Combined induction: effective fermentation procedure for heterologous secondary metabolite production in actinomycetes

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Introduction. The members of the order *Actinomycetes*, especially the genus *Streptomyces*, have the potential to produce many antibiotics and bioactive natural compounds. A recent analysis of the *Streptomyces* genome revealed the presence of almost 30 biosynthetic gene clusters for secondary metabolite production, and these data imply that a single strain of *Streptomyces* can produce over 30 secondary metabolites. However, most of these secondary metabolites cannot be isolated from the broth of *Streptomyces* pure culture.

To date, various methods have been used to induce these "silent" genes biosynthesizing the secondary metabolites. Combined culture method is a co-culture method used to activate such silent genes. When mycolic acid-containing bacteria (MACB) were co-cultured with *Streptomyces*, MACB induced secondary metabolisms in *Streptomyces*, and the profiles of secondary metabolites changed (1). We used this finding to develop a new co-culture method called the "combined culture" and discovered new antibiotics, Alchivemycins from *Streptomyces endus* S-522 (2). Here, we will show "combined induction" method, that is, the combined culture method can be applied to develop a heterologous expression host to increase the production of antibiotics.

Methods. Combined induction was performed using *Tsukamurella pulmonis* (Tp), *Rhodococcus erythropolis* (Re), and *Corynebacterium glutamicum* (Cg) as the stimulator strains. These strains are MACB, and they contain mycolic acid localized in their outer cell layer. *Streptomyces lividans* TK23 was used as the heterologous host in this study, and the heterologous expression of staurosporine, rebeccamycin, and goadsporin was analyzed. The heterologous expression vectors were the ones that had been constructed in our previous studies (3, 4, 5).

Results. *S. lividans* is known to be a conditional producer of 2 types of red pigments, actinorhodins and undecylprodigiosins. We observed that *S. lividans* produces these 2 red pigments in the combined culture with Tp, Re, or Cg. This result indicates that the secondary metabolism of *S. lividans* is stimulated by MACB. We then transformed the biosynthetic gene clusters of staurosporine from *Streptomyces* sp. TP-A0274, rebeccamycin from *Lechevarielia aerocolonigenes*, and goadsporin from *Streptomyces* sp. TP-A0584 into *S. lividans* to construct strains showing heterologous expression; the combined culture method was performed using these heterologous strains and MACB. The concentration of staurosporine, rebeccamycin, and goadsporin in the culture of the heterologous strain increased. Fig. 1 shows the increase in rebeccamycin production by the combined induction method using the 3 MACB strains. In the combined induction by Re, rebeccamycin production increased by 26 times of that observed in the pure culture. The other heterologous strains also increased the production of each metabolite by 2.5 to 26 times more than that observed for the pure cultures.

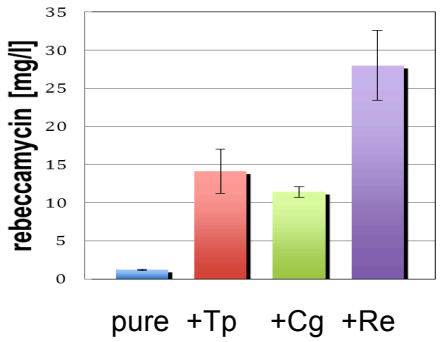

Fig.1 The amount of rebeccamycin heterologous production in the pure culture, and the combined induction with Tp, Cg, and Re. Each culture was cultivated as a liquid culture of A-3M medium at 30°C for 7 days.

Conclusions. In this study, we found that heterologous expression of secondary metabolites is activated by MACB. Here, we have established the combined culture method by using heterologous hosts as the "combined induction." Combined induction in this study implied that *S. lividans* transmitted MACB-stimulated signal to activate heterologous secondary metabolism as well as endogenous metabolism. We believe that the combined induction method will be a useful technology in isolating new secondary metabolites from actinomycetes whose genome has been analyzed.

References.

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