PENGARUH SELULASE, CAT GRAM SAFRANIN DAN POLIETILEN GLIKOL (PEG) 6000 TERHADAP HASIL ISOLASI PROTOPLAS MIKROALGA *Chlorella vulgaris*

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INTISARI

Kemajuan dibidang bioteknologi dengan penggunaan fusi protoplas memungkinkan diperolehnya hibrida-hibrida dengan tingkat heterogenitas yang tinggi Sebelum dilakukan fusi atau budidaya protoplas maka teknik isolasi protoplas harus dikuasai terlebih dahulu. Sampai saat ini, berbagai penelitian telah dikembangkan untuk mengisolasi protoplas dari alga uniseluler, akan tetapi penelitian lebih jauh mengenai isolasi protoplas dari mikroalga *Chlorella vulgaris* masih belum banyak dan optimal dilakukan. *C.vulgaris* mengandung sumber nutrisi beragam sehingga banyak digunakan sebagai pakan ikan, suplemen makanan, bahan penawar berbagai penyakit, bahan untuk biofuel dan bioremediator. Penelitian ini bertujuan untuk mengetahui konsentrasi dan waktu inkubasi enzim selulase terhadap jumlah dan viabilitas protoplas *C.vulgaris*, kemampuan PEG 6000 40% dan cat gram safranin 0,25% untuk mendeteksi jumlah protoplas dan kemampuan cat gram safranin 0,25% untuk menguji viabilitas protoplas *C. vulgaris*. *C.vulgaris* diambil saat fase eksponensial dengan kepadatan 10^6 sel/mL. Lalu disentrifugasi dengan 1000 x g selama 15 menit dan dilakukan isolasi protoplas dengan larutan enzim konsentrasi 2% dan 4% selama 10 menit, 240 menit (4 jam), 480 menit (8 jam), 720 menit (12 jam) dan 960 menit (16 jam). Selanjutnya protoplas yang terbentuk diamati dengan menambahkan PEG 6000 40% dan safranin 0,25%. Kemudian dihitung jumlah protoplas yang terbentuk dengan PEG 6000 40% dan cat gram safranin 0,25% serta dilakukan uji viabilitas protoplas dengan cat gram 4 safranin 0,25%. Hasil menunjukkan bahwa konsentrasi dan waktu inkubasi enzim selulase terhadap jumlah protoplas *C.vulgaris* adalah sebesar 4% dengan waktu inkubasi 720 menit (12 jam) sejumlah 1.444.205,08 ± 26.297,18 pps/mL dan 480 menit (8 jam) terhadap viabilitas protoplas *C.vulgaris* sebesar 67,00 ± 2,0 %, PEG 6000 40% dan cat gram safranin 0,25% mampu mendeteksi jumlah protoplas dan cat gram safranin 0,25% mampu untuk menguji viabilitas protoplas *C.vulgaris*.

Kata kunci : isolasi protoplas *C.vulgaris*, enzim selulase, safranin, PEG 6000

THE EFFECT OF CELLULASE, GRAM PAINT SAFRANINE AND POLYETHYLENE GLICOL (PEG) 6000 TO THE RESULT OF PROTOPLAST ISOLATION MICROALGAE *Chlorella vulgaris*

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ABSTRACT

Progress in the field of biotechnology with the use of protoplast fusion allows obtaining hybrids with a high degree of heterogenety. Prior to protoplast fusion, isolation techniques must be prioritized first. Until now, various studies have been developed to isolate protoplast from unicellular algae, but the advanced research of microalgae *Chlorella vulgaris* is still not much done. *C.vulgaris* contains of rich nutrients which are widely used as fish feed, dietary supplements, ingredients antidote for various diseases, materials for biofuels and bioremediator. This study aimed to determine the concentration and incubation time of celluase enzyme to the yield and viability of *C.vulgaris* protoplast, the ability of PEG 6000 40% and gram paint safranine 0,25% to detect the yield of protoplast and to determine the gram paint safranine 0,25% for testing the viability of *C.vulgaris* protoplast. *C.vulgaris* was taken during the exponential phase with a density of 10^6 cells/mL, centrifuged at 1000 x g for 15 minutes and protoplast was isolated with cellulase enzyme solution at concentration of 2% and 4% for 10 minutes, 240 minutes (4 hours), 480 minutes (8 hours), 720 minutes (12 hours) and 960 minutes (16 hours). Furthermore, protoplast formation was observed by adding PEG 6000 40% and safranine 0,25%. The viability of protoplast was detected by gram paint safranine 0,25%. The yield and viability of protoplast formed were calculated. The result showed that the concentration and incubation time of cellluase enzyme to the yield of *C.vulgaris* protoplast was 4% with 720 minutes (12 hours) accounted for 1.444.205,08 ± 26.297,18 pps/mL and 480 minutes (8 hours) to the viability of *C.vulgaris* protoplast accounted for 67,00 ± 2,0 %, PEG 6000 40% and gram paint safranine 0,25% could be used to detect the yield of protoplast and gram paint safranin 0,25% also could be used to test the viability protoplast of *C.vulgaris*.

Keywords : protoplast isolation, *C.vulgaris*, cellulase enzyme, safranine, PEG 6000