1959 2009

TRANSFORMASI PLASMID pUR5750 KE DALAM SEL MONASCUS PURPUREUS ITBCC-HD-F002 MELALUI MEDIASI BAKTERI AGROBACTERIUM TUMEFACIENS LBA1100 DAN AGL1

Tiana Milanda¹⁾, Syarif Hamdani²⁾ Marlia S. Wibowo³⁾, Tutus Gusdinar³⁾, Haryanto Dhanutirto³⁾, Philippe J. Blanc⁴⁾

¹⁾Fakultas Farmasi, Universitas Padjadjaran, Indonesia
²⁾Sekolah Tinggi Farmasi Indonesia, Indonesia
³⁾Sekolah Farmasi, Institut Teknologi Bandung, Indonesia
⁴⁾Institut National des Sciences Appliquées de Toulouse, France

ABSTRACT

Monascus purpureus is a red fungus that produces various secondary metabolites, such as pigments, monacolin K, and monascidin A. The pigments are traditionally used as food and cosmetic colorants, whereas monacolin K shows an antihypercholesterolemic activity. Monascidin A shows an antimicrobial activity, but it has carcinogenic and nephrotoxic properties. Many researches have been done to eliminate this toxic metabolite. Since monascidin A's precursor is similar to its pigments, our research was aimed to develop an efficient genetic transformation system that can be used to identify monascidin A genes. The transformation must be applied in a nonproducing pigment (albino) mutant. M. purpureus ITBCC-HD-F002, an EMS-induced albino mutant, was transformed into hygromycin B resistance using the hygromycin B phosphotransferase (hph) of Escherichia coli as the selective trait, governed by gdp promoter of Aspergillus nidulans in pUR5750 plasmid. This plasmid was transformed into spores or protoplasts from this fungus mediated by Agrobacterium LBA1100 and AGL1. The transformation efficiency was 346 ± 4.00 transformants/ 10^7 protoplast (mediated by A. tumefaciens LBA11) and 635.7 \pm 13.32 transformants/10⁷ spores (mediated by A. tumefaciens AGL1). These results indicating the highly virulent strain A. tumefaciens AGL1 was found to be more efficient in DNA transfer than The majority of transformants were mitotically stable up to five generations and the presence of hph genes were detected by PCR. In four randomly chosen transformants, single-copy integration of the marker gene at different chromosomal site were proven by Southern Blot analysis..

Key words: Transformation, pUR5750, Monascus purpureus, Agrobacterium tumefaciens

Pendahuluan

Monascus purpureus adalah kapang merah yang digunakan untuk memfermentasi beras menjadi beras angkak. Dari produk fermentasi ini telah diisolasi berbagai metabolit sekunder, diantaranya zat warna, monakolin K dan