



Supplementary Materials: Latitudinal Characteristic Nodule Composition of Soybean-Nodulating Bradyrhizobia: Temperature-Dependent Proliferation in Soil or Infection?

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Table 1. Soil property of the study sites [15].

Location	Soil type ^a	pH	NH ₄ -N (mg/kg)	P ₂ O ₅ (mg/kg)	K ₂ O (mg/kg)	Total C (g/kg)
Fukagawa	Andisol	6.0	16	472	369	5.2
Matsue	Inceptisol	6.2	12	288	86	1.2
Miyazaki	Andisol	6.4	22	160	220	4.4

^a Based on USDA classification.

Table 2. PCR ingredients for amplification of 16S rRNA and 16S-23S rRNA ITS region.

Ingredients	Amount (μL)
Reaction buffer(10X) (GENETBIO)	1.0
dNTPs mixture (2.5mM) (GENETBIO)	0.25
forward primer (12.5μM) ^a	0.4
reverse primer (12.5μM) ^a	0.4
Taq DNA polymerase (GENETBIO)	0.25
DNA template/culture	^b
MilliQ water	7.7
Total	10

^a fD1 and rP2 [17] and 1512F and 23R [21] for 16S rRNA and 16S-23S ITS, respectively. ^b A small amount of colony was directly used as template.

Table 3. PCR running conditions.

Reaction	Gene amplification	Cycle sequence
Pre-run	94 °C, 3 min	96 °C, 2 min
Denaturation	94 °C, 30 sec	96 °C, 10 sec
Annealing	50 °C, 30 sec	50 °C, 5 sec
Extension	72 °C, 1 min	60 °C, 4 min
Cycle number	30	30
Final run	72 °C, 5 min	72 °C, 5 min