49a Hydrodynamic Shear in Plant Cell Suspension Cultures: Using Biological View to Understand the Engineering Problem

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For large-scale cultivation of plant cells, the negative effects of hydrodynamic shear on cell growth and production of secondary metabolites are a concern. Early studies of shear sensitivity focused on cell damage and secondary production. A fuller understanding the mechanism of shear sensitivity and responses to shear stress using biological view may assist in the optimization of large-scale cultivation conditions. Our interests are in the mechanism of defense responses to shear stress and the pathway of the shear signal transduction, which were little attention in early studies. Additionally, we attempt to optimize the cultivation conditions by means of combining the environmental factors and the physiological effects that were caused by shear stress in scale-up bioreactors. The flow field inside the impeller-stirred bioreactor was simulated using CFD software of FLUENT. The main results include (1) 3D simulation of the velocity field, energy field and shear stress field in single-phase bioreactor; (2) 2D simulation of the distribution of the dissolved oxygen, (3) accurate simulation of the mixing state inside the impeller-stirred bioreactor. The simulations show that the shear stress and concentration of the dissolved oxygen were higher in the impeller swept and discharged regi1ons than that in the baffle and bulk regions. We can forecast the concentration of the dissolved oxygen and shear stress fields in bioreactor by this method. Experiments demonstrated that the suspension cultured cells of Taxus and Catharanthus roseus were sensitive to the shear stress with 0.16 Pa to 0.78 Pa. Additionally, the respiration action was depressed when the cells cultured in either pure oxygen or oxygen-limited conditions. However, it is still difficult to predict the physiological changes of cells under different conditions in bioreactor. We try to investigate the effects of single engineering factor on suspension cells, such as shear stress and bubble. In order to seek the quantitative relation between shear stress and biological responses of plant cells (Taxus chinesis var. mairei, Taxus cuspidate, and Catharanthus roseus), and understand the mechanism of shear sensitivity of plant cells, a Couette-type shear reactor was used to create a defined uniform shear fields. There was a critical shear stress between 0.36 Pa and 0.57 Pa for suspensions of Taxus cells. Large amount of cell debris were observed when the applied shear stress was higher than this critical value. Cell wall acting as the barrier for protecting cell also changes significantly with FTIR analysis during shear stress. The characteristic absorptive spectrum of pectin exhibits negatively changes using PCA analysis. To well-understand the mechanism of the effect of shear stress to plant cell and the mechanism of shear sensitivity of plant cell, we investigate the signal transduction during shear stress with 50-200 rpm, which has less damage to cells and phenomenal effects. It was found that Taxus cuspidata cells can sense shear rate of 95s-1 and respond with oxidative bursts. Their existed H2O2 burst in intracellular with triphasic characteristics in 6 hour as well as O2- generation in extracellular. Inhibition studies with DPI□Aazide showed that the key enzyme responsible for oxidative burst under shear rate is NADPH oxidase and contribution of peroxidase towards oxidative burst was less. Additionally, shear stress also elicits NO generation in suspension cultures of Taxus cuspidate. NO2-, a major end-product of NO, also increased gradually after shear stress. It was further demonstrated that NO production was sensitive to a nitric oxide like-enzyme (NOS) inhibitor. The interactions between NO and reactive oxygen intermediates synergize the membrane permeability and lipid peroxidation. Excessive reactive oxygen species (H2O2, O2-, NO) caused by shear stress also result in changes of the redox degree and the associated antioxidant enzymes activities in suspension cultures of Taxus cuspidata. It was found that the content of ascorbate (As) decreased significantly by 95s-1 shear rate as well as glutathione (GSH) concentration. Compared with the normal contents of suspension cells, the concentration of As and GSH did not recover within 6 hours. The ratio of As/total As increased while GSH/total GSH decreased. Additionally, there was a negative corresponding correlation coefficients between As and H2O2 with r=-0.71 as well as between GSH and H2O2 with r=-0.64. The activities of ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR) increased remarkably. Unlike other studies, glutathione S-transferase (GST) decreased during shear stress in a

short-term. This inactivation of GST partially recovered when NOS inhibitor was added in cell cultures during shear stress. Treatment of suspension culture cells and enzyme extracts of GST with reactive nitrogen species (RNS) also resulted in loss of GST activity. The results indicate that NO plays a crucial role in GST inactivation in Taxus cuspidate cells under shear stress. GST is a principal enzyme responsible for detoxification. The suppression of GST indicated that GST might be an important target for RNS to regulate the cell redox because GST is crucial for regulation of glutathione and ROI levels. Changes of redox state induced defense genes expression. In addition to serving as oxidant, H2O2 and NO burst play important roles in signal transduction, inducing activating of PAL enzyme, phenolics accumulation, and salicylic acid generation. We investigate the G-protein□ACa2+channel and phospholipase C action sequence and speed involved in the signal pathway for oxidative burst induced by shear rate. The action time for G-protein, Ca2+-channel and PLC were 10-15min, 15-25min and 20-25min respectively. Based on results above, a hypothetical model for oxidative burst in cultured Taxus cuspidata cells challenged with shear stress is schemed: Shear stress induced the activation of heterotrimeric G proteins, which may stimulates ion channels and phospholipase, the action of Ca2+ channel resulted in Ca2+ influx. The activation of PLC led to the release of inositol phosphates and diacylglycerol as the second messengers. Ca2+ influx and diacylglycerol (DG) can activate protein kinase and intracellular Ca2+ level cooperated with activated protein kinase will induce NADPH oxidase activity and engender oxidative burst. Membrane fluidity is considered a mechanical receptor. Sudden changes in membrane order can result in medium alkalization and oxygen burst. G-proteins are also activated by the increase membrane fluidity. In this mechanism, shear stress is converted into an intracellular biochemical signal by the plasma membrane. So, the age-related different physiology of membrane fluidity and H+-ATPase activity may partially contribute to the difference of cell signal intensities, transduction speed and biological responses under the shear stress. Additionally, Phospholipid bilayer peroxidation caused by oxidative burst lead to membrane rigidification, which may be regulate membrane fluidity. We investigated the mechanism of the effects of hydrodynamic shear in plant cell suspension cultures and compared age-related signal responses of suspension culture plant cells to shear stress using biological basis. Our studies provide a new sight for shear sensitivity studies and regulation of cell culture process and production of desired products in plant cell cultures.