated *S. rectivirgula* strains (data not shown).

For crude calculation of 16S rRNA genes in environmental samples, a high specificity of the employed primer system is necessary because of high amounts of 16S rRNA gene sequences from different bacteria. Therefore, the specificity of the developed primer system was evidenced by cloning analyses from environmental samples, whereas all analyzed sequences showed high sequence similarities (>99.4%) to sequences from *S. rectivirgula*.

**CONCLUSIONS**

qPCR generally seems to be a potential method for the species or genus-specific quantification in bioaerosols (Makino et al., 2001; Makino and Cheun 2003; Zeng et al., 2006; Cayer et al., 2007; Dutill et al., 2007; Oppliger et al., 2008, Fallschissel et al., 2009, Martin et al., 2010) and presumably, this method is suitable for standardization in occupational exposure measurements in future. With this study, we extend the currently existing real-time PCR approaches, with a quantification protocol for the detection of airborne *S. rectivirgula*, a non-infectious but a well-known causative of extrinsic allergic alveolitis (synonym: farmer’s lung disease).

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