International Center For Science & high Technology and Environmental Sciences
چهارمین همایش ملی بیوتکنولوژی جمهوری اسلامی ایران
کرمان مردادماه ۱۳۸٤
The 4th National Biotechnology congress
Islamic Republic of Iran
Kerman august 2005

A combination of conventional bacterial isolation and colony PCR technique for detection of *Ornythobacterium rhinotracheale* (ORT) in poultry

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Abstract

Ornythobacterium rhinotracheale (ORT) is an emerging bacterial pathogen in poultry industry. The disease is acute highly contagious, affecting chickens and turkeys. The clinical signs, duration of the disease and mortality are extremely variable and are influenced by suboptimal environmental condition. Due to the difficulties in isolation and biochemical characterization of the bacteria, development of a fast and reliable method for diagnosis of the disease and characterization of the isolate is necessary. A combination of conventional bacterial isolation technique and colony PCR method were developed as a diagnostic tool for diagnosis of ORT infection in poultry.

Key word: *Ornythobacterium rhinotracheale*, ORT, colony PCR and molecular detection

INTRODUCTION

Ornythobacterium rhinotracheale (ORT) infection is an acute highly contagious disease of chickens and turkeys. The organism has been isolated from several bird species with respiratory infections in different parts of the world. The clinical presentation, duration of the disease and mortality are extremely variable and are influenced by suboptimal environmental condition such as mismanagement, poor ventilation, overcrowding, , poor litter conditions, poor hygiene, high level of ammonia, low level of oxygen, particularly in fast growing strains of broiler, concurrent viral and bacterial infections and the state of immuncompetance and secondary infection. It has been shown that various respiratory viruses, such as turkey rhinotracheitis virus (TRT) and Newcastle disease virus (ND), have a triggering effect on the outcome of *O. rhinotracheale* infections. Initial symptoms are nasal discharge, sneezing coughing and sinusitis followed in some cases by severe respiratory distress, dyspnoea, prostration and mortality. A reduction in feed consumption and water intake may accompany the symptoms. The gross lesions may include rhinitis, tracheitis, uni- or bilateral pneumonia with a foamy fibropurulent white exudates. Pericarditis, airsacculitis, peritonitis and enteritis may also be detected. Therapeutic treatment of the disease has been found to be difficult and sometimes even impossible because many *O. rhinotracheale* strains are resistant to the regularly used antibiotics. Furthermore, economic losses, due to ORT infections are hard to be estimated, as the disease is often just slumbering with increased mortality rates of 2 - 10% and growth retardation which is difficult to be determined. Only in older turkeys losses are dramatic with mortality rates up to 50% in a few days.

MATERIALS AND METHODS

Clinical and Gross pathology examination. The clinical and post mortem examination of the cases were performed in the poultry disease section of the school of Vet Med of Ferdowsi

university of Mashhad or a private professional poultry disease clinic (The first author private clinic).

Isolation of bacteria. For isolation of *O. rhinotracheale* swabs from affected organs (air sacs, lungs and tracheae) were taken and inoculated onto sheep blood agar with10 µg/ml gentamicin as a growth inhibitor of other bacteria. The agar plates were incubated at 37°C in an atmosphere containing 5-10% CO2 for at least 72 hours. Each day the agar plates were checked for suspicious
colonies. Upon observation, these colonies were subcultured under the same conditions. All isolates were tested biochemically for the presence of oxidase and the absence of catalase.

Nucleic acid extraction. In initial experiments the genomic DNA of bacterial colonies were extracted from a 500 uL volume of the bacterial homogenates using phenol-chloroform extraction methods as described in the literature. The DNA were suspended in 30 ul TE buffer, and quality of the DNA was examined . by agarose gel electrophoresed through a 1% agarose gel.

Bacterial Colonies. Following the establishment of PCR assay, a new PCR procedure were set up to omit the DNA extraction step. Any positive suspicious colonies were picked by a tooth pick and processed without DNA extraction.

PCR assay. PCR was performed using a primer pairs designed based on the published literature, with some modification. These primers were selected to amplify a 784 bp fragment of the 16S rRNA gene of *O. rhinotracheale*. The modified primers were examined against genomic databases using blast N program. The typical PCR reactions were carried out in a 25-µl volume consisted of 1× PCR buffer, 200 µM deoxynucleoside triphosphate,0.5 µM of each primer, 1.5 mM MgCl2, 2 µl of DNA template, one unit Taq DNA polymerase. Ultrapure water was added to a total volume of 25 µl. The PCR reactions were performed on a Bio-Rad thermocycler at 93°C for 30 s, 55°C for 45 s, and 72°C for 2 min for 35 cycles. An initial denaturation of 5 min at 93°C and a final extension of 10 min at 72°C were also included in the beginning and the end of PCR cycles, respectively. The PCR products were electrophored in a 1.5% agarose gel pre-stained with ethidium bromide, and visualized by a transilluminator under ultraviolet light.

Result

Clinical and Gross pathology examination. Post mortem examination of the broiler chickens demonstrated a foamy, yellow-white, “yogurt like” exudate in the air sacs, predominantly in the abdominal air sacs and sometimes accompanied with a unilateral or bilateral purulent pneumonia, and/or exsudate in the trachea.
Isolation of bacteria. Under the circumstances of culture described, *O.rhinotracheale* developed small, grey to grey-white colonies, and always with a distinct odor, similar to the odor of butyric acid. Upon primary isolation, most *O. rhinotracheale* cultures showed great differences in the colony size from 1 to 3 mm after 48 h of incubation. When the primary cultures were subcultured, the colony size became more uniform.

PCR assay. A 784 bp amplicon was observed in the PCR with all *O. rhinotracheale* isolates. No amplification was observed in negative controls, including *E. coli* control samples.

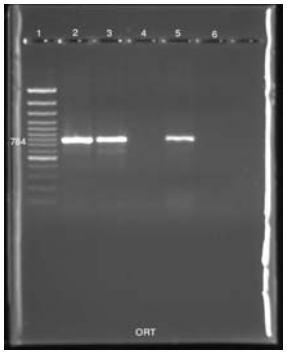

Figure 1:
Line 1: Molecular size marker 100bp
Line 2: Positive sample
Line 3: Positive sample
Line 4: Negative sample
Line 5: Positive control
Line 6: Negative control

DISCUSSION:

One of the main limitation of the ORT culture is the difficulty in isolation and biochemical characterization of the bacteria, as especially *O.rhinotracheale* is often overgrown by other bacteria. Furthermore, the process of diagnosis is time-consuming. Therefore, development of a fast and reliable method for diagnosis of the disease and characterization of the isolate is an absolute necessity. All isolates tested were positive for oxidase, and negative for catalase tests. The PCR assays employing both ORT DNA and ORT colonies as the template, worked equally and in a perfect manner. It is recommended, until the completion of validity tests, the procedure employed with the care. This PCR might also be suitable for the demonstration of *O. rhinotracheale* in e.g. eggs, faeces, dust- or tissue samples.
It has been reported that the success in direct PCR amplification of ORT colonies depend on the use of fresh cultures. The sensitivity limit of the direct PCR in those experiments has been reported to be a figure between 180 and 4500 single bacteria. In that report, it has been claimed, If cultures are allowed to grow for longer than 48 hours, the PCR would fail in most cases but it was not the case for our experiments, although the number of samples tested were limited. This paper describe a combination of conventional bacterial isolation technique and a colony PCR assay for molecular detection of *O. rhinotracheale* in poultry.

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