Dalia Martinkënaitë

Mutantø rinkinio konstravimas neesminiø *Helicobacter pylori* J99 genø nustatymui

Santrauka

Helicobacter pylori yra unikali bakterija. Ji gyvena skrandyje ir dvylikapirðtëje þarnoje bei yra viena ið daþniausiai þmogø infekuojanèiø mikroorganizmø rûðiø. *H. pylori* buvo antras mikroorganizmas, kurio genomo seka buvo pilnai iððifruota ir paskelbta. Taèiau daugelio *H.pylori* J99 atviro skaitymo rëmeliø funkcijø vis dar nëra nustatytos, be to, nëra duomenø apie jø reikðmæ bakterijos gyvybingumui. Esminio geno produktas, kuris yra bûtinas ðios bakterijos iðgyvenimui, gali tapti nauju antimikrobiniu taikiniu efektyviø vakcinø gamyboje arba gali bûti panaudotas cheminiø inhibitoriø, kurie iððauktø mikroorganizmo þûtá, kûrime.

Galutinis mûsø darbo tikslas yra nustatyti neesminius *Helicobacter pylori* J99 bakterijos genus, pritaikant naujà Biotechnologijos institute Prokariotø genø inþinerijos laboratorijoje sukurtà metodikà, kuri ágalina gana greitai ir pigiai atlikti bakterijø genomø analizæ. Magistrinio darbo tikslas buvo - sukonstruoti reprezentatyvø *Helicobacter pylori* J99 mutantø rinkiná, atlikti jo pirminæ analizæ ir sukonstruoti mutacijos taðkus genome reprezentuojanèius DNR þymenis, kuriuos ateityje numatoma panaudoti neesminiø *H.pylori* J99 genø nustatymui. Tikslo pasiekimui buvo sukonstruota reprezentatyvi *H.pylori* J99 genomo fragmentø klonoteka, kuri perdengë ðios bakterijos genomà ~175 kartus. Taip pat buvo sukonstruota speciali DNR kasetë turinti atsparumà chloramfenikolui sàlygojantá genà. Ði kasetë buvo áterpta á gautos *H.pylori* J99 klonotekos plazmides per genominës DNR fragmentus. Toks preparatas buvo naudojamas *H.pylori* J99 mutagenezei, kurios metu, DNR kasetë dël jà supanèiø klonuotø *H. pylori* J99 DNR sekø, homologinës rekombinacijos dëka, buvo perneðta ið plazmidës á *H. pylori* J99 genomà. Vëliau buvo sukonstruoti DNR kasetës ásistatymo vietà reprezentuojantys DNR þymenys. Sukonstruotus *H. pylori* J99 DNR þymenis numatoma panaudoti tolesniuose eksperimentuose, kad pasiekti pagrindiná tikslà - nustatyti kokie genai *Helicobacter pylori* J99 bakterijai yra neesminiai (visi likæ bus esminiai) augant turtingoje mitybinëje terpëje *in vitro*.

The availability of total genome sequence information from a variety of bacterial genera has facilitated a dramatic increase in loss of function analysis technologies for pathogenic species of clinical relevance. Elucidation of essential metabolic and biosynthetic pathways in pathogenic organisms is critical for identification of new antimicrobial targets. From a pharmaceutical standpoint, it is the ability to rapidly identify essential genes where loss of function is coincident with loss of viability that is driving force behind genomics-based targets validation.

The aim of this work was to construct the mutant's pool of *Helicobacter pylori* J99 for identification of nonessential genes and to prepare large amount of DNA tags representing sites for nonessential genes. To achieve this goal we constructed representable *H.pylori* J99 DNA library which was composed from 240,000 independent clones and overlap *H.pylori* J99 genome many times. In parallel, special DNA cassette containing chloramphenicol acetyl transferase gene (*cat*) which confers the chloramphenicol resistance to the *H.pylori* was constructed and inserted into cloned *H.pylori* J99 DNA fragments. At least 12,000 different clones containing correct insertions of DNA cassette were selected. Then total plasmid preparation was used for genetic transformation of *H.pylori* J99 strain. Cells that survived the allelic exchange were selected on agar media containing chloramphenicol. As allelic exchange results DNA cassette insertions were transferred from recombinant plasmids into genome of *H.pylori* J99 resulting at least 100,000 mutants. The DNA tags representing cassette insertion sites were produced using new technology developed in our laboratory.