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Effect of Rare Earth Elements on Growth Behavier of Microorganisms

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Introduction

We studied on the effects of Rare Earth Elements(REEs) on growth of *Streptomyces* sp. YB-1 on the diluted nutrient agar plates. A paper disk soaked in 0.5 M REEs (La, Nd, Sm and Yb) or other metal ions (Ni, Co, Mn and Al) was placed on the plates inoculated with *Streptomyces* sp. YB-1. After incubation of 24 hr, clear inhibition zones were formed around the disks although there were some differences in the size of inhibition zone of each element. Interestingly in the case of culture with REEs, colonies of *Streptomyces* sp. YB-1 exhibited the formation of reddish-purple pigment (Photo.1). On the other hand, Ni, Co, Mn and Al did not result in this pigmentation. The pigments seemed to be produced when concentrations of REES were close to the limit for growth of strain YB-1. Main component of the pigments has a nuclear structure similar to that of naphthoquinone (Fig.1). These results suggest that REEs might affect the physiological activity of this strain. These findings led us to study on the effects of REEs on the growth behaviors of microorganisms.

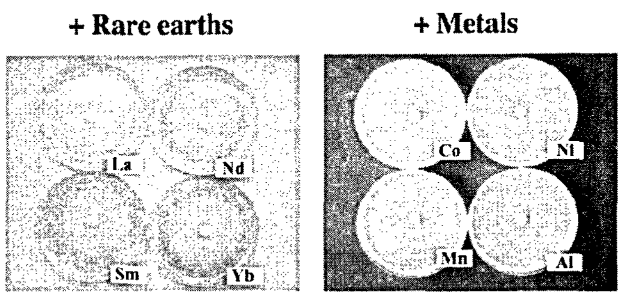

Photo. 1 Pigment production of *Streptomyces* sp. YB-1 in the presence of rare earth elements

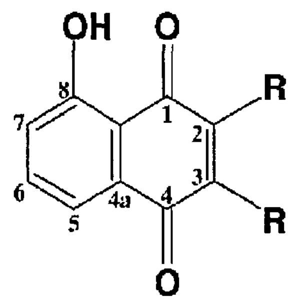

Fig. 1 Structure of main pigment produced by *Streptomyces* sp. YB-1 in the presence of rare earthe elements

1. A soil bacterium producing slime in the presence of Ce

We screened microorganisms exhibiting curious growth behavior on Ce-containing diluted nutrient agar plates. We isolated a soil bacterium whose growth was promoted in the presence of Ce, resulting in the formation of larger colonies in comparison to those in the absence of Ce. But the detail microscopic observation of the colonies revealed that the cells produced some slime around the colonies in the presence of Ce (Photo. 2). Based on morphological, physiological and biochemical characterization and phylogenetic analysis of 16S rDNA sequence, the bacterium was identified as *Bardyrhizobium* sp. (designated strain CE-3). Genus *Bradyrhizobium* is generally known to produce slime around cells in the presence of mannitol. We confirmed that strain CE-3 also produced slime in the presence of mannitol (Photo. 2). As shown in Table 2, La and Nd also induced the formation of slime by *Bradyrhizobium* sp. CE-3, and Sm caused a slight production of slime. The other REEs and the vital metals did not induceformation of slime. It is of interest that only the light REEs caused strain CE-3 to produce the slime. The mean molecular weights of both slimes were calculated to be about 1 million by gel filtration chromatography on Sepharose CL-4B. Next, we investigated to determine the chemical structure

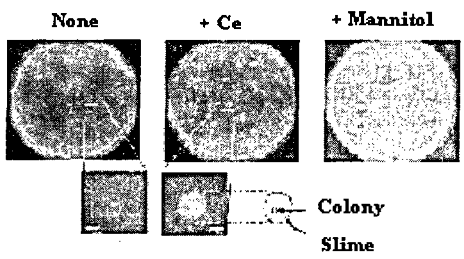

Photo. 2 Slime production of a soil bacterium in the presence of Ce and Mannitol

Table. 2 Slime production by *Bradyrhizobium* sp. CE-3 in the presence of rare earth elements and other metals

Element	Ion radius (Å)	Electronic number	Slime formation	Element	Ion radius (Å)	Electronic number	Slime formation
Y	1.040	III	-	Na	1.160	I	-
La	1.172	III	+++	Al	0.675	III	-
Ce	1.150	III,(IV)	+++	K	1.520	I	-
Nd	1.123	III	+++	Ca	1.140	II	-
Sm	1.098	III	+	V	0.780	III,(IV,V)	-
Eu	1.087	III,(II)	-	Cr	0.755	III,(II,IV)	-
Gd	1.078	III	-	Co	0.790	II,(III)	-
Tb	1.063	III,(IV)	-	Ni	0.830	II	-
Dy	1.052	III	-	Sr	1.320	II	-
Ho	1.041	III	-	Ba	1.490	II	-
Er	1.030	III	-	Rb	1.600	I	-
Yb	1.160	III,(II)	-				

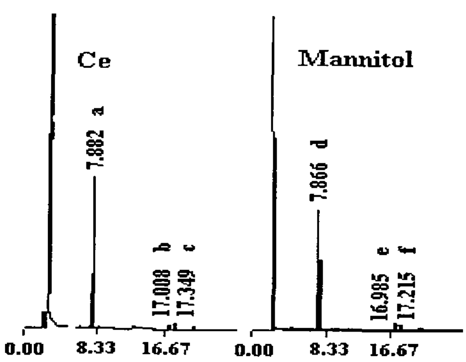

Fig. 2 GLC analysis of alditol acetates from slimes

Table. 3 GLC analysis of alditol acetates of hydrolysed slimes produced by *Bradyrhizobium* sp. CE-3 in the presence of Ce and mannitol

	Peak	Sugar	Conc.(%)
Ce (III)	a	D-Rhamnose	87.7
	b	D-Glucose	5.1
	c	D-Galactose	7.2
Mannitol	d	D-Rhamnose	90.0
	e	D-Glucose	5.2
	f	D-Galactose	4.7

of the slime. Slime produced in the presence of Ce and mannitol was collected from culture plates. The both slimes were concentrated, dialyzed against water and subjected to ^{1}H-NMR and FT-IR analyses. The results suggested that the both slimes might be chemically identical polysaccharides. TLC analysis of acid hydrolysates of the polysaccharides suggests that the polysaccharide was composed of D-rhamnose, D-glucose and D-galactose. The quantitative GLC analysis of the alditol acetates of the hydrolysates were shown in Fig. 2 and Table 3. These results revealed that both slime composed of about 90% D-rhamnose and several % of D-glucose and D-galactose. From this result, the both slimes seemed to be homopolymers of D-rhamnose (rhamnan).

2. Promotion of the growth of a soil bacterium by Sm

We isolated a soil baterium which showed 1.2-1.5 time larger colony diameter in the presence of Eu than in the absence of Eu in diluted nutrient agar plates (Photo. 3). This bacterium was identified as *Methylobacterium* sp. (designated EU-1), a facultative methylotrophic bacterium, by morphological, physiological and biochemical properties and phylogeneric analysis of 16S rDNA sequence. As shown in Fig. 3 , it is of interest that the growth of this bacterium on 1% methanol-containing diluted nutrient agar was promoted by Sm instead of Eu. The growth promotion by Sm was also recognized using diluted nutrient broth containing methanol. When ethanol, buthanol. propanol, glucose or fructose was used, the appreciable growth promotion by Sm was not observed. These results indicate that Sm might be concerned with pathway of methanol metabolism of this strain.

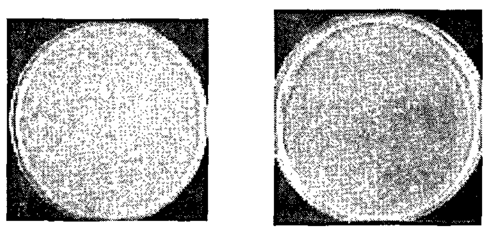

Photo. 3 Effect of Eu on the growth of a bacterium isolated from soil

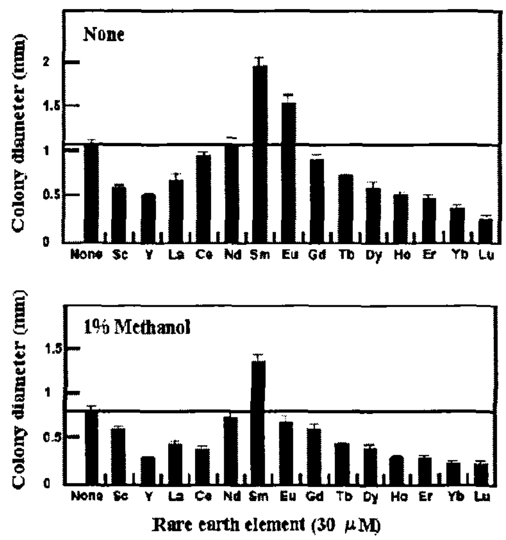

Fig. 3 Effects of rare earth elements on the growth of *Methylobacterium* sp. EU-1 in the oresence of 1% metanol.

3. Production of water-soluble bluis- purple pigment by true fungi in the presence of Sc

From 1,140 strains of oligotrophic microorganisms from108 soil samples, we isolated two fungi which produced water-soluble bluish-purple pigment in the presence of Sc (Photo. 4). Both fungi are identified as *Fusarium solani* based on morphological observation by microscope and phylogeneric analysis of nucleotide sequence of rDNA-ITS region. Although these two fungi were isolated from soils at the different sampling spots, it is very interesting that both fungi were identified as *F. solani.* As shown in Photo. 5, other *F. solani*s trains also produced the bluish-purple pigments, indicating that the pigment production by *F. solani* was universal. Among REEs (except Pm), only Sc induced the pigment production by *F. solani* SC -1 and SC-2. The other metals such as Mg, Al, Fe and Cu did not induce the pigment production of both strains.

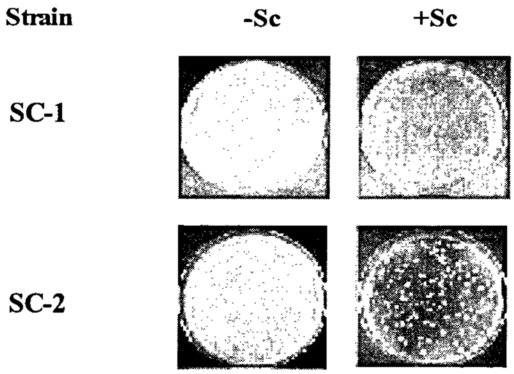

Photo. 4 Pigmentation of fungi in the presence of Sc

Strain	Production of pigment - Sc	+ Sc
F. solani 5-10	-	+
F. solani TW 10	-	+
F. solani f. sp. batatas SUF1327	-	+
F. solani f. sp. pisi SS1	-	+

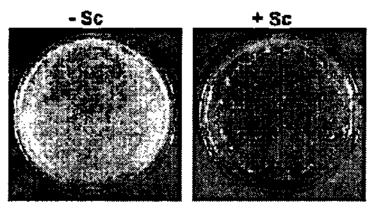

Photo. 5 Pigment production by 4 strains of *Fusarium solani* in the preasence of Sc

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