Screening of microbial secondary metabolites that act as an abscisic acid mimetic in plants

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Summary

Plants respond to high-salt and drought stresses, and induce the expression of numerous genes associated with stress tolerance. The plant hormone abscisic acid (ABA) mediates the expression of most of the salt-stress-responsive genes, as evidenced by the finding that these genes can be induced by exogenous ABA treatment. However, externally-applied ABA only induces the transient expression of ABA-responsive genes, and fails to improve salt tolerance of plants owing to its rapid metabolism to the inactive phaseic acid. It was shown that several phytopathogenic fungi such as *Botrytis cinerea* produce ABA, and that many soil microbes produce plant-growth-regulating compounds as their fermentation products, prompting us to screen for microbial secondary metabolites that act as an ABA mimetic in plants.

To discover ABA mimetic compounds from soil microbes by screening of their fermentation products, we designed a new bioassay with transgenic *Arabidopsis thaliana* plants containing *pRD29A*-firefly luciferase (*LUC*) reporter gene fusion. The *RD29A* promoter contains the ABA-responsive element and can be activated by ABA treatment or high-salt stress. The *LUC* reporter gene expression in plants can be measured noninvasively by real-time luminescence imaging, making it practical to screen a large number of test samples prepared from microbial fermentations. Through extensive screening of methanol extracts from 820 strains of *Streptomyces* sp. and 100 strains of fungi, we isolated five *Streptomyces* sp. strains that produce *RD29A* promoter activators in their fermentations. Methanol extracts from *Streptomyces* sp. TM-07, TM-55, EB-1032, EB-1042, and EB-1061 culture broths induced *RD29A-LUC* expression to a level comparable to that induced by 100 μM ABA. Among these strains, *Streptomyces* sp. TM-07 and TM-55 was selected for further purification of the *RD29A* promoter activator. The activity was found only in the culture filtrates, but not in the mycelium after filtration of their broths. The culture filtrates of each strain were successively extracted with *n*-hexane, ethyl acetate, and water-saturated *n*-butanol. The activity was found in the *n*-butanol extract, suggesting that the active compounds are relatively polar. Purification of the active compounds is now in progress.