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Fadhli Nanda Putra, Prof. Dr. drh. A.E.T.H. Wahyuni, M.Si.; Dr. drh. Bambang Sutrisno, M.P.
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Karakterisasi Molekuler *Avibacterium paragallinarum* dari Sampel Sinus Infraorbitalis Ayam Petelur yang Menunjukkan Gejala *Infectious Coryza /* Snot

Fadhli Nanda Putra
21/485516/PKH/00769

Intisari

Infectious coryza / snot merupakan penyakit unggas yang sangat merugikan peternakan ayam petelur yang disebabkan oleh bakteri *Avibacterium paragallinarum.* Berbagai metode diagnostik telah dikembangkan untuk mendeteksi penyakit ini, termasuk isolasi dan identifikasi melalui kultur bakteri dan uji biokemis. Isolasi dan identifikasi secara konvensional masih relatif sulit untuk dilakukan karena karakter bakteri membutuhkan faktor pertumbuhan tertentu. Penelitian ini bertujuan untuk melakukan isolasi dan identifikasi *A. paragallinarum* secara konvesional melalui kultur bakteri dan uji biokemis serta karakterisasi molekuler *A. paragallinarum* dari sampel klinis sinus infraorbitalis. Sampel sinus infraorbitalis dilakukan isolasi bakteri menggunakan plat agar cokelat (PAC) dan diinkubasi secara mikroaerofilik selama 24 jam pada suhu 37^{o}C. Koloni dugaan dilakukan konfirmasi pengecatan Gram untuk melihat morfologi sel bakteri. Bakteri dilakukan proses pemurnian dan di kultur pada media agar miring cokelat. Biak murni dilakukan identifikasi dengan melalui uji biokemis, seperti: katalase, oksidase, urease, indol, fermentasi karbohidrat (glukosa, laktosa, sukrosa, maltose, dan mannitol). Sampel yang sama dilakukan deteksi molekuler gen *pyr*G secara parsial (285 bp) melalui primer HPG-2 (target amplikon 500 bp) dengan *polymerase chain reaction* (PCR) dan sekuensing. Hasil sekuensing dilakukan analisis bioinformatika meliputi pemotongan, penyejajaran nukleotida, prediksi asam amino, dan konstruksi filogenetik. Hasil penelitian menunjukkan 15 sampel terisolasi dan teridentifikasi *A. paragallinarum* melalui uji biokemis. Empat dari 15 sampel (26,6%) terdeteksi *A. paragallinarum* menggunakan metode PCR dengan gen target HPG-2 (500 bp). Sekuen nukleotida dari 4 sampel positif PCR melalui *basic local alignment search tool* (BLAST) dengan data sekuen yang terdapat pada *National Center for Biotechnology Information* (NCBI) menunjukkan persen identik 98,64%-100% *A. paragallinarum*. Analisis parsial gen *pyr*G melalui konstruksi filogenetik nukleotida dan asam amino menunjukkan *A. paragallinarum* yang terdeteksi berada pada satu klaster yang sama, terpisah dari klaster *Pasteurella multocida, Avibacterium sp.,* dan *Rodentibacter pneumotropicus.* Analisis parsial gen *pyr*G pada sampel LP9 menunjukkan perubahan susunan nukleotida pada empat titik, yaitu: T-A pada urutan 1373, G-T pada urutan 1379, T-G pada urutan 1381 serta G-T pada ururan 1382 tanpa adanya perubahan dalam translasi ke asam amino. Hasil penelitian disimpulkan bahwa 15 sampel terisolasi dan teridentifikasi *A. paragallinarum* melalui uji biokemis. Kesimpulan berdasarkan karakterisasi molekuler menunjukkan 4 sampel teridentifikasi *A. paragallinarum* dengan adanya *silent mutation* pada gen *pyr*G pada sampel LP9. Sekuen parsial *pyr*G pada sampel LT-12, LT-7, dan V5 identik dengan strain MODESTO sebagai strain referen.

Kata kunci: *Infectious coryza*, snot, *Avibacterium paragallinarum*, molekuler, PCR, HPG-2.

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Molecular Characterization of *Avibacterium paragallinarum* from Infraorbital Sinus Samples of Laying Hens with Infectious Coryza Symptoms

Fadhli Nanda Putra
21/485516/PKH/00769

Abstract

Infectious coryza / snot is a poultry disease that is very detrimental to laying hens farms caused by the bacterium *Avibacterium paragallinarum*. Various diagnostic methods have been developed to detect this disease, including isolation and identification through bacterial culture and biochemical tests. Conventional isolation and identification are still relatively difficult because the character of the bacteria requires certain growth factors. This study aims to isolate and identify *A. paragallinarum* conventionally through bacterial culture and biochemical tests as well as molecular characterization of *A. paragallinarum* from clinical samples of infraorbital sinus. Infraorbital sinus samples were isolated by bacteria using a chocolate agar plate (PAC) and incubated microaerophilically for 24 hours at 37^{o}C. Presumed colonies were confirmed by Gram staining to see the morphology of the bacterial cells. The bacteria were purified and cultured on chocolate oblique agar media. Pure cultures were identified by means of biochemical tests, such as: catalase, oxidase, urease, indole, and carbohydrate fermentation test (glucose, lactose, sucrose, maltose, and mannitol). Molecular detection of the *pyr*G gene (285 bp) was carried out for the same sample using HPG-2 primer (500 bp amplicon target) by polymerase chain reaction (PCR) and sequencing. The sequencing results were subjected to bioinformatics analysis including trimming, nucleotide alignment, amino acid prediction, and phylogenetic construction. The results showed that 15 samples were isolated and identified as *A. paragallinarum* through biochemical tests. Four of the 15 samples (26.6%) detected *A. paragallinarum* using the PCR method with the target gene HPG-2 (500 bp). Nucleotide sequences from 4 PCR positive samples using the basic local alignment search tool (BLAST) with sequence data available at the National Center for Biotechnology Information (NCBI) show a 98.64-100% identical percentage of *A. paragallinarum*. Partial analysis of the *pyr*G gene through nucleotide and amino acid phylogenetic construction showed that the detected *A. paragallinarum* was in the same cluster, separate from the *Pasteurella multocida*, *Avibacterium sp*., and *Rodentibacter pneumotropicus* clusters. Partial analysis of the *pyr*G gene in the LP9 sample showed 4 SNPs (T-A at sequence 1373, G-T at sequence 1379, T-G at sequence 1381 and G-T at sequence 1382) without any change in translation to amino acids. The results of the study concluded that 15 samples were isolated and identified as *A. paragallinarum* through biochemical tests. The conclusion based on molecular characterization showed that 4 samples identified *A. paragallinarum* with silent mutations in the *pyr*G gene in LP9 samples. The sequence of partial *pyr*G gene in LT-12, LT-7, and V5 samples was identical to strain MODESTO as Reference strain.

Keywords: Infectious coryza, snot, *Avibacterium paragallinarum*, molecular, PCR