Isolasi, karakterisasi dan pemanfaatan bakteriofaga untuk identifikasi escherichia.coli O157:H7 = Isolation characterization and utilization of bacteriophage for the identification of escherichia coli O157: H7

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Abstrak

Latar Belakang: E.coli O157:H7 merupakan salah satu bakteri foodborne disease yang menyebabkan tingkat morbiditas dan mortalitas yang sangat tinggi pada manusia. Reservoar utama bakteri adalah ternak sapi. Beberapa metode telah digunakan untuk mendeteksi keberadaan E.coli O157:H7 di Indonesia namun belum ada metode identifikasi spesifik yang sekaligus dapat dimanfaatkan sebagai bahan alami dalam pengendalian E.coli O157:H7. Bakteriofaga faga adalah virus yang menginfeksi secara spesifik dan mampu melisiskan bakteri. Penelitian ini bertujuan untuk mencari faga isolat Indonesia yang akan dimanfaatkan untuk identifikasi yang spesifik terhadap E.coli O157:H7.

Metode: Sebanyak 60 isolat lokal E.coli O157:H7 yang telah dikarakterisasi secara biokemik, serologik antiserum hiperimun dan komersial dan molekuler Multiplex-PCR digunakan pada penelitian ini. Sampel untuk isolasi faga diambil dari air sungai, air sumur, limbah Rumah Potong Ayam, feses sapi, limbah dan air di peternakan sapi di wilayah Yogyakarta, Bogor, Cianjur dan Bandung. Isolat faga diuji spesifisitasnya terhadap E.coli O157:H7 dan dikarakterisasi secara molekuler PCR dan sekuensing dan pengamatan morfologi dengan Transsmission Electron Microscope. Faga yang spesifik terhadap E.coli O157:H7 digunakan untuk phagetyping terhadap 60 isolat lokal E.coli O157:H7.

Hasil: Penelitian tampak bahwa isolat lokal E.coli O157:H7 mempunyai sifat biokemik yang bervariasi yaitu 3,33 2/60 bersifat tipikal SOR-,GUD- dan 96,67 mempunyai variasi fenotipik SOR-,GUD dan SOR ,GUD . Hasil serotyping dengan antiserum hiperimun tampak 100 bereaksi positif aglutinasi sedang dengan antiserum komersial latex O157 hanya isolat yang bersifat SOR- yang menunjukkan reaksi positif 31,67 . Semua isolat lokal E.coli O157:H7 tampak mempunyai gen spesifik penyandi faktor virulensi yaitu rfbE LPS O157 , fliCh7 flagella H7 , eaeA intimin , hlyA haemolysin , stx 1 Shiga toxin 1 dan stx 2 Shiga toxin 2 . Sebanyak 22 faga telah diisolasi dari 187 sampel dan diperoleh 10 isolat yang bersifat spesifik terhadap E.coli O157:H7. Selanjutnya dibedakan ke dalam 3 tipe yaitu T4, HK dan Lambda. FagaT4 adalah famili Myoviridae, faga HK dan Lambda adalah famili Siphoviridae. Faga T4 isolat Indonesia paling banyak mengidentifikasi isolat lokal E.coli O157:H7 yaitu 56,67 34/60 , faga HK mengidentifikasi 8.33 5/60 isolat lokal E.coli O157:H7 dan faga lambda hanya mengidentifikasi 3.33 2/60 isolat lokal E.coli O157:H7.

Kesimpulan: Faga isolat lokal Indonesia T4, HK dan Lambda dapat digunakan untuk mengindentifikasi isolat E.coli O157:H7 asal Indonesia.

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Background E. coli O157 H7 is one of foodborne disease bacteria which causes the high morbidity and mortality in humans. The main reservoir of this bacterial strain is cattle. Several methods have been used to detect the existence of E. coli O157 H7 in Indonesia but there is no specific identification method that can

also be used as a natural agent to control E. coli O157 H7. Bacteriophage phage is a virus which infects specifically and is able to lyse bacteria. This study aimed to explore the Indonesian phage isolates which would be used for the specific identification of E. coli O157 H7.

Method Sixty local isolates of E. coli O157 H7 characterized through biochemical, serological hyper immune antiserum and commercial and molecular Multiplex PCR method were used in this study. Samples for the phage isolation were retrieved from water in the river, water in the well, chicken slaughter house waste, cow feces, waste and water at cattle farms in Yogyakarta, Bogor, Cianjur and Bandung. Phage isolates were tested their specificity to E. coli O157 H7 and characterized by molecular method PCR and sequencing and morphological observation with Transmission Electron Microscope. Specific phages to E. coli O157 H7 were used for phage typing on 60 local isolates of E. coli O157 H7.Results This study showed that local isolates of E. coli O157 H7 had varied biochemical characteristics 3.33 2 60 were typical SOR , GUD and 96.67 had phenotypic variations SOR , GUD and SOR , GUD.

Results of serotyping with hyper immune antiserum 100 reacted as positive agglutination, while with O157 commercial latex antiserum, only isolates having SOR characteristic showed positive reaction 31.67 . All local isolates of E. coli O157 H7 had specific virulence factor encoding genes namely rfbE LPS O157 , fliCh7 flagella H7 , eaeA intimin , hlyA haemolysin , stx 1 Shiga toxin 1 and stx 2 Shiga toxin 2 . Twenty two phages were isolated from 187 samples and obtained 10 isolates that specifically characterize to E. coli O157 H7. Further distinguished into three types T4, HK and Lambda. The T4 phage was family Myoviridae, HK and Lambda phage were family Siphoviridae. Indonesian isolates of T4 phage identified local isolates of E. coli O157 H7 at the highest percentage that was 56.67 34 60 , HK phage identified 8.33 5 60 local isolates of E. coli O157 H7 and lambda phage only identified 3.33 2 60 local isolates of E. coli O157 H7.

Conclusion Phages of Indonesian local isolates T4, HK and Lambda could be used to identify E. coli O157 H7 isolates from Indonesia.