Production of valuable compounds using cultured plant cells and plant immunity–activating endophytes

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Research objective

Cultured plant cells typically grow faster than whole plants and can be grown stably indoors without being affected by climate or weather, making them an effective means of producing plant bioactive compounds, such as phenolics and terpenoids. However, considering the number of genes involved in the synthesis of secondary metabolites in cultured plant cells, only a small proportion of phytochemicals have been produced to date, and many of these genes remain dormant. Recently, new attempts have been made to induce gene expression in cultured plant cells by co-culturing them with microorganisms, which is expected to induce dynamic metabolic changes through cell-cell interactions. Fungi have often been used for co-cultivation with cultured plant cells. However, there have been few reports on the use of bacteria for co-cultivation with cultured plant cells. Previous studies have shown that co-cultivation with microorganisms increases the production of certain secondary metabolites in cultured plant cells. However, the effects of microorganisms on the growth and death of cultured plant cells and the details of their interactions have not yet been fully elucidated.

We previously isolated endophytic bacteria that activate immunity in *Brassicaceae* plants. *Delftia* sp. BR1R-2 and *Arthrobacter* sp. BR2S-6 were isolated from *Brassica rapa* var. *perviridis*. *Pseudomonas* sp. RS1P-1 was isolated from *Raphanus sativus* var. *hortensis*. When these three strains were grown endogenously in *Arabidopsis* plants, they improved resistance against plant pathogens. These three endophytic bacteria also enhanced the elicitor-induced production of reactive oxygen species in tobacco BY-2 cells.[1-3]

In this study, we investigated the interactions between bacteria and cultured plant cells. We analyzed the effects of strain BR1R-2 on the growth and metabolism of BY-2 cells in coculture. The findings obtained in this study will be useful for the application of plant immunity-activating endophytic bacteria in the production of bioactive compounds by cultured plant cells.

Methods

The solution containing strain BR1R-2 (OD_{600} of 0.2, 1 mL) was inoculated into the liquid medium (100 mL) containing BY-2 cells after 24 h of cultivation, and these cells were co-cultured on a rotary shaker (120 rpm) at 28°C for 96 h. Growth of cultured plant cells was measured by wet weight, and cell death was assayed using Evans Blue reagent. BY-2 cells

were lyophilized and then dissolved in methanol. Metabolites were extracted from the cells in methanol by sonication and analyzed by HPLC.

Results

Effects of co-culture with strain BR1R-2 on growth of BY-2 cells

First, we examined the effects of the co-culture with *Delftia* sp. BR1R-2 on tobacco BY-2 cell growth, which is a commonly cultured plant cell line. When BY-2 cells were cocultured with the plant pathogen *Pectobacterium carotovora* subsp. *carotovora*, which was used for comparison, the cultured plant cells turned gray over time. Furthermore, this plant pathogen inhibited the growth of BY-2 cells and caused the death of cultured plant cells (Figure 1). In addition, *Escherichia coli*, which is unrelated to plants, negatively affected the growth of BY-2 cells. These phenomena may be due to the fact that BY-2 cells were attacked by potential pathogenic factors of *P. carotovora* and *E. coli* or that the strong defense responses of BY-2 cells against these bacteria led to the death of the BY-2 cells themselves. In contrast, it was revealed that strain BR1R-2, which is an endophytic bacterium isolated from *Brassica rapa* var. *perviridis,* exerted almost no adverse effects on the growth of BY-2 cells. During this process, the BY-2 cells turned ochre, suggesting that metabolic changes were induced in the cultured plant cells (Figure 1). It was also confirmed that the strain BR1R-2 grew during co-culture. Strain BR1R-2 is reportedly able to grow inside plants without inhibiting plant growth.[1] Additionally, we found that strain BR1R-2 did not inhibit the growth of cultured plant cells, demonstrating that this strain is a potentially useful bacterium for co-culture.

Effects of co-culture with strain BR1R-2 on metabolism of BY-2 cells

Since the color change of BY-2 cells in the presence of strain BR1R-2 implies that metabolic changes were induced, we examined the effects of co-culture with strain BR1R-2 on the metabolism of BY-2 cells. Metabolites were extracted from BY-2 cells cocultured with BR1R-2 cells and subjected to HPLC analysis. In the HPLC chromatograms, the peak pattern of the metabolites changed significantly in the co-culture compared to the culture in the absence of BR1R-2, with a decrease in peak 1 and an increase in peaks 2 to 6 (Figure 2). The product corresponding to peak 1 was identified as *N*-caffeoylputrescine based on the HPLC retention time, UV-visible absorption spectrum, and LC-MS. The structures of peaks 2‑6 are currently being analyzed in detail. These results indicate that the strain BR1R-2 induces metabolic changes in cultured plant cells without inhibiting their growth.

Conclusion

Our study demonstrates that plant immunity-activating endophytic bacteria can induce metabolic changes in cultured plant cells without inhibiting growth. We hypothesized that

these endophytic bacteria are gently recognized by plant cells and may induce moderate immune responses without inhibiting the growth of cultured plant cells or whole plants. These bacteria provide new options for altering the metabolic profiles of cultured plant cells. We are currently conducting comparative analyses of different endophytic bacteria and cultured plant cells.

References

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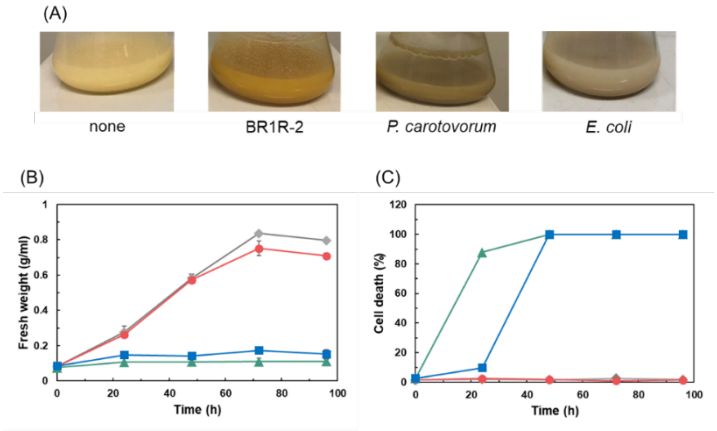

Figure 1 Effects of co-culture with strain BR1R-2 on growth of BY-2 cells. BY-2 cells were co-cultured with bacteria. (A) photographs after 96 hours of co-culture, (B) growth of BY-2 cells, (C) cell death rate of BY-2 cells. Gray diamonds, no bacteria; red circles, BR1R-2; green triangles, *P. carotovorum*; blue squares, *E. coli*.

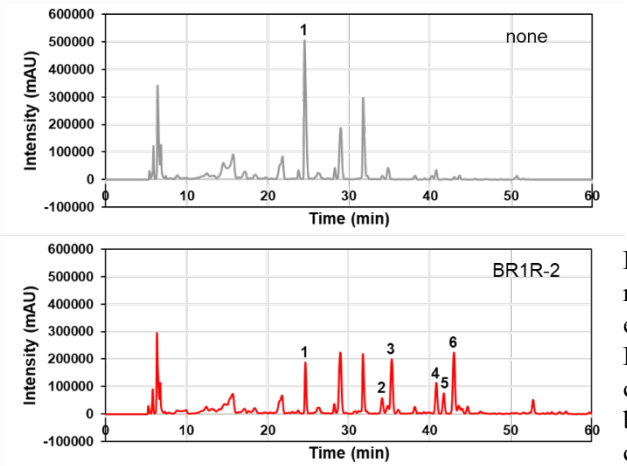

Figure 2 Effects of co-culture with strain BR1R-2 on metabolism of BY-2 cells. Metabolites were extracted from BY-2 cells co-cultured with strain BR1R-2 and analyzed by HPLC. The upper chromatogram, BY-2 cells cultured without bacteria; the lower chromatogram, BY-2 cells co-cultured with strain BR1R-2.