Development of bioassay for pathogenecity testing of Ureaplasma urealyticum as part of host-pathogen communication

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Abstrak

Bioesei Ureaplasma urealyticum perlu dikembangkan sebagai teknik untuk mendeteksi dan mendeterminasi faktor patogen bakteri, sehingga patogenesis penyakit yang disebabkan infeksi ureaplasma dapat dipahami dengan baik. Tahap pertama penelitian ini adalah mengembangkan metoda untuk kultivasi dan verifikasi ureaplasma; kultivasi pada media padat maupun cair dapat digunakan untuk mendeteksi ureaplasma dalam sampel yang diperiksa. Meskipun demikian teknik PCR yang mengamplifikasi DNA bakteri menggunakan primer yang meliputi gen urease (ure) lebih memberikan konfirmasi yang akurat tentang keberadaan U. urealyticum. Untuk memahami patogenisitas ureaplasma, uji expresi gen menggunakan gen reporter yang berfungsi sebagai penanda (marker) expresi dapat digunakan untuk membuktikan aktifitas gen yang membawa sifat patogen bakteri. Gen iceC dapat digunakan sebagai gen reporter untuk mendeterminasi patogenisitas ureaplasma karena gen ini mempunyai keunggulan sangat sensitif, mudah dideteksi dan diukur aktifitasnya menggunakan uji pembekuan es (ice nucleation assay). Patogenesis penyakit juga dapat dipantau dengan uji mutagenesis in vitro, dimana gen kompeten untuk patogenisitas bakteri diinaktifkan dengan menginsersikan gen penanda (marker) dalam proses transformasi bakteri. IgA1 protease merupakan enzim ureaplasma yang menentukan patogenisitas dan diperlukan untuk kolonisasi bakteri pada situs infeksi, sehingga identifikasi gen iga dan uji aktifitas IgA1 protease juga sangat menunjang pemahaman tentang patogenesis penyakit yang disebabkan infeksi ureaplasma. Dalam penelitian ini gen iga putatif Mycoplasma genitalium digunakan sebagai acuan untuk melacak gen iga U. urealyticum. Amplifikasi DNA ureaplasma dengan PCR menggunakan primer yang didesain kompatibel dengan gen iga putatif M. genitalium dilanjutkan sikuensing DNA, membuktikan adanya homologi sikuens nukleotida 100% seperti yang terekam pada data acuan (referensi). IgA1 protease U. urealyticum adalah enzim seluler yang tidak disekresikan; ini dibuktikan dengan aktifitas enzim yang terdeteksi didalam sel, bukan di media kultur. IgA1 protease telah terbukti merupakan protein integral membran sel dan digunakan untuk merusak IgA dipermukaan mukosa jaringan sehingga memungkinkan bakteri untuk mengkolonisasi mukosa dan menginduksi patogenesis penyakit. Penemuan ini mempunyai implikasi luas pada penanganan penyakit yang meliputi diagnosis dan terapi infeksi ureaplasma. (Med J Indones 2005; 14: 204-14)

<hr><i>Bioassay of Ureaplasma urealyticum is necessary for detection as well as determination of pathogenic factors in order to understand the pathogenesis of diseases associate with ureaplasma infection. Cultivation and verification of ureaplasma is the first step of this study in the purpose of discovering sensitive method for ureaplasma detection. Cultivation of ureaplasma either in liquid or in solid media are able to detect the existence of ureaplasma in samples analyzed. However, application of PCR using specific primers to be compatible with urease gene (ure) would confirm the presence of ureaplasma. The pathogenicity of ureaplasma is potentially monitored using reporter gene as a marker for gene expression. IceC was chosen as reporter gene for ureaplasma pathogenic determination as the gene has great sensitivity, easily detectable and quantitated in simple method of ice nucleation assay. Transposon 916 (Tn916) was

selected as a vector for iceC gene to transform ureaplasma. The application of recombinant Tn916-iceC which is considered as pUI, allow detection of ureaplasma activities when transform ureaplasma is tested by ice nucleation assay. It was expected that ureaplasma transformation is the manifestation of mutagenesis which interfere genes responsible for bacterial pathogenicity, in order pathogenesis of bacterial infection to be analyzed accurately. IgA1 protease is considered to be an important factor for ureaplasma pathogenicity as the enzyme is required for successful colonization. Identification of iga gene and determination of IgA1 protease activity are important for understanding the pathogenesis of ureaplasma infection. Putative iga gene of Mycoplasma genitalium was used as a reference to identify the presence of iga nucleotide sequence in U. urealyticum. Convincing evidence were obtained after PCR amplification of ureaplasma DNA using primers designed to be compatible with putative iga gene of M. genitalium followed by the discovery of 100% sequence homology of amplified ureaplasma iga gene and iga gene of M. genitalium mentioned in establish data. IgA1 protease activity of U. urealytium has been detectable in the cell rather than in media culture, suggesting that IgA1 protease is not secreted out of cell. It was proofed that IgA1 protease is membrane bound enzyme capable of digesting IgA1 in mucosal tissues of various organs and considered as potential virulence factor for ureaplasma that cause disease or gain entry to mucosal membrane. The existence of IgA1 protease activity in bacterial plasma membrane would have implication in ureaplasma management such as diagnosis and therapy of ureaplasma infection. (Med J Indones 2005; 14: 204-14)</i>