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液中乾燥法による徐放型含栄養塩マイクロカプセルの調製と性能

横田　祐司，石橋　一二，山田　勝利，田中　重信
J.L. PONDEVIDA，L.G. DOMINGUEZ，B.M. LALUSIS，
C.G. PIGAO，R.A. PANLASIGUI

液中乾燥法による含栄養塩マイクロカプセルの調製と性能に関する研究を行った。硫酸アンモニウム（ＡＳ）あるいは硫酸アンモニウムを吸着させた活性炭（ＡＣＡＳ）をマイクロカプセルの芯物質とし，エチルセルロースをマイクロカプセルの壁膜物質としてそれぞれ用いた。

マイクロカプセルの収率はＡＣＡＳの方がＡＳよりも高かった。調製したマイクロカプセルの水中における栄養塩の溶出試験の結果，ＡＣＡＳの方がＡＳを用いた場合よりも徐放性の高いことが明らかになった。また，壁膜の厚さによってもその徐放性を制御出来ることがわかった。

キーワード：マイクロカプセル，液中乾燥法，栄養塩，徐放性，活性炭

フライアッシュをケイ酸原料とする緩効性ケイ酸カリ肥料の製造

山田　勝利，野田　良男，石橋　一二，
R. CHAIWATTANANONE，R. WUNGDHEETHUM，
B. SUTTISONK，P. MATA

タイのメモ石炭火力発電所から排出する性状の異なる２種類のフライアッシュをケイ酸原料として，緩効性ケイ酸カリ肥料化について検討した。

キーワード：緩効性肥料，フライアッシュ，ケイ酸カリウム

内燃型流動炉を用いた緩効性ケイ酸カリ肥料の製造

山田　勝利，L.G. DOMINGUEZ，L.A. MANALO，
B.Y. MERCADO，A.T. MALLILLIN，C.G. PIGAO，
細田　英雄，桑垣　　整，石橋　一二

農産廃棄物である籾殻の農業への循環利用を考え，籾殻中のケイ酸とドロマイトを主原料とする緩効性ケイ酸カリ肥料の製造を試みた。原料の配合割合，焼成条件等と可溶化率（0.5mol·dm^{-3}塩酸に対するケイ酸の溶出量），ク溶化率（２％－クエン酸に対するカリウムの溶出量），水溶性カリウム量を比較検討した。

キーワード：緩効性ケイ酸カリ肥料，籾殻，ドロマイト，焼成

新しく分離された炭化水素を唯一の炭素源として生育する好アルカリ性炭化水素資化性菌 *Corynebacterium* sp. の特性について

池田　光二，中島　健二，湯本　勲

炭化水素を唯一の炭素源として合成培地で増殖する微生物を土壌から分離した。分離菌株は，絶対好気性，非運動性，グラム陽性，異染顆粒を形成した。そして非抗酸性で芽胞を形成しなかった。菌体壁にはメソ-ジアミノピメリン酸，アラビノウス，ガラクトースを含み，グリカン部N-アシル型はアセチル基であった。分離菌株はカタラーゼ陽性，オキシダーゼ陰性でDNAのGCmol%が70.8%であった。これらのテストに従って分離菌株は *Corynebacterium* 属と選定した。本分離菌株はpH 6.2～10.2の pH 範囲で同程度生育を示し，このpH範囲でのダブリングタイムは4～6時間であった。分離菌株はNa^+の添加によって増殖のラグタイムが短く成るが，pH 7.2とpH 10.2の両方でNa^+を必須としなかった。分離菌株は炭化水素の他に，酢酸，グルコース，フルクトースを唯一の炭素源として合成培地で増殖することができる。本菌体内に含まれるチトクロウムの含量は，分光学的分析から好アルカリ性 *Bacillus* 属の細菌に比べて10分の1以下であった。以上の結果，本分離菌は従来研究されてきた *Bacillus* 属の好アルカリ性細菌とは異なったアルカリ環境適応特性を持つものと考えられる。

キーワード：通性好アルカリ性菌，炭化水素，コリネバクテリア，ナトリウムイオン，チトクロウム

マウスNADPH-チトクロウムP450還元酵素：クローニングと酵母内での発現

扇谷　悟，神力　就子，鎌滝　哲也，石崎　紘三

ddYマウスより単離したNADPH-チトクロウムP450還元酵素の推定アミノ酸配列はラットの同酵素の推定アミノ酸配列と98.4%という高い相同性を有していた。特に，チトクロームP450との相互作用に係わっていると推定される酸性残基のクラスターは，哺乳動物のチトクロームP450還元酵素の一次構成上，進化の過程で非常によく保存されていた。また，非コード領域をすべて欠失させたマウスチトクロームP450還元酵素のcDNAを用いることにより，酵母内でのマウスチトクロームP450還元酵素を発現させ，酵素活性を検出することが出来た。

キーワード：チトクロームP450還元酵素，cDNAクローニング，塩基配列，発現，マウス肝，酵母

<Abstracts>

Preparation and Performance of Slow Release Microcapsules Containing Nutrient by Complex Emulsion Method

Yuji YOKOTA, Katsuji ISHIBASHI, Katsutoshi YAMADA, Shigenobu TANAKA,
J.L. PONDEVIDA, L.G. DOMINGUEZ, B.M. LALUSIS,
C.G. PIGAO and R.A. PANLASIGUI

A study on the preparation of nutrient containing microcapsules by complex emulsion method was conducted. Ammonium sulfate (AS) and ammonium sulfate adsorbed on activated carbon (ACAS), as core materials, and ethylcellulose as wall substance, were used. The yield of microcapsules using ACAS was higher than that obtained using AS. Comparison of the dissolution rate of nutrient in water from the microcapsules indicates that ACAS is more suitable than AS as core material for slow release microcapsule.

Key Words : Microcapsule, Complex emulsion method, Nutrient, Slow release, Activated carbon

Studies on the Slow-Release Type Potassium Silicate Fertilizer from Fly Ash

Toshikatu YAMADA, Yoshio NODA, Kazuji ISHIBASHI
R. CHAIWATTANANONE, R. WUNGDHEETHUM, B. SUTTISONK and P. MATA

Using two kinds of fly ash samples, production test of the slow-release type potassium silicate fertilizer at 800～950℃ for 10～40 minutes in air with potassium carbonate, calcium hydroxide and magnesium carbonate was conducted. From the experiment we obtained following results:

1) The solubility of SiO_2 in 0.5 mol · dm^{-3} HCl solution ranges from 53 to 92% and the solubility of K_2O in 2% citric acid ranges from 60 to 75% depending on the calcination condition.
2) By heat treatment at 950℃ for 20 minute, the silicate compounds clearly changed to $K_2Al_2O_8$ (or $K_2(Al, Fe)_2Si_2O_8$), K_2MgSiO_4 and α'-Ca_2SiO_4. These compounds are solouble in 0.5 mol · dm^{-3} HCl and 2 % citric acid.
3) The addition of a suitable amount of K_2CO_3 and $MgCO_3$ was favorable for the increase of the solubility of SiO_2 in 0.5mol · dm^{-3} HCl solution and the solubility of K_2O in 2 % citric acid.

Key Words : Slow release type fertilizer, Pottasium silicate, Fly ash

Studies on the Production of Slow Release Potassium Silicate Fertilizer Using the Internal Heat Type Fluidized Bed Reactor

Katsutoshi YAMADA, L.G. DOMINGUEZ, L.A. MANALO,
B.Y. MERCADO, A.T. MALLILLIN, C.G. PIGAO,
Hideo HOSODA, Hitoshi KUWAGAKI, Katsuji ISHIBASHI

A study on the production of slow release type fertilizer utilizing rice husks as SiO_2 source was undertaken. The mixture of rice husks, dolomitic limestone as CaO and MgO source and K_2CO_3 was granulated using molasses as binder. The granules were calcined in the internal heat type fluidized bed reactor using sawdust as heat sustaining source. The effect of CaO/SiO_2 mole ratio,calcination temperature and residence time on the solubility of the product was investigated. The mixture with CaO/SiO_2 mole ratio 1.15 calcined at 800℃ for 20minutes produced a potassium silicate fertilizer with 24.4% 0.5M HCl soluble K_2O but 3.1%water soluble K_2O. Production of the slow release type fertilizer in the form of potassium silicate was found to be technically feasible.

Key Words : Slow release potasssium silicate fertilizer, Rice husk, Dolomite, Calcination

Isolation and Characterization of a Novel Facultatively Alkaliphilic Bacterium, *Corynebacterium* sp. Grown on n-Alkanes

Koji IKEDA, Kenji NAKAJIMA and Isao YUMOTO

A novel facultatively alkaliphilic bacterium that grows on a chemically defined medium containing *n*-alkanes as the sole carbon source was isolated from soil. The isolate was obligately aerobic, non-motile, gram-positive, and formed metachromatic granules. It was not acid-fast and did not form endospores. The cell wall contained meso-diaminopimelic acid, arabinose, and galactose; the glycan moiety of the cell wall contained acetyl residues. The bacterium was catalase-positive, oxidase-negative, and the G+C content of DNA was 70.8 mol%. According to these tests, the isolate was assigned to the genus *Corynebacterium*. The bacterium grew well between pH 6.2 to 10.2 and the doubling time in this pH range was 4-6 h. For the growth of the isolate, added Na^+ in the culture medium stimulated growth, but was not indispensable at both pH 7.2 and pH 10.2. In addition to hydrocarbons, the isolate was able to grow on a chemically defined medium containing acetate, glucose, or fructose as the sole carbon source. Analyses of reduced *minus* oxidized difference spectrum of whole cells showed that the bacterium only possess less than one tenth amounts of total cytochromes compared with *Bacillus alcalophilus*. The above results suggested that the bacterium has characteristics different than of the alkaliphilic *Bacillus* previously described.

Key Words : Facultatively alkaliphilic bacterium, *n*-alkanes, *Corynebacterium*, Na^+, Cytochrome

Mouse NADPH-cytochrome *P*-450 Oxidoreductase: Molecular Cloning and Functional Expression in Yeast

Satoru OHGIYA, Nariko SHINRIKI, Tetsuya KAMATAKI and Kozo ISHIZAKI

We published isolation of a mouse NADPH-cytochrome P-450 oxidoreductase cDNA and afterward ascribed the cDNA to the guinea-pig instead of the mouse [Ohgiya, S. et al. (1992) Biochim. Biophys. Acta 1171, 103-105 and *Corrigendum* (1993) Biochim. Biophys. Acta 1174, 313]. We report here nucleotide and deduced amino acid sequences of an NADPH-cytochrome P-450 oxidoreductase cDNA isolated from the ddY mouse. The mouse cytochrome P-450 oxidoreductase shares 98.4 % identity with its rat counterpart. In particular, clusters of acidic residues that presumably participate in interaction with cytochrome P-450 are highly conserved in primary structures of mammalian cytochrome P-450 oxidoreductases. The mouse cytochrome P-450 oxidoreductase was functionally expressed in yeast using a modified cDNA clone lacking whole noncoding regions

Key Words : Cytochrome *P*-450 reductase, cDNA cloning, Nucleotide sequence, Expression, Mouse liver, Yeast

Preparation and Performance of Slow Release Microcapsules Containing Nutrient by Complex Emulsion Method*1

(Key Words : Microcapsule, Complex emulsion method, Nutrient, Slow release, Activated carbon)

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1. Introduction

From the view point of global environment protection, development of afforestation technology has been conducted for those lands desolated by destruction of tropical rain forests, excessive cattle grazing, and slash-and-burn farming. One of the technology is a method by effective fertilization.

Unlike the phosphate and potassium fertilizer, a large amount of the nitrogen fertilizer is lost when leached and denitrified in the soil. Also,excessive application of nitrogen brought by fast release causes fertilizer burn. Recently, in order to deal with these defects, slow release nitrogen fertilizer such as insoluble nitrogen compound and coated nitrogen fertilizer have been produced.

Microencapsulation is one of the slow release technique which has been applied widely in the pharmaceutical industry (Ishibashi, 1984), biotechnology (Higashide, 1979), and other related fields, but in the field of agriculture, applications involve only microencapsulated pesticides and encapsulated vegetable fat in cattle feeds.

In this paper, microcapsules were prepared by complex emulsion method using AS and ACAS as core material and ethylcellulose as wall substance. Their yield, particle size distribution, and the dissolution of core components are discussed from the view point of applying to slow release fertilizer. Gradual release of the nutrients for a long period of time is an essential factor for an effective fertilization.

This paper discusses the application of the microencapsulation technology in the field of agriculture.

2. Materials and Methods

2. 1 Preparation of ACAS

Figure 1 shows the flow diagram of the preparation of ACAS. Fifty (50) grams of AS (Kanto Chemical Co.,Inc.) as adsorbate was dissolved in two (2) liters of distilled water, and the solution was adjusted to pH 7.0. Then,

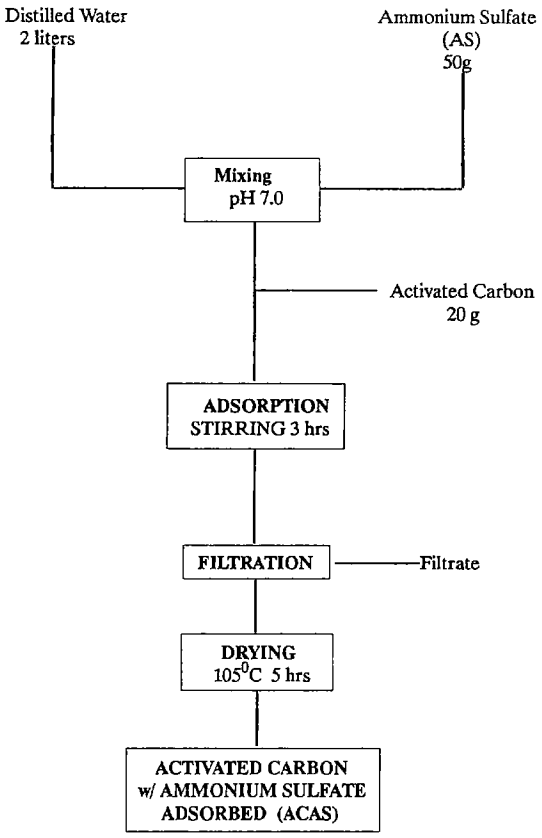

Fig. 1 Fow diagram of the preparation of activated carbon with ammonium sulfate adsorbed (ACAS).

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twenty (20) grams of activated carbon powder (Kanto Chemical Co. Inc.) passing 350 mesh size (previously dried at 105℃ for 5 hours) was added to the solution and stirred for three (3) hours. The internal surface area and adsorptive capacity for methylene blue of this activated carbon were 1,010㎡/g and 363.5mg/g, respectively. The activated carbon in the solution was filtered using glass fiber filter paper under vacuum and dried for five (5) hours at 105℃. The quantity of AS adsorbed on the activated carbon was measured by the increase in weight of the activated carbon. For this study, AS adsorbed on activated carbon at 50mg/g was used as core material.

2. 2 Preparation of Microcapsule

Figure 2 shows the flow diagram of microencapsulation by complex emulsion method. Ethylcellulose (Kanto Chemical Co.Inc.) as wall substance was dissolved in 250ml of dichloromethane (Kanto Chemical Co.Inc.) at concentration of 1.5, 3.0 and 6.0% (w/v). Various amounts of core material (AS or ACAS) was dispersed in the solution and maintained in uniform suspension by constant agitation for 20 minutes. Then, the suspension was poured slowly into a 3.0 liter vessel containing 1.0 liter of 4%(w/v) gelatin (Kanto Chemical Co.Inc.) solution to form uniform droplets under different stirring velocity. The temperature during the process was maintained at 30℃. Figure 3 shows the apparatus used in the complex emulsion method. After the mixed solution had been totally added, the temperature was gradually increased to 40℃. The solvent evaporates and a rigid ethylcellulose film forms around the core material. The microcapsules obtained after stirring overnight was collected in 280 mesh sieve and washed in water repeatedly to remove the remaining solvent and gelatin. The microcapsules were dried in a vacuum oven for 20 hours at 60℃.

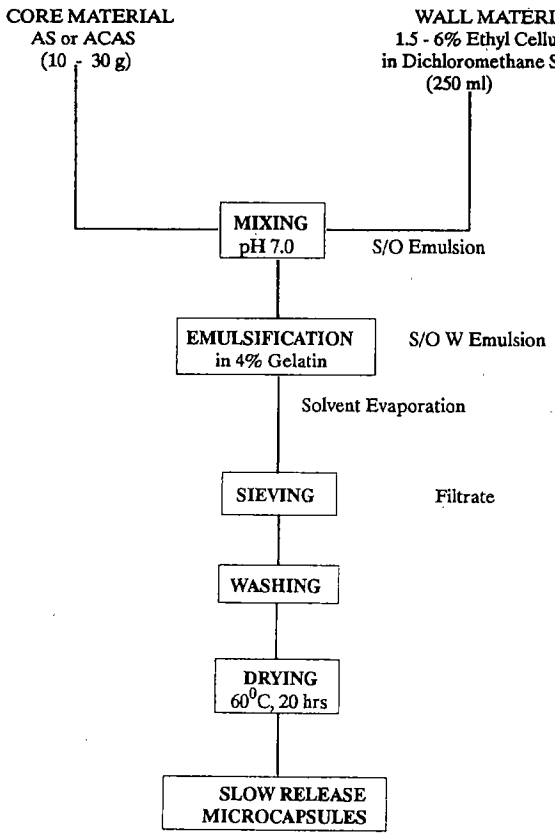

Fig. 2 Flow diagram of microencapsulation by complex emulsion method.

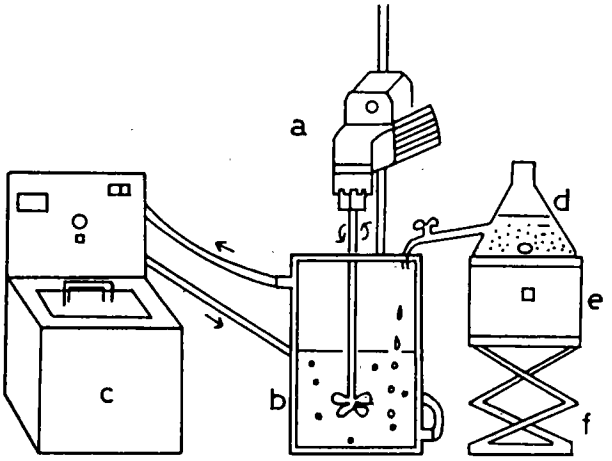

Fig. 3 Apparatus of for microencapsulation:
a. Stirrer
b. Stainless Steel Reactor
c. Thermostat
d. Core Material Dispersion in Polymer Solution
e. Magnetic Stirrer
f. Laboratory Jack

2. 3 Dissolution Test for NH_4^+ Release

Two (2) grams of the microcapsules were added to one (1) liter of distilled water, the pH of which was initially adjusted to 7.0 in a screw cap bottle. The bottle was placed in an incubator maintained at 30℃. Five (5) ml aliquot was taken at fixed times, then filtered and the NH_4^+ concentration was determined.

2. 4 Experimental Analysis

The concentration of NH_4^+ was determined by Automated Phenate Method using the Technicon Auto Analyzer II. Internal surface area of activated carbon was measured by Quantasorb OS-81 (Quantachrome Co.) and

calculated by means of the BET method. Methylene blue adsorbability of activated carbon was measured using the method introduced by Ishibashi et.al. (Ishibashi et. al., 1973).

2. 5 Particle Size Analysis

Particle size analysis of the microcapsules was carried out using stainless steel U.S. Standard sieves. One hundred grams of microcapsules were placed on the top sieve and the set of sieves was placed in a sieve shaker and shaken for 30 minutes. The microcapsules retained on each sieve were weighed. Then cummulative percentage retained on a probability scale versus the average particle size retained on each sieve was determined in order to get the average particle size (50% size) for each batch of microcapsules.

2. 6 Scanning Electron Microscope (SEM)

The photograph of the microcapsules containing ACAS as core material was taken by JEOL JSM T-20 using 100X and 3,500X magnifications.

3. Results and Discusion

3. 1 Yield

Table 1 shows the percentage yield of microcapsules prepared under varying concentrations of ethylcellulose and weight of AS as core material. Experiments with increased ethylcellulose concentration produced an increasing product yield; on the other hand, increasing core material weight resulted in the decrease of product yield. Ethylcellulose is the polymer selected as the wall material to coat the core materials (AS) used in the preparation of microcapsules. It can be seen that with an increase of the amount of ethylcellulose a greater amount of microcapsules is produced while an increase of the amount of core materials produce less amount of microcapsules, the excess core materials going with the discarded liquor.

However, in any case, the yield was low because the core material is a water soluble substance and the emulsification process was not so efficient producing microcapsules ranging from 8 to 51% yield only.

The product yields from the experiments using ACAS as core material shown in Table 2 were ranged from 97 to 100% under the conditions used. The activated carbon adsorbed the core material and prevented the loss of the nutrients during the process and therefore produced higher yield in comparison with the experiment using AS. It can be seen that the method using ACAS is more efficient compared with the method using AS for the core material of microcapsules.

Table 1. Effect of Etcell Concentration and Weight of AS on the Yield of Microcapsules Produced.

Etcel Conc.% (w/v)	Wt. of AS (g)	Yield (%)
1.5	10	17.7
1.5	15	15.6
1.5	30	8.4
3.0	10	34.7
3.0	15	31.6
3.0	30	18.1
6.0	10	51.5
6.0	15	43.1
6.0	30	30.2

Note: Stirring Velocity = 280 rpm.

Table 2. Effect of Etcell Concentration and Weight of ACAS on the Yield of Microcapsules Produced.

Etcel Conc.% (w/v)	Wt. of AS (g)	Yield (%)
3.0	10	99.8
3.0	20	98.8
3.0	30	98.9
6.0	10	97.8
6.0	20	96.6
6.0	30	100.0

Note: Stirring Velocity = 500 rpm.

Legend: Etcell - Ethyl Cellulose
AS - Ammonium Sulfate
Y - Yield of Microcapsules
ACAS - Activated Carbon with Ammonium Sulfate Adsorbed

3. 2 Dissolution Test

Microcapsules with different core materials like AS and ACAS were investigated in order to determine whether there was a relationship between the dissolution rate of AS and ACAS and the microcapsules prepared with varying amount of core material.

Figure 4 shows the result of dissolution test conducted on microcapsules prepared using 10, 15, 30 grams of AS as core material and 6% ethylcellulose as wall substance. It can be observed that the increasing core material weight resulted in the increased NH_4^+ concentration.

However, under any condition, the microcapsules showed rapid release characteristics as such that NH_4^+ concentration reaches its maximum value within 2 or 3 hours of dissolution test. The nutrient content of the microcapsules was released rapidly in the few hours of the dissolution test because the rate of release depends on the amount of coating of the microcapsules.

Figure 5 shows the result of dissolution test conducted on microcapsules prepared using 10, 20 and 30 grams of ACAS as core material and 6% ethylcellulose as wall substance. It can be seen that the amount of the nutrient release increased as the amount of the core material weight was increased.

The dissolution curve of the microcapsules using ACAS as core material exhibited gradual release pattern when the maximum amount of nutrient released by the microcapsules was attained during the 20 days period.

A significant difference exists in the dissolution tests conducted between those microcapsules using AS and ACAS as shown in Figures 4 & 5. It proves that the method using ACAS is more efficient than when AS is used. The type of core material also greatly influences the rate of nutrient release in the microcapsule.

3. 3 Particle Size Distribution

The particle size distribution of the microcapsules obtained at varying ethylcellulose concentration of 3 to 6% (w/v) and core material weight (ACAS) of 10 to 30 grams with a stirring velocity of 500 rpm is shown in Figure 6. This figure shows that the increase of the core material weight from 10 to 30 grams gives a narrower range of microcapsule size distribution. The average particle size of the microcapsules obtaind ranged from 0.5 to 1.0 mm. However, varying concentration of the ethylcellulose has no significant effect on the particle size distribution of the product.

3. 4 SEM

Figure 7 shows the SEM photograph of microcapsules prepared using ACAS as core material. The photograph of the microcapsule was taken using 100x magnification and the pore structure was taken at 3500x magnification. It describes the porous structure of the microcapsule wall. This pore structure was caused by solvent evaporation during the microencapsulation process and in these pores, the nutrients were diffused during the

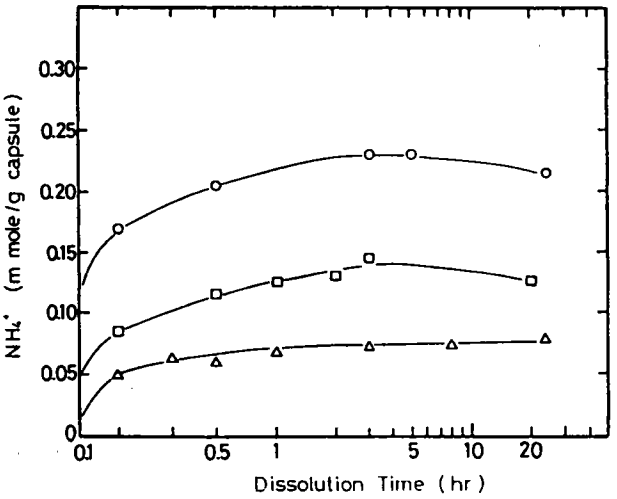

Fig. 4 Dissolution of NH_4^+ from microcapsules prepared using 6% ethylcellulose solution at various weights of AS: △, 10g; □, 15g; ○, 30g.

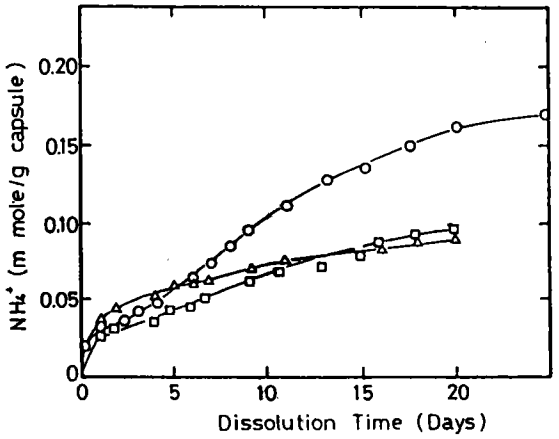

Fig. 5 Dissolution of NH_4^+ from microcapsules prepared using 6% ethylcellulose solution at various weights of AS: △, 10g; □, 20g; ○ 30g.

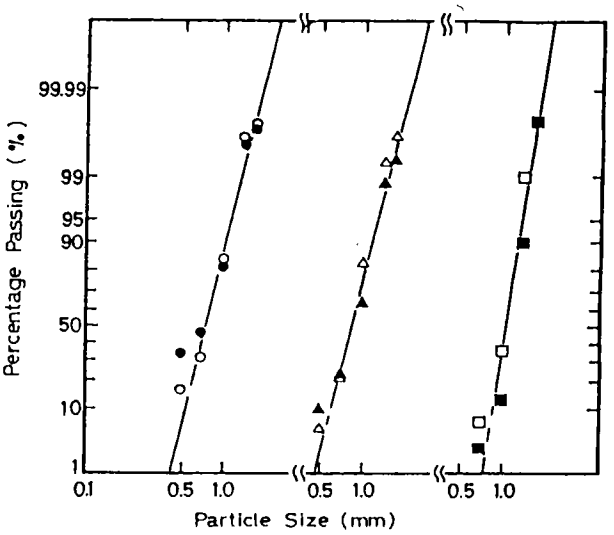

Fig. 6 Correlation between the particle size of the microcapsules and varying ethylcellulose concentration/core material weight:
○, 3%,10g △, 3%,20g □, 3%,30g
●, 6%,10g ▲, 6%,20g ■, 6%,30g

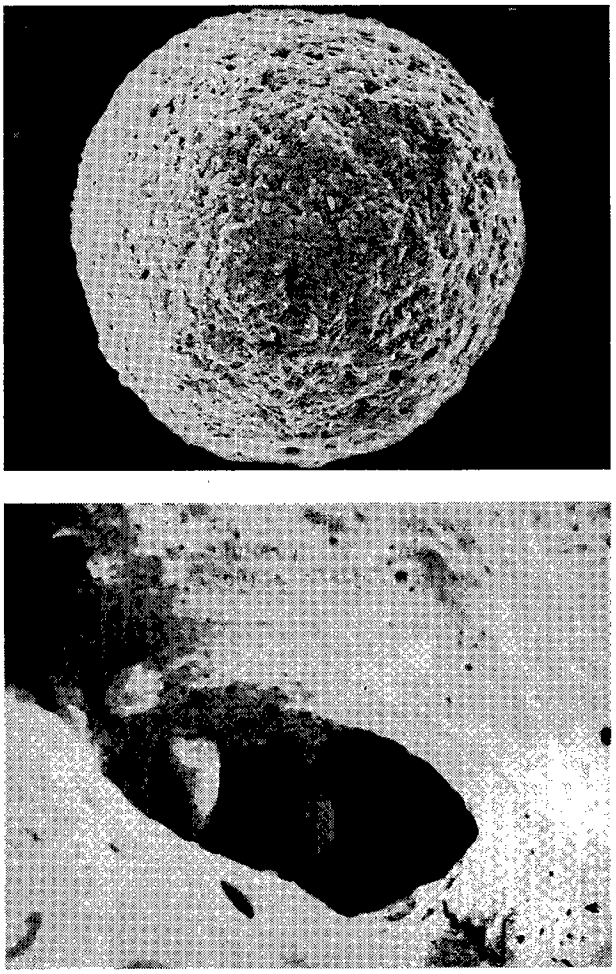

Fig. 7 SEM photograph of microcapsules prepared using ACAS.

dissolution test of the microcapsules. The pore structure of the microcapsule is another factor that may influence the dissolution rate of the samples.

4. Conclusion

Nutrient containing slow release microcapsules were prepared by complex emulsion method using AS and ACAS as core materials and ethylcellulose as wall substance. The use of ACAS as core material was found effective as evidenced by the higher yield obtained and the gradual dissolution rate of nutrient in water.

Promising results have been obtained for the preparation of nutrient containing slow release microcapsules by complex emulsion method using ACAS as core material. However, further studies for different wall substance to obtain gradual dissolution rate will be considered.

Acknowledgement

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フライアッシュをケイ酸原料とする緩効性ケイ酸カリ肥料の製造*1

（キーワード：暖効性肥料，フライアッシュ，ケイ酸カリウム）

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1．は　じ　め　に

メモ（Mae Moh）火力発電所（ランパン州）は，総出力2,025MW(1991年）でタイ国の総発電量の約25％を占める。発電用燃料は自国のメモ褐炭を使用し，石炭の年間使用量は1,600万t／年である。燃焼灰の総排出量は約480万t／年で，その内フライアッシュが約80％の380万tを占めている。

これらの大量の燃焼灰は一部分が造成，埋立に利用されているのみで，大部分は石炭採掘後に埋め戻されているのが現状である。フライアッシュの有効利用法には緩効性ケイ酸カリ肥料，セメント混和剤，建材等がある。緩効性ケイ酸カリ肥料[1]は我が国が1978年に世界で初めて開発したもので，現在商業化されている[2]。しかし，発電所から排出される種々のフライアッシュの肥料化に関する研究や特許もあり[3)-10)]，その他，数多くの研究例をとっても一様に肥料化するのは難しく，その製造法が確立されているとはいえない。その理由は，使用炭の種類，火力発電所の運転条件等によって，フライアッシュの化学組成および物理的性質に大きな差異があり，あるいは，貯蔵条件によって化学組成が変質するためと考える。

本研究は，上記火力発電所から排出するフライアッシュの緩効性ケイ酸カリ肥料製造の焼成条件，添加物の効果を検討した。

2．メモ火力発電所のフライアッシュ

採取した8種類のフライアッシュの化学組成を**Table 1**に示す。化学組成の分析方法は蛍光X線によるファンダメンタルパラメーター法（FP法）を用いた。蛍光X線は島津製作所製SXF-1200型，X線管 DEG-76H，Rn，X線パワー40kW－70mAで測定した。

主成分はSiO_2，CaO，Al_2O_3，Fe_2O_3，MgO，K_2O，Na_2O，SO_3で，他にTiO_2，MnO，P_2O_5，BaO等の微量成分が含まれる。成分的にはSiO_2，CaO，Al_2O_3，Fe_2O_3の含有量に大きな差異が認められる。この理由は，選炭技術によるものと考えられるが，はっきりとしたことは分からない。また，SO_3含有量が約5～30％と日米の～4％に比べて非常に高い。ガラス質成分は多いもので78～85％，少ないもので47～53％である。ガラス質成分の定量はOhlbeg, StricklerらのX線回折によるガラス質定量方法によった[11)]。

肥料化製造試験には，肥料化に重要な成分と考えられるSiO_2，CaO，ガラス質（非晶質の）の含有量に差があるフライアッシュ（FA）No.1およびNo.2を用いた。

FA No.1，FA No.2の走査電子顕微鏡写真（日本分光製，JSM-T20型）を**Fig. 1**に示す。FA No.1はほぼ完全な球状をしているものが大部分であるが，FA No.2は形状が不整で表面が部分的に溶融した形跡が認められる。

Fig. 2に両フライアッシュの熱分析（理学電気製，8112RH型）の減量曲線および示差熱曲線を示す。FA No.1の約700℃までの約4％の減量（TG）は，フライアッシュ中の残留未燃炭素の燃焼および$Ca(OH)_2$の分解によるものである。温度1,150℃以上での減量は，主に$CaSO_4$の分解による。FA No.2は，温度1,050℃以下では残留未燃炭素および鉱物の分解による減量が認められない。温度1050℃以上での減量は，FA No.1の場合と同様に主として$CaSO_4$の分解によるものである。

X線回折パターンを**Fig. 3**に示す。両フライアッシュに共通して同定できる鉱物は，$Al_6Si_2O_{13}$，α-SiO_2，CaO，$CaSO_4$，Fe_2O_3である。また，FA No.2には$CaCO_3$，$Ca(OH)_2$の鉱物が認められるがFA No.1では認められない。FA No.1の回折線2θ＝35～15°はFA No.2よりブロードであることからも，かなりガラス質成分に富んでいることが分かる。したがって，FA No.1の場合は上記の鉱物のかなりの部分がガラス質中に分散して存在しているものと思われる。以上の結果から，FA No.2は，FA No.1に比べると高温または長時間の熱履歴を受けたものと考えられる。

3．実　験　方　法

3・1　焼成用試料調整

フライアッシュはフルイを通して60mesh以下の粒径

*1. 資源と素材，Vol.110，No.6（1994）pp.493-498 より転載（資源・素材学会より転載許可）
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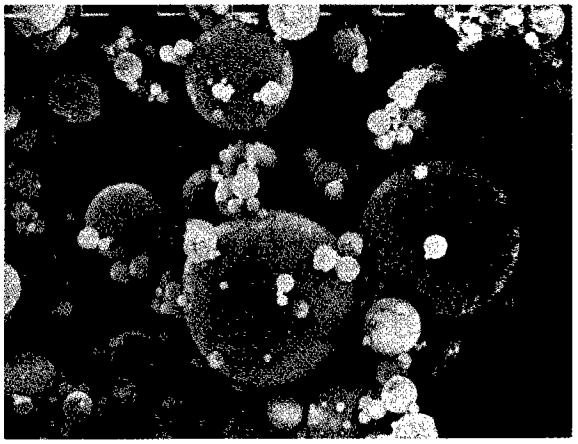
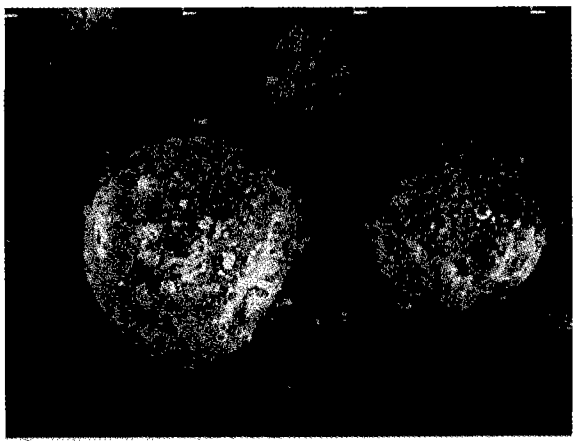
Table 1 Chemical composition of fly ash.

Constituents (%)	Fly ash No.							
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8
SiO_2	21.26	46.50	48.60	33.70	40.78	25.90	17.80	25.50
Al_2O_3	12.60	27.00	25.90	17.60	22.50	14.50	6.67	11.40
Fe_2O_3	15.80	10.50	10.30	9.71	10.10	10.70	16.90	20.20
CaO	16.60	4.01	4.21	18.70	7.43	21.70	19.80	13.20
MgO	4.59	2.63	1.70	2.52	3.79	3.59	3.75	5.06
K_2O	1.11	2.27	2.26	1.67	2.06	1.41	0.62	1.12
Na_2O	2.32	1.04	0.76	0.79	1.20	1.41	2.47	1.99
SO_3	23.50	4.99	5.03	14.60	10.20	18.90	31.20	20.10
Glass	78 - 85	47 - 53	70 - 73	70 - 75	68 - 70	50 - 55	63 - 70	75 - 80



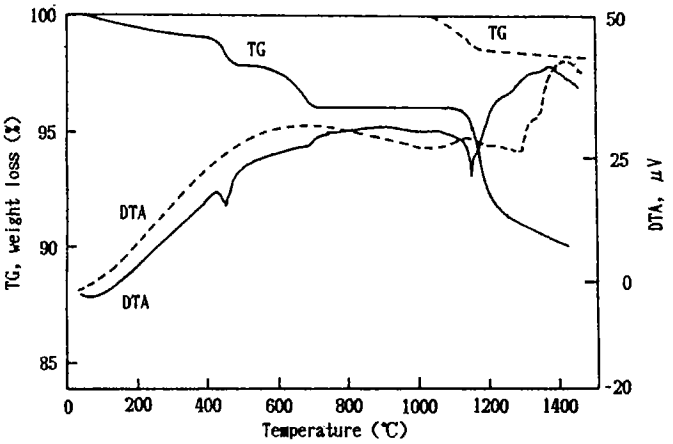
Fly ash No. 1 Magnification : × 1,000 L 100 μmL



Fly ash No. 2 Magnification : × 300 L 1,000 μmL

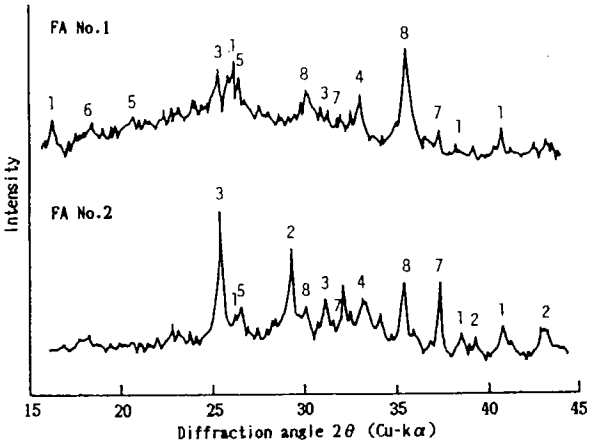
Fig. 1 Scanning electoron micrographs of Thai fly ash.

のものを使用した。FA No.1，FA No.2の両フライアッシュに，肥料三要素の一つであるカリウム（特級試薬K_2CO_3を使用）をK_2O換算で20%を混合調整し，適性焼成温度，焼成時間を検討するための供試試料とした。さらに，可溶化率，クエン酸溶化率を高める目的で，カルシウム（特級試薬$Ca(OH)_2$を使用）をCaO換算で5～30%，カリウムをK_2O換算で5～30%およびマグネシウム（特級試薬$MgCO_3$を使用）をMgO換算で2～15%の範囲で添加量を変えて調整し，その効果を調べるための供試試料とした。



Ref. sample : α - Al_2O_3. Program rate : 10 ℃/min,
Atmosphere : air, — : FA No. 1, — — : FA No. 2

Fig. 2 Thermal analysis of Thai fly ash.



1 : $Al_6Si_2O_{13}$, 2 : $CaCO_3$, 3 : $CaSO_4$, 4: α - Fe_2O_3,
5 : α - SiO_2, 6 : $Ca(OH)_2$, 7: CaO, 8 : Fe_3O_4

Fig. 3 X-ray diffraction pattern of Thai fly ash.

3・2 焼成試験

上記の混合試料10gを直径5 cm,深さ1 cmの磁製皿に入れ，あらかじめ設定した所定の温度（800～950℃）に保持したシリコニット電気炉で，所定の時間（10～40分）焼成した。焼成後は直ちに炉から取り出して空気中で冷

却した。

3・3　焼成物の溶出試験

焼成物は公定肥料分析法[12)13)]により，粉砕して60meshのフルイを通した試料1gに0.5mol·dm^{-3}塩酸，2％クエン酸または水を加え，30℃で1時間振とう（回転数：30～40回転／min）して各成分を溶出させた後速やかに常温に戻し，一定量まで水を加えて直ちに乾燥ろ紙でろ過した。次いで塩酸可溶性SiO_2（可溶化率）（％）を求めるために0.5mol·dm^{-3}塩酸で溶出したろ液をイオンクロマトグラフ装置（ダイオネックス製2020i型）で測定した。また2％クエン酸および水で溶出したろ液を原子吸光光度計（日立製作所製，170-30型）で測定し，K_2Oクエン酸溶出率（ク溶化率）（％）およびK_2O水溶出率（％）とした。

3・4　焼成物の生成鉱物の同定

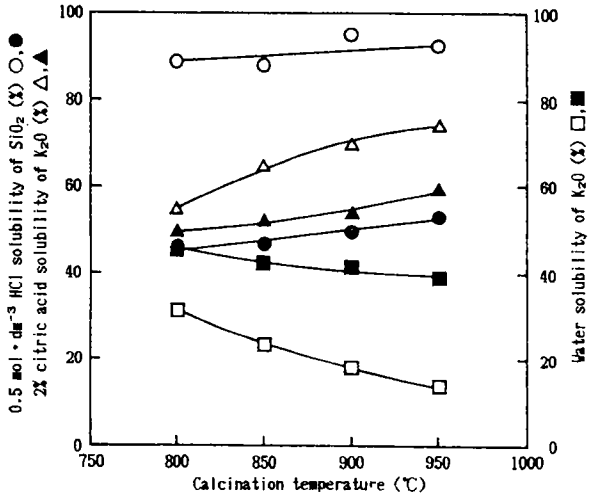
X線回折装置（理学電気製ガイガーフレックス）用いて，焼成物および0.5mol·dm^{-3}塩酸，2％クエン酸溶出の残渣物の回折線を測定し，焼成物中の鉱物の同定と酸溶出試験結果との関係を推察した。

4．結果と考察

4・1　焼成温度と可溶化率およびク溶化率の関係

K_2CO_3をK_2O換算で20％混合して調整したFA No.1（K_2O：22.11％，CaO：11.60％），FA No.2（K_2O：22.27％，CaO：4.01％）の焼成温度と可溶化率およびク溶化率の関係を**Fig. 4**に示す。

可溶化率，ク溶化率は，FA No.1，FA No.2ともに焼成温度が高くなると微増し，水溶性カリウム（％）は低くなる。FA No.1の場合，焼成温度950℃，焼成時間20分の条件で可溶化率は92％，ク溶化率は75％，水溶性カリウムは14％で，FA No.2の場合の可溶化率は53％，ク溶化率は60％，水溶性カリウムは39％である。

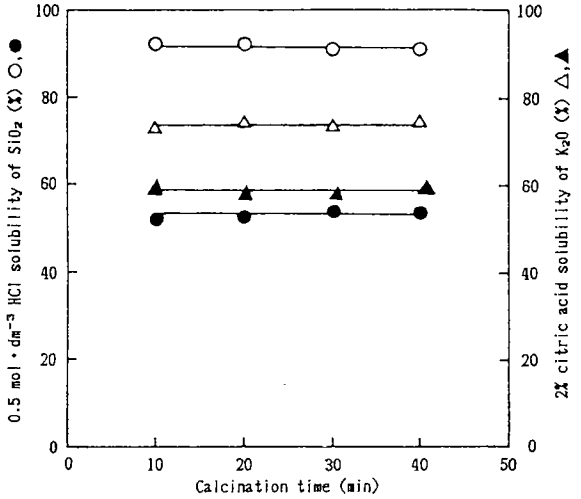


○, △, □: FA No. 1, ●, ▲, ■: FA No. 2, calcination time: 20 min

Fig. 4　Change of SiO_2 and K_2O solubility by calcination

4・2　焼成時間と可溶化率およびク溶化率の関係

両フライアッシュの焼成物の焼成時間と可溶化率およびク溶化率の関係は，**Fig. 5**に示すように10～40分の範囲でほとんど差がないので，以後の焼成試験はすべて20分で行った。



○, △ 950℃: FA No. 1, ●, ▲: FA No. 2, calcination temperature: 950℃

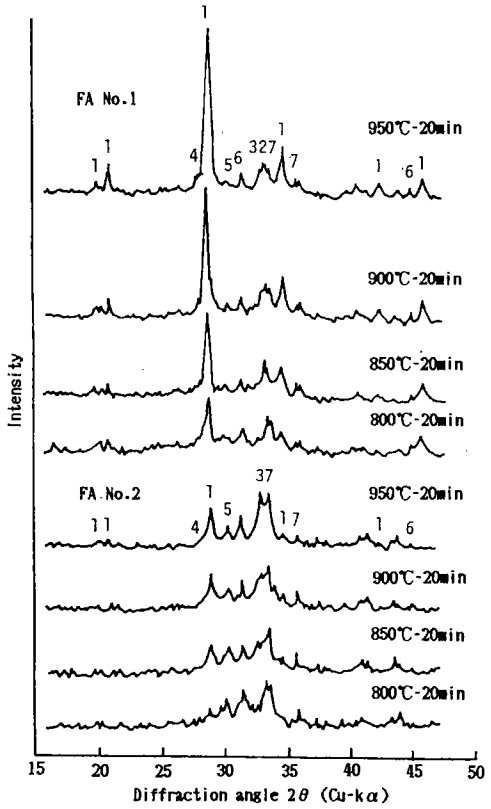
Fig. 5　Change of SiO_2 and K_2O solubility by calcination

4・3　生成鉱物とその溶解性

焼成反応によって生成する各種鉱物のX線回折を**Fig. 6**に示す。焼成物の主要な鉱物の最強ピークは，$2\theta=28.55°$，$2\theta=33.3°$および$2\theta=32.9°$に認められそれぞれ，$K_2Al_2Si_2O_8$（あるいはAl_2O_3の一部をFe_2O_3が置換した$K_2(Al, Fe)_2Si_2O_8$が生成している可能性がある。しかし，この鉱物は$K_2Al_2Si_2O_8$のX線回折線とほとんど重なるために同定が不可能である），α'-Ca_2SiO_4，K_2MgSiO_4に一致する。これらの鉱物の回折線強度は焼成温度が高いほど明瞭となる。その他に生成する鉱物として，回折線強度が強くないが$K_2MgSi_3O_8$（28.45°），$Ca_2MgSi_2O_7$（31.1°），K_2CaSiO_4（31.6°），$Ca_2Al_2SiO_7$（31.4°）が認められる。

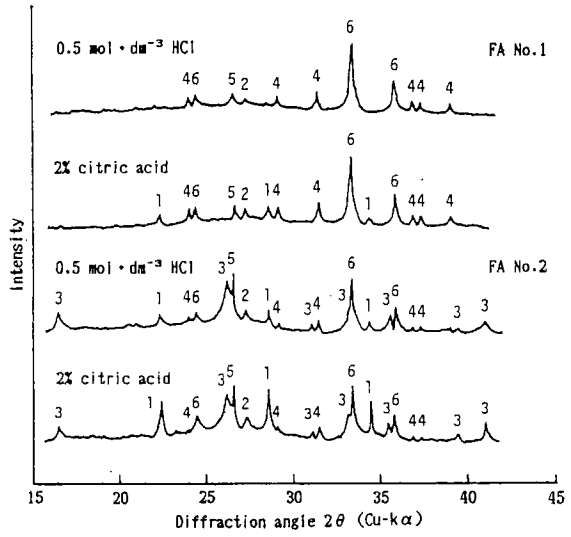
0.5mol·dm^{-3}塩酸，2％クエン酸溶出残渣物のX線回折を**Fig. 7**に示す。酸溶出残渣物には，未反応のα-SiO_2，α-Fe_2O_3，$Al_6Si_2O_{13}$の他に焼成反応で生成した$K_2MgSi_3O_8$，$K_2Al_2Si_4O_{12}$，$Ca_2Al_2O_3$が認められる。Fig. 6と比較すると，これらの鉱物は酸に難溶あるいは不溶性である。しかし，FA No.1，FA No.2の酸溶出残渣物の回折線を比較すると，$K_2MgSi_3O_8$は0.5mol·dm^{-3}塩酸にはかなり溶ける鉱物と考えられる。

主な酸溶解性生成鉱物の回折強度と焼成温度との関係



1 : $K_2Al_2Si_2O_8$ or $K_2(Al, Fe)_2Si_2O_8$, 2 : K_2MgSiO_4,
3 : α'- Ca_2SiO_4, 4 : $K_2MgSi_3O_8$, 5 : $Ca_2MgSi_2O_7$,
6 : K_2CaSiO_4, 7 : α - Fe_2O_3

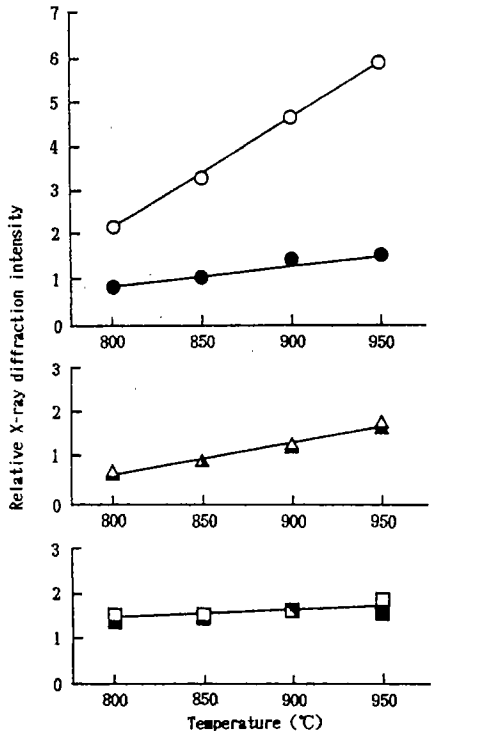
Fig. 6 X-ray diffraction pattern of the calcined products with varying calcination temperature.



1 : $K_2MgSi_3O_8$, 2 : $K_2Al_2Si_4O_{12}$, 3 : $Al_6Si_2O_{13}$, 4 : $Ca_2Al_2O_3$,
5 : α - SiO_2, 6 : α - Fe_2O_3

Fig. 7 X-ray diffraction pattern of the 0.5mol・dm⁻³ HCl and 2% citric acid insoluble residue of the produsts obtained at 950℃-20min.

を**Fig. 8** に示す。$K_2Al_2Si_2O_8$, α'-Ca_2SiO_4 は焼成温度が高くなるとともに回折強度が強くなり，$K_2Al_2Si_2O_8$ は特にFA No.1 の場合に顕著である。これは結晶性の



○, △, □ : FA No. 1, ●, ▲, ■ : FA No. 2,
○, ● : $K_2Al_2Si_2O_8$, △, ▲ : α'- Ca_2SiO_4, □, ■ : K_2MgSiO_4

Fig. 8 Formation of valious compounds by calcination.

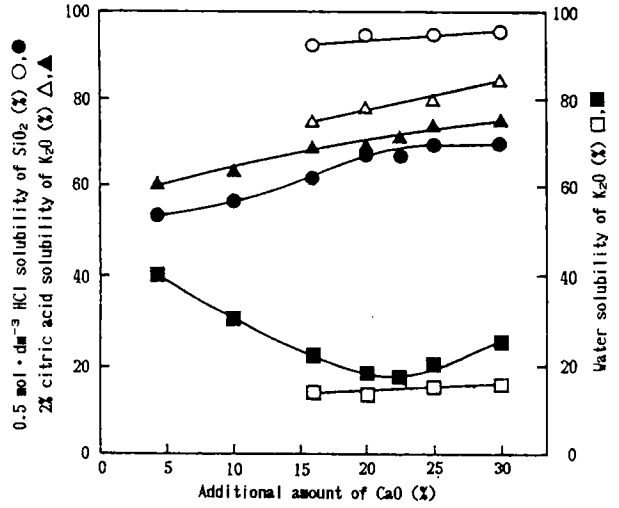
SiO_2, $Al_2Si_2O_{13}$ に比べて，ガラス質中に反応性の高い2鉱物が多く含まれているためと考えられる。また，K_2MgSiO_4 の回折強度は，両フライアッシュともにほとんど変化が認められなかった。

以上の結果から，焼成温度800～950℃におけるフライアッシュと添加したK_2CO_3との反応は，種々の成分系で起こっている。しかし，ケイ酸を含む可溶性鉱物の主な生成反応は，K_2O-Al_2O_3-SiO_2 の3成分系，K_2O-MgO-SiO_2 の3成分系および CaO-SiO_2 の2成分系である。一方，カリウムを含むク溶性鉱物の主な生成反応は K_2O-Al_2O_3-SiO_2 の3成分系，K_2O-MgO-SiO_2 の3成分系である。カリウムおよびカルシウムを含む生成鉱物としては K_2CaSiO_4 が認められが，Fig. 6 と Fig. 7 から判断するとこの鉱物は溶出試験に用いた酸には溶解しないと推察される。したがって，カリウムを含むク溶性鉱物の生成反応にはカルシウムがほとんど関与していないと考えられる。

4・4 カルシウム添加の効果

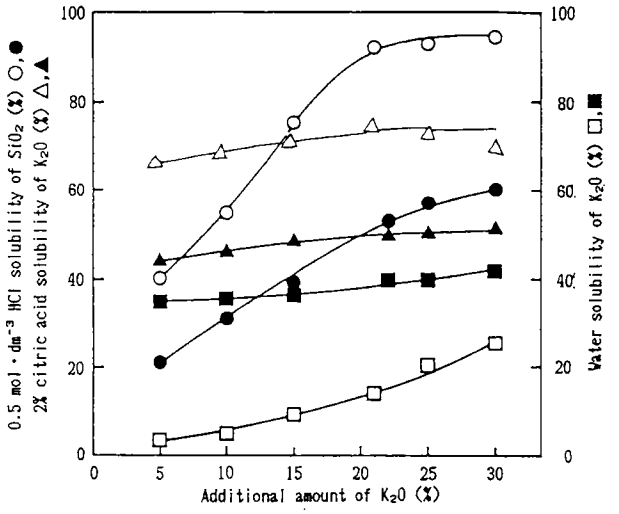
可溶化率およびク溶化率を高める目的で，カルシウム添加の効果を検討した。両フライアッシュにK_2CO_3をK_2O 換算で20％を加え，$Ca(OH)_2$をCaO換算で5～30％まで変えて調整した各混合物を950℃，20分の条件で焼成し，その結果を**Fig. 9** に示す。

FA No.2 焼成物の可溶化率とク溶化率，FA No.1 焼



○, △, □: FA No. 1, ●, ▲, ■: FA No. 2, calcination temperature: 950 ℃, calcination time: 20 min

Fig. 9 Effect of the addition of CaO(%)added on the SiO_2 and K_2O solubility.



○, △, □: FA No. 1, ●, ▲, ■: FA No. 2, calcination temperature: 950 ℃, calcination time: 20 min

Fig. 10 Effect of the addition of K_2O(%)added on the SiO_2 and K_2O solubility.

成物のク溶化率はCaO添加量が増すと増加し，添加量30％では約10％向上した。しかし，FA No.1の可溶化率に対してはカルシウム添加の効果が低い。これは，その可溶化率が92％に達しているため，未反応のSiO_2量が少ないためであろう。また，フライアッシュ中のAl_2O_3，MgOの含有量にも関係している。FA No.2の水溶性カリウム（％）は，カルシウム添加量が増すと減少するが，さらに増すと反対に水溶性カリウム（％）が増加する。FA No.1の場合も同様に，カルシウム添加量が約15％以上では微増する。これらの詳細な原因については今後の検討課題とした。

4・5　カリウム添加の効果

カルシウム添加の場合と同様の目的で，カリウム添加の効果を検討した。両フライアッシュにK_2CO_3をK_2O換算で5～30％まで変化させて混合し，950℃，20分の条件で焼成した。その結果を**Fig.10**に示す。

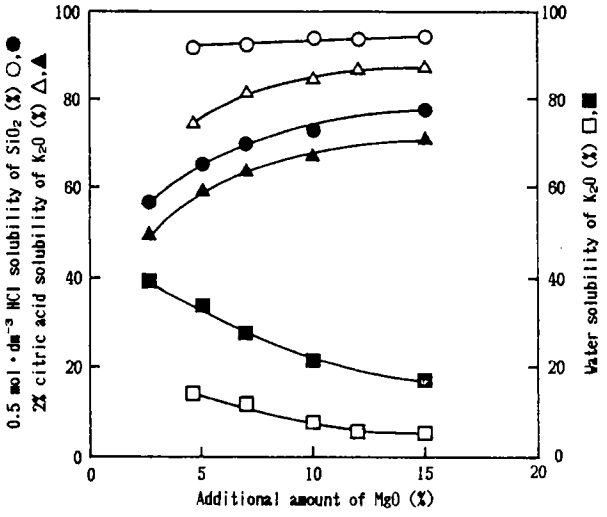
両フライアッシュの可溶化率は，カリウム添加の効果が認められるが，ク溶化率に対してはその効果が低い。また，水溶性カリウムは，添加量が増すとともに高くなる。この理由は明らかでないが水溶性カリウムすなわち未反応のカリウムが多く残っていることから推測すると，カリウムを含むク溶性鉱物の生成に必要なMgOの絶対量あるいはフライアッシュ中のMgOおよびAl_2O_3の存在形態による反応性に関係していると考えられる。したがって，SiO_2，K_2CO_3に対してMgO，Al_2O_3の含有量が少ないフライアッシュの場合には，MgO，Al_2O_3を添加して$K_2Al_2Si_2O_8$，K_2MgSiO_4を多く生成させてク溶化率を高め，反対に水溶性カリウム（％）を低くさせることが可能と思われる。

以上，カルシウム，カリウム添加の効果および酸溶解性生成鉱物のX線回折（Fig. 8）の結果から推察すると，FA No.1の主反応は，カルシウムが関与しない$K_2Al_2Si_2O_8$とK_2MgSiO_4が生成する反応である。また，カルシウム添加実験で，FA No.1の水溶性カリウム（％）の増加量（Fig. 9）がFA No.2より低いのは，主にカリウムが関与する上記の反応であることと一致している。一方，FA No.2は，カリウムが反応に関与しないケイ酸塩鉱物であるα'-Ca_2SiO_4，$Ca_2MgSi_2O_7$，$Ca_2Al_2SiO_7$，$Ca_2MgSi_2O_7$が多く生成する反応である。したがって，FA No.2のク溶化率が低く，反対に水溶性カリウム（％）が高い理由は，上記の鉱物の生成にSiO_2の多くが消費されるためにカリウムと反応するSiO_2が少なくなり，フライアッシュのガラス質中に存在する未反応のK_2CO_3の量が増えるためであろう。これは，フライアッシュ中に含有するケイ酸，アルミニウム，カルシウムの存在形態とその含有量による反応性の違いと考えられる。

4・6　マグネシウム添加の効果

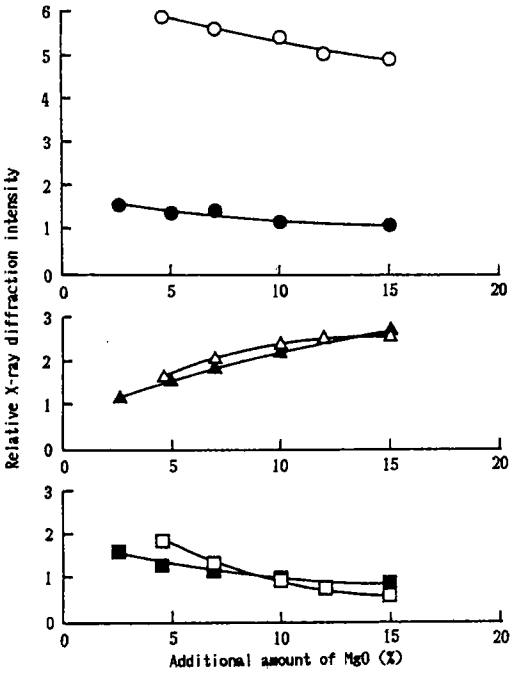
$MgCO_3$を用いてMgO換算で～15％まで変化させて混合し，950℃，20分の条件で焼成した。その溶出試験結果を**Fig.11**および主な酸溶解性生成鉱物のX線回折強度を**Fig.12**に示す。

FA No.1可溶化率はマグネシウム添加の効果がほとんど認められないが，FA No.2では添加量15％で約78％に達した。ク溶化率はマグネシウム添加量が増すと増加し，添加量15％でFA No. 1の場合に87％，FA No.2の場合に71％に達した。水溶性カリウム（％）は，マグネシウム添加量15％でFA No.1の場合に6％およびFA No.2の場合に17％に減少し，マグネシウム添加の効果が大きいことが判明した。この結果は**Fig.12**に示すよう



○, △, □: FA No. 1, ●, ▲, ■: FA No. 2, calcination temperature: 950 ℃, calcination time: 20 min

Fig. 11 Effect of the addition of MgO(%)added on the SiO_2 and K_2O solubility.



○, △, □: FA No. 1, ●, ▲, ■: FA No. 2,
○, ●: $K_2Al_2Si_2O_8$, △, ▲: K_2MgSiO_4, □, ■: α-Ca_2SiO_4

Fig. 12 Formation of valious compounds by calcination.

に$K_2Al_2Si_2O_8$とα'-Ca_2SiO_4の回折線強度が弱くなっているに対して，K_2MgSiO_4の回折強度が強くなっていることと一致する。すなわち，$K_2Al_2Si_2O_8$とα'-Ca_2SiO_4の生成量が減少するために，添加したマグネシウムと未反応のSiO_2，K_2CO_3が反応してK_2MgSiO_4の生成量が増加するためである。

以上，緩効性ケイ酸カリ肥料製造に関する焼成条件や添加物の効果について検討した結果について述べたが，緩効性ケイ酸カリ肥料製造の重要な主目的は植物に対して不可給態であるフライアッシュ中のSiO_2を可給態にして植物が吸収しやすくすることと，水に溶けやすく溶脱，流亡性（3～10%）の通常のカリ肥料（塩化カリウム，硫酸カリウム）を水に難溶性で雨水，潅水による溶脱，流亡をできるだけ少なく，性状を安定にして肥効に持続性を持たせることにある。したがって，水溶性カリウム（%）が反対に高くなる焼成条件，原料の配合条件は好ましくないといわれている。

実験結果を総括すると，FA No.1タイプが肥料化しやすいといえる。しかし，ク溶化率をより高めるには化学成分としてFA No.2と同程度のSiO_2，Al_2O_3含有量であることが望ましい。また，マグネシウムを添加することによって水溶性カリウム（%）を低くしク溶化率を高めることができる。

5．ま　と　め

タイのメモ火力発電所から排出する性状の異なる2種類のフライアッシュを試料として肥料化について検討した結果，以下の知見が得られた。

1）カルシウム含有量（CaO：16.60%),ガラス質成分（78～85%）が多いFA No.1は，可溶化率が92%，ク溶化率が75%に達した。しかし，カルシウム含有量（CaO：4.01%）とガラス質成分（47～53%）が少ないFA No.2では，可溶化率が53%，ク溶化率が60%であった。

2）可溶性，ク溶性を示す主な生成鉱物は，$K_2Al_2Si_2O_8$，α'-Ca_2SiO_4およびK_2MgSiO_4である。

3）カルシウム含有量が少ないFA No.2の場合はカルシウムを添加して水溶性カリウム（%）を低くさせることができる。しかし，CaOが約22%以上では反対に高くなる。

4）カリウム添加は，両フライアッシュともに可溶化率を向上させるが，添加量を増すほど水溶性カリウム（%）が高くなる。

5）水溶性カリウム（%）を低くするには，マグネシウムを添加するのが有効である。

6）今後，得られた知見を基に緩効性ケイ酸カリ肥料の品質をさらに向上させるために，フライアッシュの性状や生成鉱物の性質および添加物の効果を考慮して，原料の配合と焼成の最適条件を検討する予定である。

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Studies on the Production of Slow Release Potassium Silicate Fertilizer Using the Internal Heat Type Fluidized Bed Reactor*1

(Key Words : Slow release potassium silicate fertiliger, Rice husk, Dolomite, Calcination)

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1. Introduction

The demand for fertilizer in agricultural countries like the Philippines has in recent years been on the increasingly upward trend side by side with the increased need to maximize production of crops particularly rice. To fill this great demand, the production of fertilizer has been increased in many parts of the world and studies are being conducted to improve the qualities of the existing ones.

This present research has for its objective the utilization of the readily available natural resources of the country such as dolomitic limestone and rice husks for the production of a new type of slow release potassium silicate fertilizer. This involves the development of a heat treatment process wherein the granulated mixture of rice husks, dolomitic limestone and potassium carbonate (K_2CO_3) were subjected to heat treatment in the fluidized bed reactor under the established CaO/SiO_2 mole ratio, calcination temperature, calcination time to produce a potassium silicate fertilizer.

The recent fertilizer technology marks the advent of the slow release type potassium silicate fertilizer which has the unique characteristics of controlled nutrient release, such that single fertilizer application results in sustained fertilization without danger of fertilizer burns. The advantages of this type of fertilizer includes savings on labor, reduced possibility of fertilizer burns and reduction in element losses through slow release at a rate corresponding to the needs of the crop.

2. Materials and Methods

2. 1 Characterization of Raw Materials

Rice Husks

Rice husks, a cellulosic fibrous, nondigestible by-product from the milling of paddy rice and which is an agro-waste, was used as SiO_2 source. Unmilled rice yields about 20% by weight of rice husks, which on combustion lose behind about 21-24% of ash composed essentially of silica (SiO_2). Raw rice husks used in the study was of the mixed variety obtained from rice mill in Carmona, Cavite.

Dolomitic Limestone

The dolomitic limestone used in the study was obtained from the Philippine Mining Corporation, Cebu City.

Molasses

Molasses, a by-product from the processing of sugarcane was procured from Paniqui, Tarlac. The molasses was used as binder in the granulation of raw materials.

2. 2 Raw Material Preparation

Pulverized samples of rice husks and dolomitic limestone were mixed with K_2CO_3 on varying proportion based on CaO/SiO_2 mole ratio. The mixture was mixed in the kneader, then passed thru an extruder. The extrudatex was subsequently passed through the pelletizer and the granules obtained were dried in the tray drier to a moisture level of 10%.

2. 3 Calcination Procedure

Calcination of granulated dried samples was carried out in a 15.5cm diameter fluidized bed reactor as shown in Figure 7. The bed was preheated by the fludizing gas from the heater with

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the temperature controlled at 500℃. Sawdust which has heating value of 4400kcal/kg was used to sustain the heat needed to attain the desired temperature of the bed. When the desired temperature was attained, the granulated sample was charged to the reactor and controlled at varying reaction time by feeding sawdust at 2.8 to 3.5kg/hr.

2. 4 Analysis Procedure

Chemical Analysis

The chemical composition of rice husks, dolomitic limestone and molasses were determined by Atomic Absorption Spectrophotometry (AAS) (2). Preparation of sample prior to analysis by AAS was done by acid decomposition using teflon digestion vessel. Digestion was done in a muffle furnace at temperature of 125℃ for two hours. Digested sample solution was allowed to cool down then saturated boric acid was added before diluting it to 100 ml mark. Sample solution was analyzed for its chemical composition using AAS.

Thermal Analysis

The thermal decomposition of rice husks, dolomitic limestone and K_2CO_3, etc. were determined from both thermogravimetric (TG) and differential thermal analysis (DTA) using Rigaku Thermal Analyzer TAS-100(5)

X-Ray Diffraction Analysis

Rice husks, dolomitic limestone and products, etc., were each subjected to X-ray diffraction analysis using Rigaku X-ray Diffractometer Model Geigerflex. The X-ray beam source was Copper (Cu), while filter is Nickel (Ni). Acceleration voltage was 40 kV, current applied 20 mA.

Identification of the compounds were done by comparison of the diffraction pattern with the American Society for Testing Materials (ASTM) powder diffraction files (4)

Dissolution Analysis

Dissolution tests on the products were carried out using 0.5M hydrochloric acid (HCl), 2% citric acid and water according to the official methods of analysis of fertilizers (1&2).

1. 0.5M HCl and 2% citric acid soluble components

One gram of pulverized and dried calcined products was weighed accurately in a 250ml volumetric flask. One hundred fifty (150) ml 0.5M HCl or 2 % citric acid solution was added at 30-40℃. The solution was shaken for one hour at 30-40 revolutions/min (rpm) in a vertical shaker while keeping the tempeature at 30-40℃ during extraction, then cooled promptly and diluted to the mark with water, and filtered immediately through a dry filter paper. Sample solution was analyzed for its percent acid soluble SiO_2, K_2O and MgO components using AAS.

2. Water soluble components

One gram of pulverized and dried calcined products was weighed accurately in a 250 ml volumetric flask. Two hundred (200)ml water was added at 30-40℃ then shaken for one hour at 30-40 revolutions/min (rpm) keeping temperature during extraction at 30-40℃. Sample was allowed to cool, then was filtered immediately through a dry filter paper. Sample solution was analyzed for percent water soluble SiO_2, K_2O, CaO and MgO components.

3. Results and Discussions

3. 1 Characterization of Raw Materials

Chemical Analysis

Table 1 summarizes the chemical composition of rice husks, dolomitic limestone and molasses as determined by TG and AAS analyses. The table shows that dolomitic limestone contains mostly CaO and MgO with traces of other components. CaO/MgO mole ratio was 2.3 which is higher compared with pure dolomite's CaO/MgO mole ratio of 1.39. The dolomitic limestone used in this study is a non-metallic mineral containing 90.9% dolomite, a double carbonate of calcium and magnesium, $CaMg(CO_3)_2$ and 4.7% calcite ($CaCO_3$).

Replicated analyses of a mixture of rice husks from different varieties (R-40, R-66, C-4, San Domeng) using AAS gave a range of SiO_2 percent composition of 20-24%.

Chemical analysis of molasses using AAS confirmed the presence of inorganic constitu-

Table 1. Chemical Composition of Raw Materials

Components	Rice Husk	Dolomitic Limestone	Molasses
SiO_2,%	21-24	1.00	0.10
CaO,%	0-12	38.00	0.36
MgO,%	0-10	16.30	0.31
K_2O,%	0.93	0.16	2.39
Al_2O_3,%	0.20	0.10	0.10
Na_2O,%	0.13	0.24	0.10
LOI,%	76.69	45.60	75.72

ents in very small amounts causing very negligible effect on the original composition of the mixture and in the heat treatment reaction.

The ignition loss of dolomitic limestone and rice husks was also determined by the Japanese Industrial Standard(JIS) method using a muffle furnace at 925℃.

Thermal Analysis

The thermal decomposition of rice husks and dolomitic limestone were determined from both TG and DTA using a Al_2O_3 as the reference sample.

Figure 1 shows the thermal decomposition of rice husks under air atmosphere. The DTA curve describes that this reaction is an exothermic decomposition reaction. The three (3) peaks corresponds to the evolution of cellulosic and

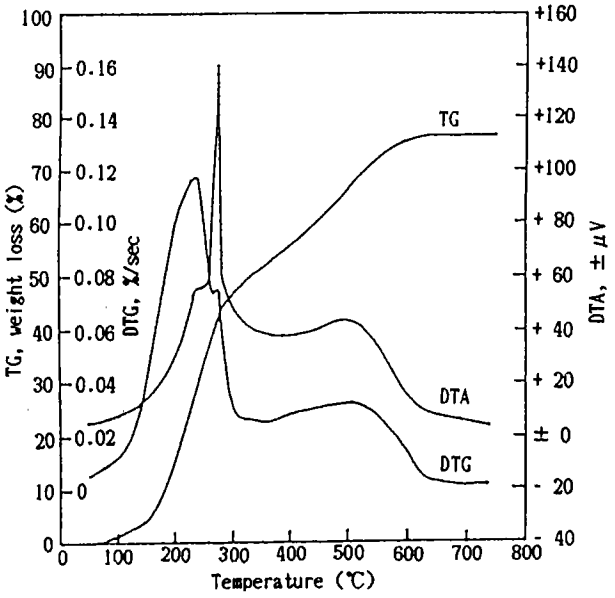


Fig. 1 Thermal analysis of the mixed rice husks

hemicellulosic matter at 200-340℃ and the degradation of lignin matter at 350-520℃.

Figure 2 shows the thermal decomposition curve of dolomitic limestone in the previous study(3) and the present study under air and CO_2 atmosphere. The curves shows a single step reaction. The decomposition of $CaCO_3$ and $MgCO_3$ in the dolomitic limestone used in the present study exist simultaneously at 808℃ in air atmosphere.

Under CO_2 atmosphere, howerer, the decomposition of these components occur at different temperatures as shown by the two DTA peaks. $MgCO_3$ decomposes faster at 793℃ while $CaCO_3$ decomposes at 886℃. Different results on DTA of this dolomite was obtained compared to

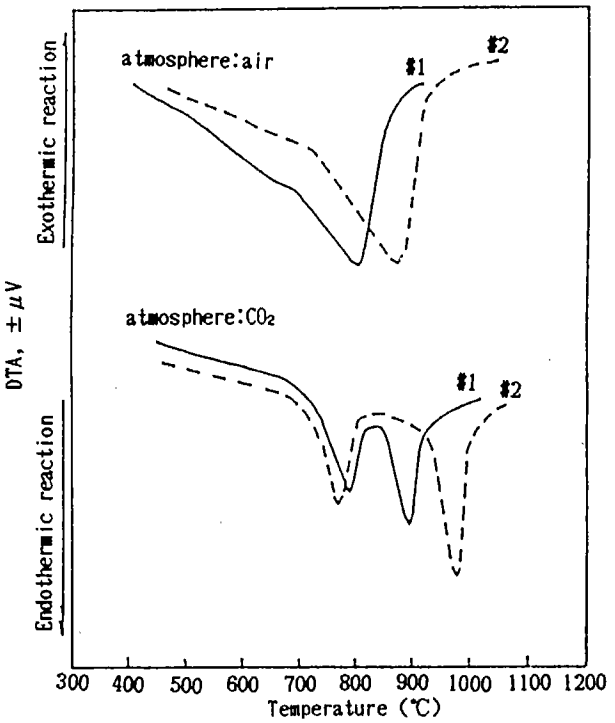


Fig. 2 Thermal analysis of the dolomitic limestone

the dolomite in the previous study(3). The DTA curve results indicates that the dolomitic limestone used in the previous study had a different composition compared to the dolomitic limestone used in the present study.

The decomposition reactions are summarized as follows:

Under air atmosphere;

	Sample #1	Sample #2[3]
$CaMg(CO_3)_2$		
$\cdots MgO+CaO+CO_2$	808℃	875℃

Under CO_2 atmosphere;

	Sample #1	sample #2[3]
$CaMg(CO_3)_2$		
$\cdots MgO+CO_2+CaCO_3$	793℃	770℃
$MgO+CaCO_3$		
$\cdots MgO+CaO+CO_2$	886℃	975℃

Figure 3 shows the effect of the addition of potassium carbonate (K_2CO_3) on the thermal decomposition of dolomitic limestone (sample #1). With the addition of K_2CO_3, the decomposition of the mixture starts at 600℃ and finishes at 746℃ regardless of the amount of K_2CO_3 added. This indicates that the presence of K_2CO_3 lowers the decomposition temperature of the mixture. Moreover, the presence of the K_2CO_3 shortens the decomposition time.

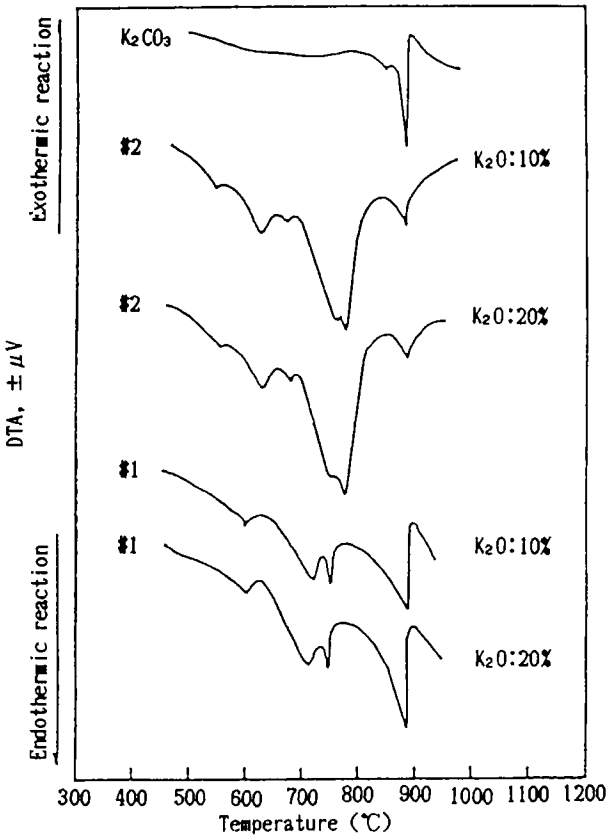


Fig. 3 Effect of addition of potassium carbonate on the thermal decomposition of dolomitic lime stone

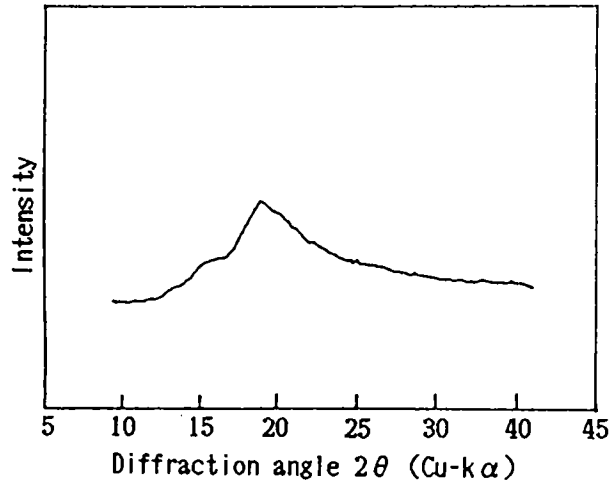


Fig. 4 X-ray diffraction pattern of the mixed rice husks

3. 2 X-ray Diffraction Analysis

Figure 4 illustrates the X-ray diffraction pattern of raw rice husks. Rice husks contain amorphous silica, which can be seen in the crystal habit of the SiO_2.

Figure 5 shows the X-ray diffraction pattern of dolomitic limestone and shows X-ray peaks

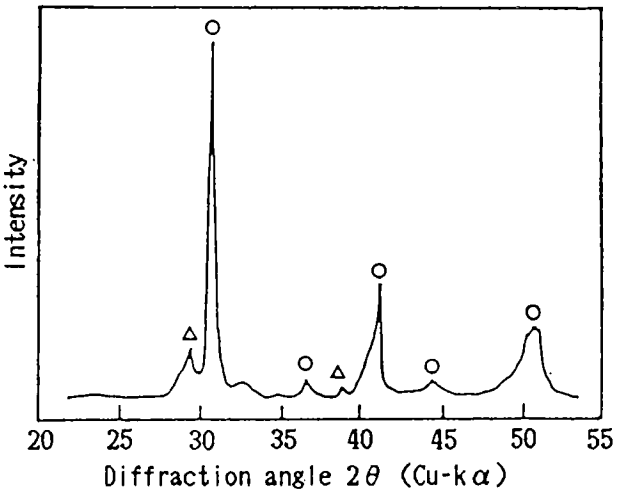


Fig. 5 X-ray diffraction pattern of the dolomitic limestone
○:$CaMg(CO_3)_2$, △:$CaCO_3$

corresponding to dolomite $CaMg(CO_3)_2$ and calcite ($CaCO_3$). The pattern shows that dolomite are the major components and calcite are detected in small quantities that corresponds to the properties of a limestone.

4. Experimentation

As shown in Table 2, a series of mixtures (No.1-6) were prepared in varying proportions of rice husk and dolomitic limestone with the addition of K_2CO_3. Samples containing varying mole ratio of CaO/SiO_2 1.05, 1.15, 1.30, 1.40, 1.60 and 1.81 were prepared as calculated based on the known concentration of SiO_2, in the rice husk, CaO, MgO in the dolomitic limestone, and K_2O in K_2CO_3 as determined previously. The sample preparation is illustrated in Figure 6.

The heat treatment was carried out using the internal heat type fluidized bed reactor as shown in Figure 7.

The fluidized bed reactor was adapted in this

Table 2. Chemical Composition of Granulated Samples

Experiment No.	Mole Ratio CaO/SiO_2	SiO_2 %	CaO %	MgO %	K_2O %
1	1.05	33.2	32.6	14.0	20.02
2	1.15	31.5	33.6	14.4	20.50
3	1.30	28.8	34.9	15.0	21.30
4	1.40	27.6	36.1	15.5	20.80
5	1.55	25.3	36.6	15.7	22.40
6	1.81	23.3	39.2	16.8	20.40

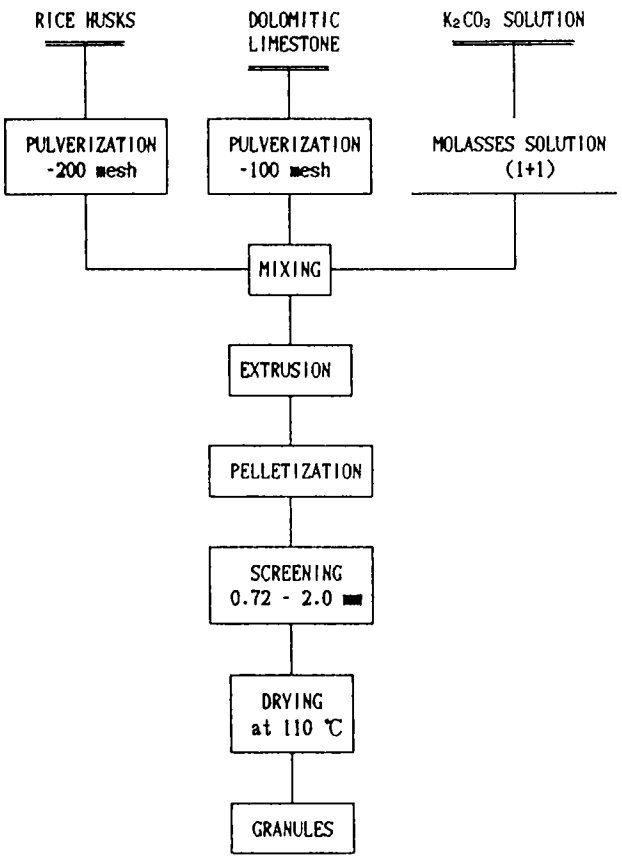
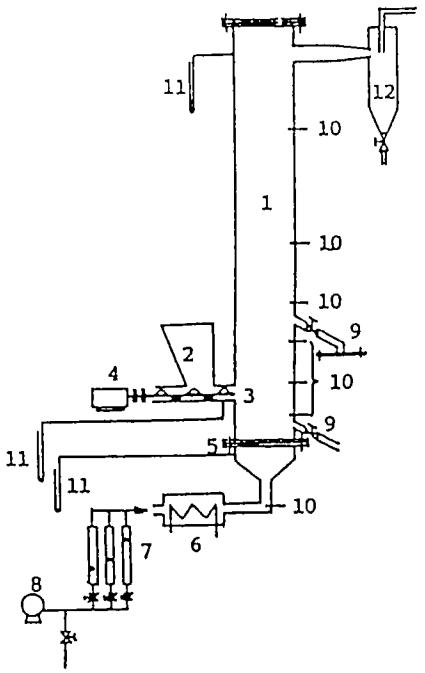


Fig. 6 Schematic diagram of sample preparation



LEGEND

1. Fluidized bed reactor	7. Flow meter
2. Hopper	8. Blower
3. Screw feeder	9. Product take off valve
4. Gear motor	10. Thermocouple
5. Perforated plate	11. Manometer
6. Preheater	12. Cyclone

Fig. 7 Schematic diagram of the internal heat type fluidized bed reactor

study owing to its great advantage over the fixed bed such as that of the muffle furnace. Foremost among this, is the fact that uniform bed temperature is easily controlled in the fluidized bed due to the intense agitation of the sample particles during fluidization. Also, the rate of heat transfer is fast in the fluidized bed because of the circulating movement of these solid particles. Under comparable condition, fluidization causes a smaller pressure drop than fixed bed operation. The charging of samples and discharging of products is convenient in fluidized bed, thus minimizing the losses of sample particularly due to rapid activity.

The internal heat type fluidized bed reactor make use of sawdust as fuel which is a much cheaper heat source than either LPG or electricity, etc.. High temperatures required for the heat treatment are quick to attain because of fast heat transfer rate.

4. 1 Effect of CaO/SiO_2 Mole Ratio

A series of experiments were conducted to determine the best conditions that would be suitable for the production of the slow release type potassium silicate fertilizer. The effect of varying mole ratio of CaO/SiO_2 was studied with the end view of maximizing the content of acid soluble SiO_2 in the product and minimizing its content of water soluble K_2O.

The sample mixtures prepared at varying CaO/SiO_2 mole ratios are ranging from 1.05-1.80 as shown in Table 2. The mixture contain 23.3 to 33.3% SiO_2 and the granulated sample was calcined at 800℃ for 20 minutes.

Results of dissolution test of samples show that the mole ratio 1.15 CaO/SiO_2 exhibits the maximum acid soluble SiO_2. The maximum acid soluble SiO_2 attained is 16% in 0.5M HCl and 11.5% in 2% citric acid. In Figure 8, acid soluble K_2O is not affected by the variation in mole ratio while the water soluble K_2O increase as the mole ratio increased.

4. 2 Effect of Varying Calcination Temperature

Further experiments on heat treatment was studied on sample mixtures containing a mole ratio of CaO/SiO_2 1.15. The samples were calcined at varying temperatures (700, 750, 800,

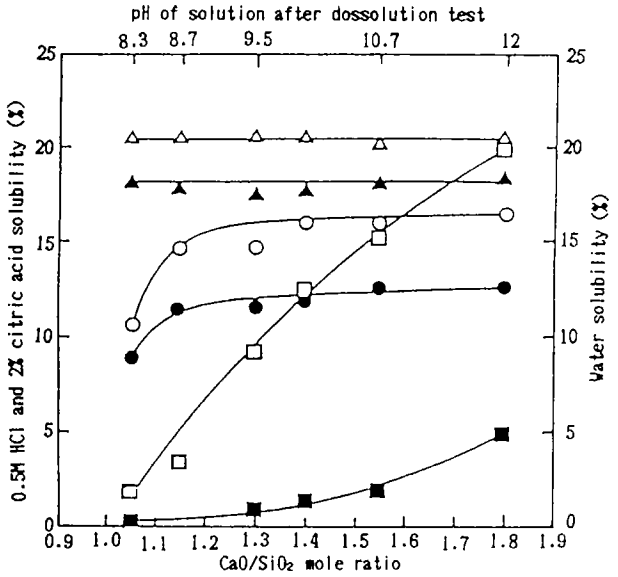


Fig. 8 Effect of varying CaO/SiO_2 mole ratio on 0.5M HCl, 2% citric acid and water soluble SiO_2 , K_2O
K_2O:20.5%
calcination temperature:800℃,
calcination time:20 min,
○ :0.5M HCl soluble SiO_2,
● :2% citric acid soluble SiO_2,
■ :water soluble SiO_2,
△ :0.5M HCl soluble K_2O,
▲ :2% citric acid soluble K_2O,
□ :water soluble K_2O

850, 900℃) for 20 minutes, to determine the effect of temperature in K_2O, SiO_2, CaO and MgO solubility in water, 0.5M HCl and 2% citric acid.

The influence of calcination temperature on the acid soluble components of the product is illustrated in Figure 9-1 and 9-2. Figure 9-1 shows that both 0.5M HCl soluble SiO_2 and K_2O components exhibit a maximum amount at 800 ℃, while slight variation in 0.5M HCl soluble CaO and MgO components are visible at various temperatures.

Similarly, in Figure 9-2 it can be seen that in 2% citric acid soluble SiO_2 and K_2O components exhibit a maximum amount at 800℃ and almost no significant effect in 2% citric acid soluble CaO and MgO components.

The maximum solubility of the product calcined at 800℃ can be explained by the formation acid soluble components like K_2CaSiO_4, K_2MgSiO_4 and $a\alpha$ (or $a'\alpha$) Ca_2SiO_4 (Figure 10) are being formed. A decreasing solubility of the product calcined at 850 to 900℃ was observed due to the crystalline property of such compounds when treated to higher temperatures.

Moreover, the effect of calcination temperature variation on water soluble components is illustrated in Figure 9-3. The water soluble SiO_2 and K_2O contents show a decreasing trend with increase in calcination temperature, whereas, the water soluble CaO exhibits a maximum

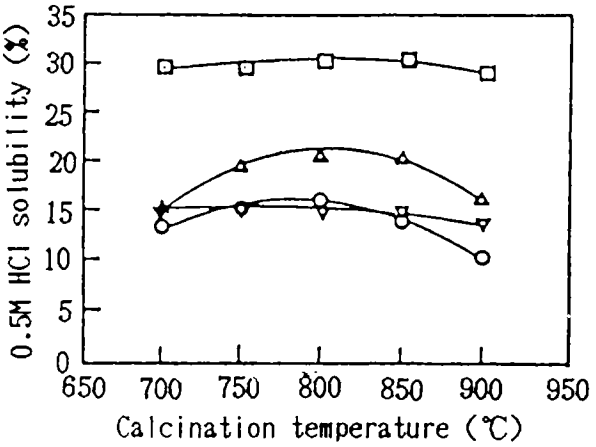


Fig. 9-1 Effect of varying calcination temperature on 0.5M HCl soluble SiO_2, K_2O, CaO, and MgO

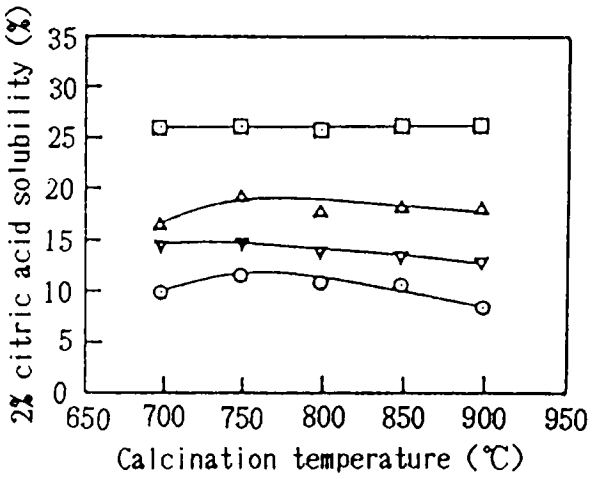


Fig. 9-2 Effect of varying calcination temperature on 2% citric acid soluble SiO_2, K_2O, CaO, and MgO

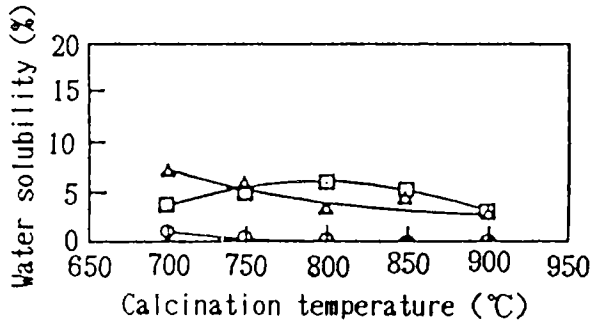


Fig.9-3 Effect of varying calcination temperature on water soluble SiO_2, K_2O and CaO

CaO/SiO_2 mole ratio:1.15, K_2O:20.5%
calcination time:20 min
○ : SiO_2, △ :K_2O, □ :CaO, ▽ :MgO

amount but neglible water soluble MgO at 800 ℃.

Figure 10 shows the X-ray diffraction pattern of the products obtained from the samples with CaO/SiO_2 mole ratio:1.15, containing 20.5% K_2O calcined at 700℃, 750℃, 800℃, 850℃, and 900℃ for 20 minutes. The presence of silicate compounds from the sample treated at 800℃, 850℃, and 900℃ is clearly indicated by the peaks corresponding to K_2CaSiO_4, K_2MgSiO_4, aα (or a'α) Ca_2SiO_4 and other silicate compounds which were not identified in 700℃ and 750℃.

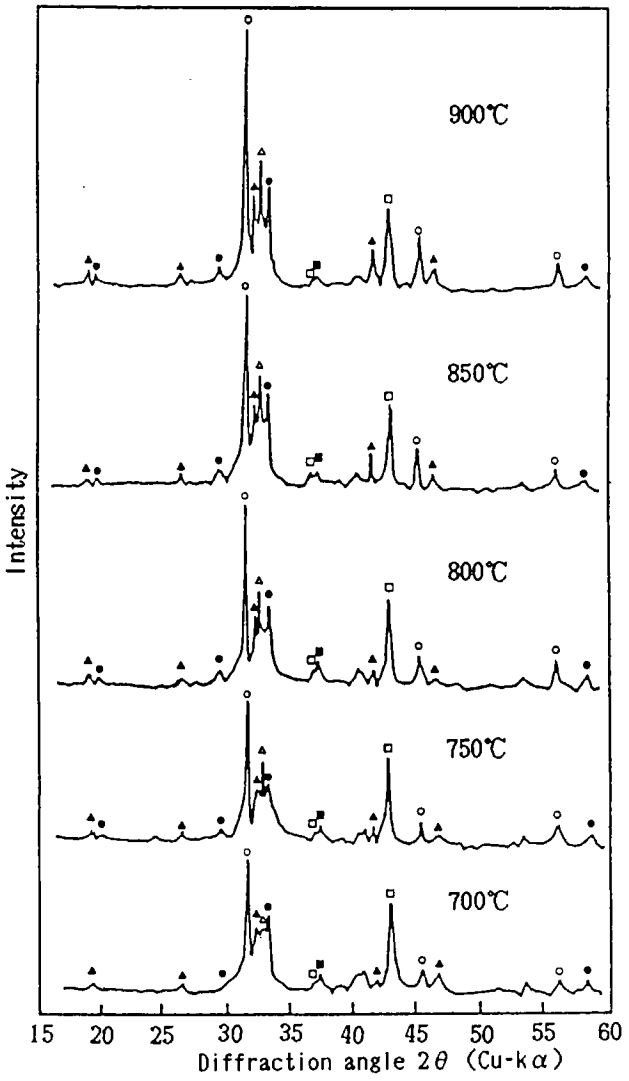


Fig. 10 X-ray diffraction pattern of the calcined products with varying calcination temperature
CaO/SiO_2 mole ratio:1.15, K_2O:20.5%, calcination time:20 min.,
○ :K_2CaSiO_4, ● :K_2MgSiO_4,
△ :α-(or α'-) Ca_2SiO_4
▲ :CaO, □ :MgO, ■ :unknown

4. 3 Effect of Calcination Time

The effect of Calcination time on the formation of acid soluble and water soluble components was investigated in the products with CaO/SiO_2 mole ratio 1.15 and calcined at 800 ℃ for 10, 20, 30, 40, 50 minutes calcination time.

The solubility trend of 0.5M HCl soluble components in the products at different calcination time is shown in Figure 11-1. The graph illustrates that the 0.5M HCl soluble SiO_2 and K_2O both increases as the reaction time was extended further from 20 to 50 minutes which resulted to the formation of acid soluble potassium silicate compound.

The 0.5M HCl soluble CaO and MgO both exhibit decreasing trend with increasing calcination time. Longer calcination time facilitates the formation of crystalline calcium and magnesium compounds which results to less

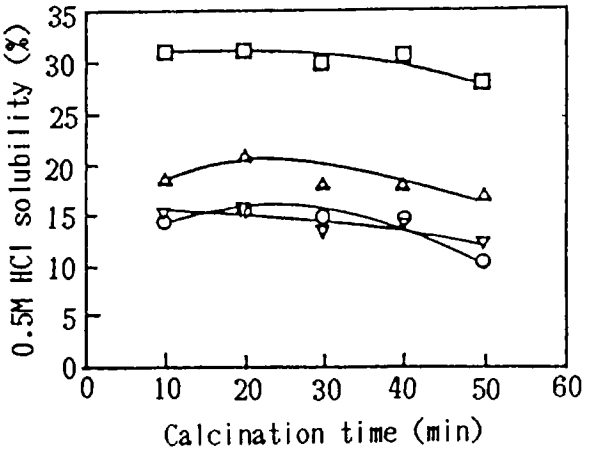


Fig. 11-1 Effect of varying calcination time on 0.5M HCl soluble SiO_2, K_2O, CaO, and MgO

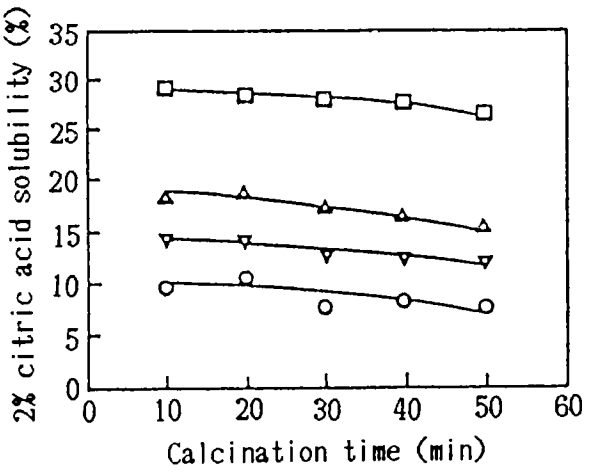


Fig. 11-2 Effect of varying calcination time on 2% itric acid soluble SiO_2, K_2O, CaO, and MgO

acid soluble CaO and MgO.

Figure 11-2 shows the decreasing trend of the 2% citric acid soluble SiO_2, K_2O, CaO and MgO contents of the sample with increase of calcination time.

However in Figure 11-3, the water soluble components like K_2O, CaO and Si_2O was observed to decrease as the calcination time is increased.

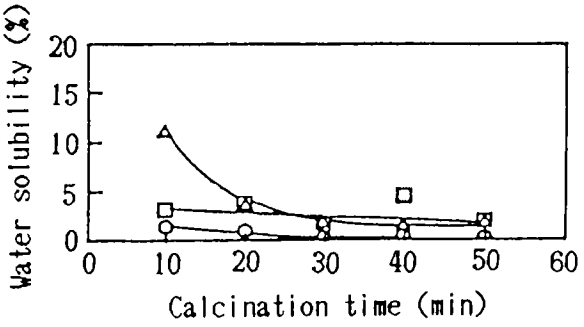


Fig.11-3 Effect of varying calcination time on water soluble SiO_2, K_2O and CaO

CaO/SiO_2 mole ratio:1.15, K_2O:20.5% calcination temperature:800℃,

Conclusion

This study shows that a slow release type potassium silicate fertilizer was produced from rice husk, dolomitic limestone and K_2CO_3. Results of the experiments proves that calcination of the granules using the INTERNAL HEAT TYPE FLUIDIZED BED REACTOR is suitable and of great advantage compared to the fixed bed (muffle furnace) for the production of potassium silicate fertilizer.

The fertilizer exhibits a high degree of solubility in 0.5M HCl and 2% citric acid and very low solubility in water (Table 3), taking into consideration the optimum conditions established.

Table 3. Dissolution Analysis of the Postassium Silicate

Mole Ratio, CaO/SiO_2 =1.15
Calcination Temperature, ℃ =800
Residence Time, minutes =20

Components	0.5M HCL Soluble	2% Citric Acid Soluble	Water Soluble
SiO_2, %	16.09	10.68	0.33
K_2O, %	24.44	17.49	3.16
CaO, %	32.56	27.06	4.92
MgO, %	15.09	14.11	<0.01

Acknowledgment

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Isolation and Characterization of a Novel Facultatively Alkaliphilic Bacterium, *Corynebacterium* sp. Grown on n-Alkanes*1

(Key Words : Facultatively alkaliphilic bacterium, *n*-alkanes, *Corynebacterium*, Na^+, Cytochrome)

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1. Introduction

Alkaliphilic bacteria have been studied for their bioenergetic characteristics, which include negative ΔpH and special solute transport systems, as well as for their industrial potential (Krulwich and Guffanti 1989; Horikoshi 1991). These studies have shown that combined action of a proton-extruding respiratory chain and secondary Na^+/H^+ antiporters play an important role in the regulation of cytoplasmic pH during growth at pH 10 and above. The net acidification of the intravesicular space (or cytoplasm) results in a Na^+-gradient ($Na^+_{out}>Na^+_{in}$). Solute uptake and motility are dependent on this Na^+-gradient (Krulwich et al. 1988, 1990). Therefore, there are several reports on the requirement of added Na^+ for the growth of alkalophiles (Krulwich et al. 1982,1990; Guffanti et al. 1986). Another interesting point is the bioenergetic property caused by the cytoplasmic pH of the alkaliphilic bacteria being lower than the ambient pH (pH 10; Krulwich et al. 1988). The "reversed" gradient of protons lowers the proton electrochemical potential generated in the memebrane of alkaliphiles. Several alkaliphilic *Bacillus* species possess high concentration of membrane-associated cytochromes (Lewis et al. 1980; Guffanti et al. 1986; Yumoto et al. 1991). It has been suggested that an abundance of cytochromes may be an essential strategy to pump out protons with optimal efficiency (Krulwich 1986). However, most of these studies focused on the bacteria belonging to aerobic *Bacillus* species. Most of these bacteria had been isolated using medium containing carbohydrates or organic acids as the carbon source. The objective of our work is to contribute to a better understanding of alkaliphiles belonging to a genus other than *Bacillus*. In this investigation, we isolated a novel facultatively alkaliphilic bacterium, *Corynebacterium* sp. K-171 that can grow on chemically defined medium containing *n*-alkanes as the isolate and the differences between the isolate and previously described alkaliphilic *Bacillus* species are reported here.

2. Materials and Methods

2. 1 *Isolation of the n-alkane-utilizing alkaliphilic bacterium*

Bacteria were isolated from oil-contaminated soils using synthetic medium (AT medium) consisting of *n*-tetradecane [1% (vol/vol)], KNO_3, (5 g, KH2PO4, (0.5 g), $MgSO_4 \cdot 7H_2O$, (0.5 g), $FeSO_4 \cdot 7H_2O$, (0.01 g), $CaCl_2 \cdot 2H_2O$ (0.02 g), $MnSO_4 \cdot nH_2O$ (0.001 g), $ZnSO_4 \cdot 7H_2O$ (0.0005 g) in 1l 100 mM $NaHCO_3$- Na_2CO_3 buffer(pH 10.2) in deionized water. After one week of aerobic incubation at 27℃, four strains were isolated. Among them, strain K-171, which had the best growth rate, was used in this study.

2. 2 *Characterization*

For identification of the isolate, a basal medium (pH 7.2) consisting of polypeptone (Nihon Seiyaku, Tokyo; 10 g) meat extract (Kyokuto, Tokyo; 10 g), NaCl, (10 g) in 1l deionized water, or 100 mM $NaHCO_3$-Na_2CO_3 (pH 10.2) buffer containing medium was used. Cultures were incubated at 27℃and tested by the methods of Yamada and Komagata (1972a) and Barrow and Feltham (1993), unless otherwise stated. Cell division was observed by the method of Komagata et al. (1969). AT medium (described above) containing 1% sugar instead of 1% *n*-tetradecane was used for the Hugh-Leifson O-F test. (Hugh and Leifson 1953). Thymol blue (0.03 g/1) was used as an indicator for acid production at pH 10. Utilization of carbohydrates (1%), organic acids (1%), and hydrocarbons (1%) was tested by using AT medium without *n*-tetradecane at pH 10(100mM $NaHCO_3$-Na_2CO_3).

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*2 Bioscience and Chemistry Division.

For the determination of G+C content, DNA was prepared by the method of Marmur (1961). The G+C content was determined by HPLC analysis (Tamaoka and Komagata 1984). Identification of *meso*-diaminopimeric acid and sugars in the cell wall was performed by TLC (art. 5552 DC-Alufoline Cellulose; Merck Darmstadt, Germany) (Yamada and Komagata 1970; Staneck and Roberts 1974). The glycolate test was done by the method of Uchida et al. (1977). On the basis of these morphological, cultural, and physiological characteristics of the isolate, the bacterium was assigned according to Yamada and Komagata (1972a,b), Jones and Collins 1986), and Holt et al. (1994).

2. 3 Microscopy

For light microscopy of cellular morphology, a gram stained smear of the isolate grown on AT agar medium (AT medium 1.5% agar containing) at pH 10.2 for 72h was examined at 1,000×magnification. For thin sections, early stationary phase cells grown in acetate instead of *n*-tetradecane-containing AT medium or AT medium at pH 10.2 were harvested by centrifugation at 10,000×*g* for 20 min. The cells obtained were fixed with osmium tetraoxide, stained with uranyl acetate, dehydrated in an ethanol series, and embedded in epoxy resin. Sections were cut using an ultramicrotome and observed under a transmission electron microscope (Hitachi H-800) operated at 75kV.

2. 4 Growth experiment

Growth experiments were performed using L-shaped test tubes containing 10 ml medium over a pH range of 6.2 to 10.2 with gentle shaking at 27℃. Growth was estimated by monitoring the OD_{660}. AT medium containing 100 mM Na_2HPO_4-NaH_2PO_4 or 100 mM $NaHCO_3$-Na_2CO_3 buffer, at pH 6.2 to 8.2 or pH 9.2 to 10.2 respectively was used. For experiments in which cells were grown without added sodium, 100 mM of K_2HPO_4-KH_2PO_4 or $KHCO_3$-K_2CO_3 buffer, at pH 7.2 or pH 10.2, respectively, was used. Contaminating Na^+ in the media from other salts was 4.28 mM 5% μm and 0.11 μM, at pH 7.2 and pH 10.2, respectively. To avoid contamination of Na^+ from the preculture, the inoculum (0.1 ml) was transferred twice to potassium-containing medium (10 ml).

2. 5 Preparation and spectroscopic analysis of whole cells

The isolate, K-171, was grown in a 500 ml-flask containing 250 ml of AT medium at pH 10.2 on a reciprocating shaker at 145 rpm at 27℃. The cells were harvested at the early stationary phase of the growth by centrifugation at 10,000 ×*g* for 20 min, washed with 50 mM Tris-HCl buffer(pH 8.0) containing 20 mM $MgSO_4$ and then suspended in the same buffer. The final cells content for measurement was 6.3 mg dry weight/ml. For comparison, cells of *Bacillus alcalophilus* JCM 5262 were used. The bacteria were grown in a 500-ml flask containing 250 ml of medium consisting of polypeptone (Nihon Seiyaku; 10 g), yeast extract (Kyokuto; 1.5 g), glucose (1.0 g), K_2HPO_4(0.1 g), $MgCl_2 \cdot 6H_2O$ (0.1 g) and 1 ml metal mixture [EDTA(3.5 g), $ZnSO_4 \cdot 7H_2O$(3.0 g), $FeSO_4 \cdot 7H_2O$(10 g), $MnSO_4 \cdot nH_2O$(2.0 g), $Co(NO_3)_2 \cdot 6H_2O$(2.0 g), H_3BO_3(1.0 g) in 1 l distilled water] in 1 l 100 mM $NaHCO_3$-Na_2CO_3 buffer (pH 10) on a shaker as described above. Cells were harvested and cell suspensions were prepared as described above. The final content of the cells for the analysis was 6.1mg dry weight)/ml. Spectrophotometric measurements were performed with a Shimadzu UV-3000 spectrophotomater using 1-cm light path cuvetttes. The suspensions in the cuvettes were stirred to prevent cells from settling. The dithionite-reduced minus ferricyanide-oxidized difference spectra were recorded at 18℃ to determine the cytochrome content in whole cells. The following wavelength pairs with the corresponding-difference millimolar extinction coefficients were used: cytochrome *a*, $\Delta \varepsilon_{600-615}$ =11.7 $mM^{-1} \cdot cm^{-1}$ (Ludwig and Schatz 1980); cytochrome, *b* $\Delta \varepsilon_{558-575}$=17.5$mM^{-1} \cdot cm^{-1}$ (Jones and Pool 1985); and cytochrome *c*, $\Delta \varepsilon_{553-537}$ =22.7$mM^{-1} \cdot cm^{-1}$ (Fee et al. 1980). The dithionite-reduced *minus* oxidized ferricyanide-oxidized difference spectrum at 77K was measured with a Simadzu MPS-2000 spectrophotometer using 2 mm light path cuvettes. The cells in 100 mM Tris-HCl buffer (pH 8.0) containing 50% glycerol, was reduced with dithionite and oxidized with ferricyanide, and crystalized by liquid nitrogen. The final cells content for the analysis was 3.1 mg dry weight/ml.

3. Results and Discussion

Characteristics of the isolate, strain K-171, that were tested in this study are shown in Table 1. and a light micrograph of the isolate grown on AT medium at pH 10.2 for 72 h is

shown in Fig.1a.The isolate, shown to have a size of 0.6−1.0×0.7−1.4 μm, was non-motile, gram-positive, non-acid-fast, non-spore forming, had snappingtype division, formed metachromatic granules, and was strictly aerobic. Cell walls contained *meso*-diaminopimeric acid, arabinose, and galactose. The glycan moiety of the cell wall contained acetyl residues. The isolate was also catalase-positive, oxidase-negative, and the G+C content of the DNA was 70.8 mol%. According to these characteristics, the isolate was assigned to the genus *Corynebacterium* The growth temperature range was 10−35℃ and the optimum temperature range was 24−31℃. Two alkaliphilic *Corynebacterium* strains, no. 93-1 and no. 150-1, have been previously described (Kobayashi and Horikoshi, 1980; Kobayashi et al. 1980). Strain K-171, differs from these two strains in metachromatic granule formation in the cell, G+C content of DNA, liquefaction of

Table 1 Comparison of characteristics of alkaliphilic *Corynebacterium* sp. K-171 with those of alkaliphilic *Corynebacterium* sp. no. 93-1and no. 150-1 (Kobayashi and Horikoshi 1980; Kobayashi et al. 1980). (DAP diaminopimelic acid; ND not determined)

	K-171	no. 93-1	no. 150-1
Morphological characteristics			
Gram stain	Positive	Positive	Positive
Acid-fast stain	Negative	Negative	Negative
Motility	Negative	Positive	Negative
Flagellation	Negative	Peritrichpus	Negative
Size (μm)	0.6–1.0 × 0.7–1.4	0.8–1.0 × 2.0–3.0	0.7–0.8 ×1.0–3.0
Spore formation	Negative	Negative	Negative
Metachromatic granule	Positive	Negative	Negative
Type of cell division	Snapping	Snapping	Snapping
Pleomorphism	Not distinct	Not distinct	Not distinct
Cultural characteristics (pH 10)			
Nutrient broth	+	+	+
Nutrient agar slant	+	+	+
Growth temperature (°C)	10–35	20–40	15–40
Growth in presence of			
5% NaCl	−	+	+
7% NaCl	−	+	+
Chemical characteristics			
Major peptidoglycan amino acid	*meso*-DAP	*meso*-DAP	*meso*-DAP
Major cell wall sugars	arabinose, galactose	ND	ND
Glycan moiety of cell wall	acetyl type	ND	ND
DNA G+C content (%)	70.8	65.8	52.0
Physiological characteristics (pH 10)			
Reduction of nitrate	+	+	+
Indole production	−	−	−
H_2S production	+	−	+
Liquefaction of gelatin	−	+	+
Hydrolysis of casein	−	+	+
DNase	−	ND	ND
Oxidase	−	+	+
Catalase	+	+	+
Acid from carbohydrates			
D-Arabinose	−	−	+
D-Fructose	−	+	+
D-Galactose	−	−	+
D-Glucose	−	+	+
Inositol	−	−	−
D-Mannitol	−	+	+
D-Raffinose	−	−	−
Sucrose	−	−	−
L-Rhamnose	−	−	−
D-Xylose	−	+	+

gelatine, hydrolysis of casein, oxidase test and acid formation from carbohydorates (Table 1). Accordingly, it is considered that the isolate is a novel alkaliphilic *Corynebacterium* species. Utilization of organic acid, sugar, and hydrocarbons by the isolate was tested at pH 10.2 (Table 2). The isolate utilized only acetate among the organic acid salts. Among the sugars, the isolate utilized only glucose and fructose effectively. Among the hydrocarbons, *n*-alkanes (C_{13}-C_{16}) and pristane were utilized effectively, but cycloalkanes and aromatic hydrocarbons were hardly utilized. To our knowledge, the isolate is the first example of a hydrocarbon utilizing alkaliphilic bacterium.

Electron micrographs of ultra-thin cell sec-

Table 2 Utilization of substrates by the alkaliphilic *Corynebacterium* sp. K-171 at pH 10.2

Hydrocarbons		Organic acid		Carbohydrates	
n-Dodecane	–	Sodium acetate	+	D-Arabinose	–
n-Tridecane	+	Sodium citrate	–	D-Fructose	+
n-Tetradecane	+	Sodium formate	–	D-Galactose	–
n-Pentadecane	+	Sodium lactate	–	D-Glucose	+
n-Hexadecane	+	Sodium succinate	–	Inositol	–
n-Eicosane	+	Sodium DL-malate	–	D-Mannitol	–
n-Tetracosane	+	Sodium L-glutamic acid	–	D-Raffinose	–
n-Octacosane	+			Sucrose	–
n-Dotriacontane	–			D-Rhamnose	–
Pristane	+			D-Xylose	–
Cyclododecane	–				
Fluorene	–				
Antracne	–				
Pyrene	–				

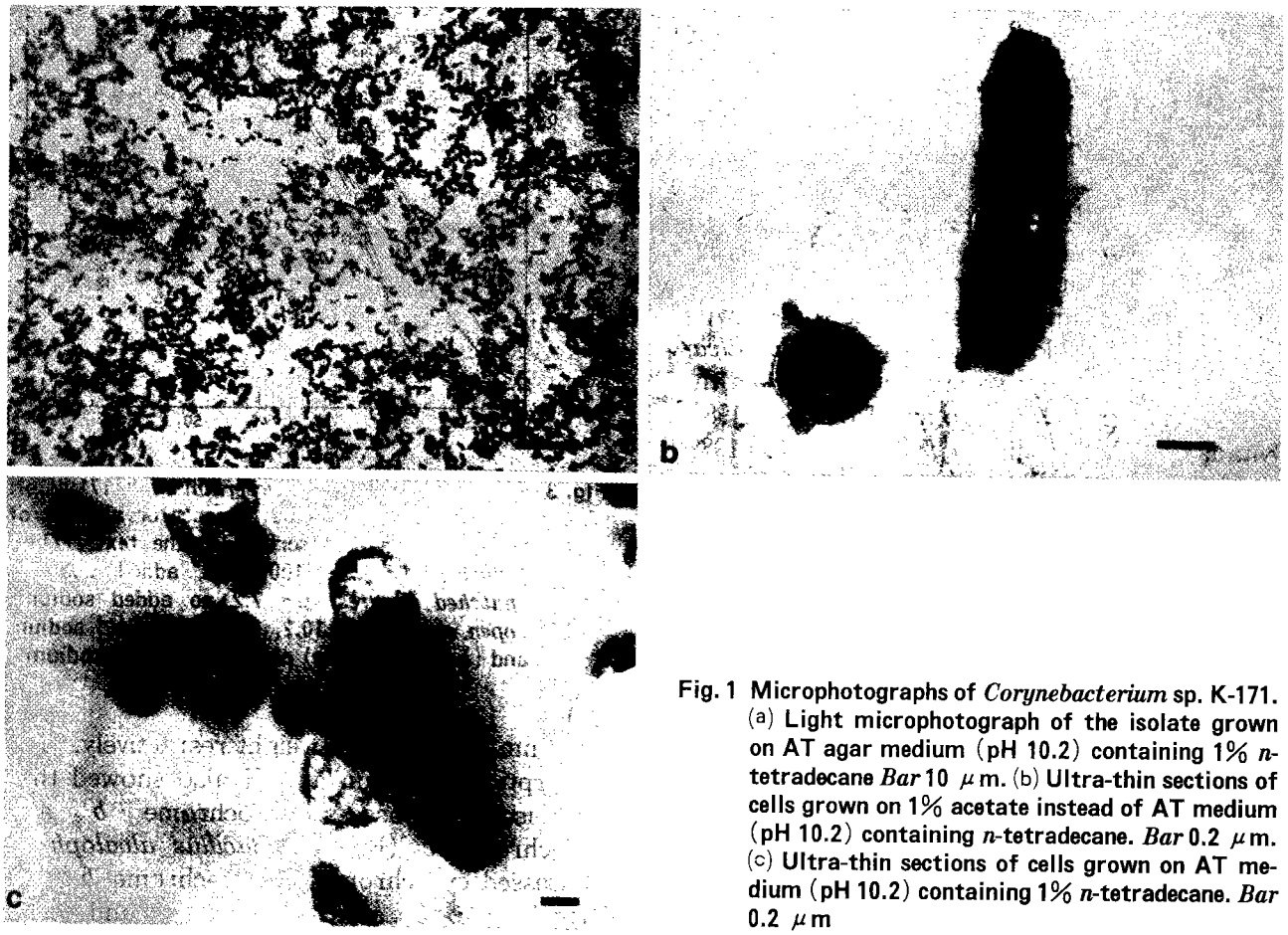

Fig. 1 Microphotographs of *Corynebacterium* sp. K-171. (a) Light microphotograph of the isolate grown on AT agar medium (pH 10.2) containing 1% *n*-tetradecane *Bar* 10 μm. (b) Ultra-thin sections of cells grown on 1% acetate instead of AT medium (pH 10.2) containing *n*-tetradecane. *Bar* 0.2 μm. (c) Ultra-thin sections of cells grown on AT medium (pH 10.2) containing 1% *n*-tetradecane. *Bar* 0.2 μm

tions of the isolate grown at pH 10.2 are shown in Fig. 1b, c. When the isolate was grown on acetate in stead of *n*-tetradecane-containing AT medium, the cell surface was very thick (ca. 0.03 μ m, Fig.1b) compared with cells frown in *n*-tetradecane-containing medium (ca. 0.01 μ m, Fig.1c). On the other hand, when the isolate was grown on n-tetradecane-containing AT medium, many inclusions, possibly hydrophobic substances, were observed in the cytoplasmic space (Fig,1c). Similar phenomena have been observed in other bacteria (de Andres et al. 1991; Morikawa and Imanaka 1993). Growth of the isolate at various pHs in media containing *n*-tetradecane as the carbon source was tested (Fig. 2). The isolate grew well over a broad range of pH and the doubling time was similar over the pH range (ca. 4-6 h). The shortest doubling time of the isolate was 4 h at pH 8.2, much longer than that of the alkaliphilic *Bacillus firmus* RAB (43 min) and *Bacillus alcalophilus* (40 min, Guffanti and Hicks 1991). The experiment shown was performed with a preculture grown at pH 10.2, but the result was similar when the preculture was grown at pH 7.2. The isolate lowered the ambient pH to 8.2 when grown at pH 10.2, and raised the pH to 7.4 when grown at pH 7.2. This phenomenon been also reported for an *alkaliphilic Bacillus* (Horikoshi 1991).

Dependency of growth on added Na^+ was also tested with the isolated (Fig 3). The lag period of growth became longer when the isolate was grown on medium without added Na^+. Growth was stimulated by added Na^+, but addition of Na^+ was not indispensable for growth. There are some alkaliphiles (e.g., *Bacillus alcalophilus*) that do not seem to added Na^+ in the medium for growth (Aono and Horikoshi 1983; Kitada and Horikoshi 1987; Krulwich et al. 1982, 1990). However Krulwich et al. (1988) reported that these alkaliphiles were actually dependent on $Na^+$for growth and demonstrated this by rigorous exclusion of Na^+ from defined media and the use of plastic flasks. It is still possible that the present isolate requires the small amounts of Na^+ found as contaminants in the medium without added Na^+(see "Materials and methods").

Cytochrome content of the bacterial whole cells was estimated (Fig.4) and compared to that of cells of the obligately alkaliphilic bacterium *Bacillus alcalophilus*. The isolate contained cytochrome *b* and cytochrome *c* at 0.028 and

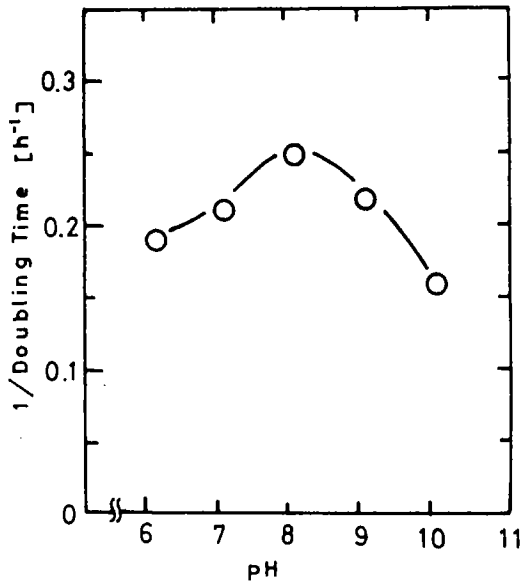

Fig. 2 Effect of pH on the doubling time (h^{-1}) of *Corynebacterium* sp. K-171 grown on 1% *n*-tetradecane as described in the text.

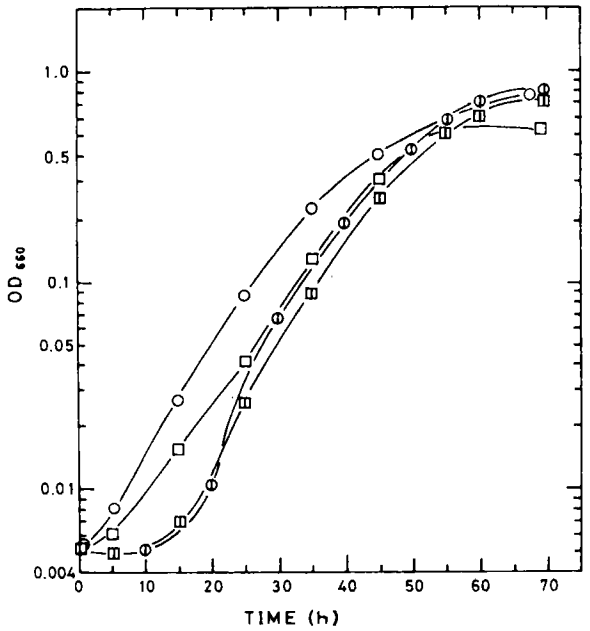

Fig. 3 Growth of the *Corynebacterium* sp. K-171 at pH 7.2 and pH 10.2 in the presence or absence of added sodium as described in the text. (*Open squares*) pH 7.2, 100 mM added sodium; (*hatched squares*) pH 7.2 no added sodium; (*open circles*) pH 10.2, 100 mM added sodium and (*hatched circles*) pH 10.2 no added sodium

0.018 nm/mg cell dry weight respectively. The absorption spectrum at 77K also showed that the isolate possess cytochrome *b* and cytochrome *c* (Fig. 5). *Bacillus alcalophilus* possessed cytochrome *a*, cytochrome *b*, and cytochrome *c* at 0.056, 0.290, and 0.244

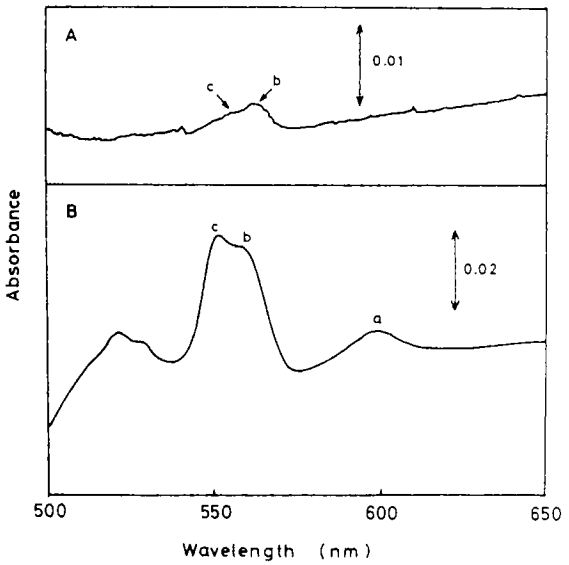

Fig.4 Difference spectra of the *Corynebacterium* sp. K-171 and *Bacillus alcalophilus*. Reduced *minus* oxidized samples were recorded as described in the text. (A) Spectrum of the isolate grown at pH 10.2; (B) Spectrum of *Bacillus alcalophilus* grown at pH 10. Each peak was identified as follows; *a* cytochrome *a*; *b* cytochrome *b*; and *c*, cytochrome *c*

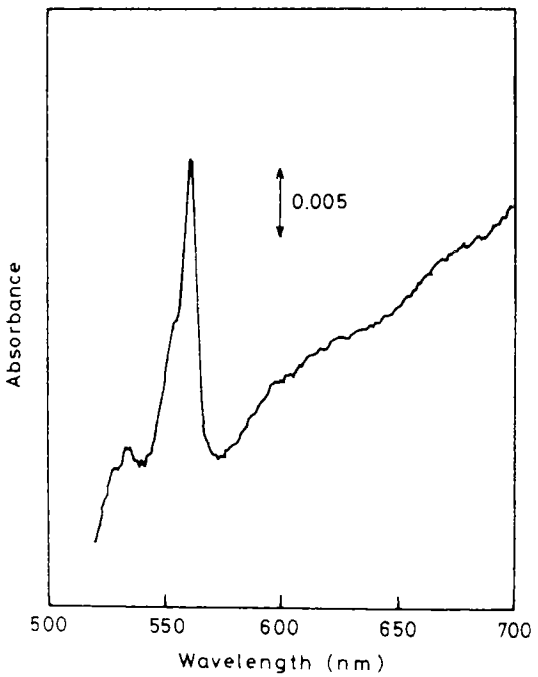

Fig.5 Difference spectrum of *Corynebacterium* sp. K-171 at 77K. Reduced minus oxidized sample was recorded as described in the text

nmol/mg cell dry weight, respectively. These results showed that *Bacillus alcalophilus* possess 13.4-folds higher amounts of total cytochromes than the isolate.

The above results suggest that the isolate has characteristics different from those of alkaliphilic *Bacillus* in growth aspects and cytochrome content. Energy-producing processes, solute transport systems, and cell components such as cell wall and membrane lipids of the isolated alkaliphilic bacterium, *Corynebacterium* K-171, will be elucidated by further studies.

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Mouse NADPH-cytochrome *P*-450 Oxidoreductase: Molecular Cloning and Functional Expression in Yeast*1

(Key Words : Cytochrome *P*-450 reductase, cDNA cloning, Nucleotide sequence, Expression, (Mouse liver); (Yeast))

Satoru OHGIYA*2, Nariko SHINRIKI*2, Tetsuya KAMATAKI*3 and Kozo ISHIZAKI*2

NADPH-cytochrome *P*-450 oxidoreductase (P450 reductase; EC 1.6.2.4) is an important component of the microsomal mixed-function monooxygenase system [1]. P450 reductase catalyzes the transfer of electrons from NADPH to P450 that is responsible for metabolism of endogenous as well as exogenous compounds [2]. Although P450 is composed of a number of isozymes [3], P450 reductase is thought to be unique [4] and able to interact with all microsomal P450 isozymes. Primary structures of P450 reductases have been identified through nucleotide sequences of their cDNAs isolated from the rat [5,6], rabbit [7], human [8], *Saccharomyces cerevisiae* [9], *Candida tropicalis* [10], *Schizosaccharomyces pombe* [11], mung bean [12] and *Arabidopsis thaliana* [12], and by protein sequencings of P450 reductases purified from the pig [13,14] and trout [15]. While we had published isolation of a mouse P450 reductase cDNA named MSr2, we found thereafter that MSr2 had to be ascribed to a guinea-pig clone [16]. We accordingly screened mouse liver cDNA libraries and isolated a cDNA encoding mouse P450 reductase from a liver cDNA library of the ddY mouse. Sequence alignment of amino acids of P450 reductases among various species demonstrates strong conservation of acidic residues. The mouse P450 reductase was functionally expressed in *Saccharomyces cerevisiae* by removal of whole noncoding regions from the isolated cDNA prior to insertion into an expression vector.

A male ddY mouse was treated with a single intraperitoneal dose of 3-methylcholanthrene (200mg/kg). The liver was excised 20h after the administration. Poly $(A)^+$ RNA was isolated according to standard protocols [17], followed by synthesis of cDNA using oligo (dT) as a primer. The constructed cDNA library was screened using a *Pst* I fragment of pFP105, the rabbit P450 reductase cDNA [7]. By sequential rescreenings, a cDNA clone containing a complete open reading frame was isolated and designated dR25. dR25 is 2457 bp long and encodes 678 amino acids with a calculated molecular weight of 77 043 (Fig. 1).

Two plasmids were constructed for expression in yeast. For construction of an expression plasmid, dR25 was digested with restriction endonuclease *Kpn* I and *Afl*III to partly remove noncoding regions (see Fig. 1). Subsequently, both termini of the digested dR25 were converted into *Hind*III sites by blunting and *Hind*III linker ligation. The *Hind*III fragment thus obtained was inserted at the proper orientation into the yeast expression vector pAAH5 to construct the expression plasmid. The other expression plasmid was prepared as follows: the coding region of dR25 was amplified from the dR25 cDNA by PCR [18] with oligonucleotide primers containing a *Hind*III site just upstream from the translation initiation codon or just downstream from the stop codon; forward (GAAAGCTTATGGGGGACTCTCACGAA), and reverse (GGAAGCTTCTAGCTCCATACATCCAG). A PCR mixture containing 0.2 mM dNTPs, 0.2 μM each of primer and 25 U/ml of *Pfu* DNA polymerase (Stratagene) was subjected to 30 cycles of amplification; 1 min at 94 ℃, 2 min at 55 ℃ and 4 min at 72 ℃. The amplified DNA was di-

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The nucleotide sequence data reported in this paper appear in the DDBJ/EMBL/GenBank Nucleotide Sequence Databases with the accession number 'D17571', definition 'mouse mRNA for NADPH-cytochrome P450 oxidoreductase'.

The species and strain data of MSr2 have been corrected in the DDBJ/EMBL/GenBank Nucleotide Sequence Databases (accession number 'D10498').

Abbreviations: P450 reductase, NADPH-cytochrome *P*-450 oxidoreductase; P450, cytochrome *P*-450; bp, basepairs; PCR, polymerase chain reaction; FMN, flavin mononucleotide.

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                                                    CGCGCGGTGTGTGATCTGGTCGGTACCGAGGAGCGCAGGTTGTGTCACCAAC      52

ATGGGGGACTCTCACGAAGACACCAGTGCCACAGTGCCTGAGGCAGTGGCTGAAGAAGTGTCTCTATTCAGCACAACGGACATTGTTCTG                142
 M  G  D  S  H  E  D  T  S  A  T  V  P  E  A  V  A  E  E  V  S  L  F  S  T  T  D  I  V  L      30

TTTTCTCTCATCGTGGGGGTCCTGACCTACTGGTTCATCTTTAAAAAGAAGAAAGAAGAGATACCGGAGTTCAGCAAGATCCAGACAACG              232
 F  S  L  I  V  G  V  L  T  Y  W  F  I  F  K  K  K  K  E  E  I  P  E  F  S  K  I  Q  T  T      60

GCCCCACCTGTCAAAGAGAGCAGCTTCGTGGAAAAGATGAAGAAAACGGGAAGGAACATTATTGTATTCTATGGCTCCCAGACGGGAACC              322
 A  P  P  V  K  E  S  S  F  V  E  K  M  K  K  T  G  R  N  I  I  V  F  Y  G  S  Q  T  G  T      90

GCGGAGGAGTTTGCCAACCGGCTGTCCAAGGATGCCCACCGCTATGGGATGCGGGGCATGTCTGCAGACCCTGAAGAGTATGACTTGGCC              412
 A  E  E  F  A  N  R  L  S  K  D  A  H  R  Y  G  M  R  G  M  S  A  D  P  E  E  Y  D  L  A      120

GACCTGAGCAGCCTGCCTGAGATCGACAAGTCCCTGGTAGTCTTCTGCATGGCCACATACGGAGAAGGCGACCCCACCGACAACGCGCAG              502
 D  L  S  S  L  P  E  I  D  K  S  L  V  V  F  C  M  A  T  Y  G  E  G  D  P  T  D  N  A  Q      150

GACTTCTATGATTGGCTGCAGGAGACTGACGTGGACCTCACGGGTGTCAAGTTTGCTGTGTTTGGTCTCGGGAACAAGACCTATGAGCAC              592
 D  F  Y  D  W  L  Q  E  T  D  V  D  L  T  G  V  K  F  A  V  F  G  L  G  N  K  T  Y  E  H      180

TTCAACGCCATGGGCAAGTATGTGGACCAGCGGCTGGAGCAGCTTGGCGCCCAGCGAATCTTTGAGTTGGGCCTTGGTGATGACGACGGG              682
 F  N  A  M  G  K  Y  V  D  Q  R  L  E  Q  L  G  A  Q  R  I  F  E  L  G  L  G  D  D  D  G      210

AACTTGGAAGAGGATTTCATCACATGGAGGGAGCAGTTCTGGCCAGCTGTGTGCGAGTTCTTCGGGGTGGAAGCCACTGGGGAGGAGTCG              772
 N  L  E  E  D  F  I  T  W  R  E  Q  F  W  P  A  V  C  E  F  F  G  V  E  A  T  G  E  E  S      240

AGCATCCGCCAGTACGAGCTCGTGGTCCACGAAGACATGGACACAGCCAAGGTGTACACGGGTGAGATGGGCCGTCTGAAGAGCTACGAG              862
 S  I  R  Q  Y  E  L  V  V  H  E  D  M  D  T  A  K  V  Y  T  G  E  M  G  R  L  K  S  Y  E      270

AACCAGAAACCCCCCTTCGATGCCAAGAATCCATTCCTGGCTGCTGTCACCACGAACCGGAAGCTGAACCAAGGCACTGAGAGGCATCTA              952
 N  Q  K  P  P  F  D  A  K  N  P  F  L  A  A  V  T  T  N  R  K  L  N  Q  G  T  E  R  H  L      300

ATGCACCTGGAATTGGACATCTCAGACTCCAAGATCAGGTATGAATCTGGAGATCACGTGGCTGTGTACCCAGCCAACGACTCCACCCTG             1042
 M  H  L  E  L  D  I  S  D  S  K  I  R  Y  E  S  G  D  H  V  A  V  Y  P  A  N  D  S  T  L      330

GTCAACCAGATTGGGGAGATCCTGGGGGCTGACCTGGATGTCATCATGTCTCTAAACAATCTCGATGAGGAGTCGAATAAGAAGCATCCG             1132
 V  N  Q  I  G  E  I  L  G  A  D  L  D  V  I  M  S  L  N  N  L  D  E  E  S  N  K  K  H  P      360

TTCCCCTGCCCCACCACCTACCGCACGGCCCTCACCTACTACCTGGACATCACTAACCCGCCACGAACCAACGTGCTCTACGAGCTGGCC             1222
 F  P  C  P  T  T  Y  R  T  A  L  T  Y  Y  L  D  I  T  N  P  P  R  T  N  V  L  Y  E  L  A      390

CAGTACGCCTCAGAGCCCTCGGAGCAGGAACACCTGCACAAGATGGCGTCCTCCTCCGGCGAGGGCAAGGAGCTGTACCTGAGCTGGGTG             1312
 Q  Y  A  S  E  P  S  E  Q  E  H  L  H  K  M  A  S  S  S  G  E  G  K  E  L  Y  L  S  W  V      420

GTGGAGGCCCGGAGGCACATCCTAGCCATTCTCCAAGACTACCCGTCCCTGCGGCCACCCATCGACCACCTGTGCGAGCTCCTCCCGAGG             1402
 V  E  A  R  R  H  I  L  A  I  L  Q  D  Y  P  S  L  R  P  P  I  D  H  L  C  E  L  L  P  R      450

CTGCAGGCCCGCTACTATTCCATTGCCTCGTCGTCTAAGGTCCACCCCAACTCCGTGCACATCTGCGCCGTGGCTGTGGAGTATGAAGCG             1492
 L  Q  A  R  Y  Y  S  I  A  S  S  S  K  V  H  P  N  S  V  H  I  C  A  V  A  V  E  Y  E  A      480

AAGTCTGGACGAGTGAACAAGGGGGTGGCCACCAGCTGGCTTCGGACCAAGGAACCAGCAGGAGAGAATGGCCGCCGGGCCCTGGTCCCC             1582
 K  S  G  R  V  N  K  G  V  A  T  S  W  L  R  T  K  E  P  A  G  E  N  G  R  R  A  L  V  P      510

ATGTTCGTCCGCAAGTCCCAGTTCCGCTTGCCTTTCAAGCCCACCACACCTGTTATCATGGTGGGCCCCGGCACTGGGGTTGCCCCTTTC             1672
 M  F  V  R  K  S  Q  F  R  L  P  F  K  P  T  T  P  V  I  M  V  G  P  G  T  G  V  A  P  F      540

ATGGGCTTCATCCAGGAGCGCGCTTGGCTTCGAGAGCAAGGCAAGGAGGTCGGAGAGACGCTGCTCTACTACGGCTGCCGGCGCTCGGAT             1762
 M  G  F  I  Q  E  R  A  W  L  R  E  Q  G  K  E  V  G  E  T  L  L  Y  Y  G  C  R  R  S  D      570

GAGGACTATCTGTACCGCGAGGAGCTGGCGCGCTTCCACAAGGACGGCGCCCTCACGCAGCTTAATGTGGCCTTTTCCCGTGAGCAGGCC             1852
 E  D  Y  L  Y  R  E  E  L  A  R  F  H  K  D  G  A  L  T  Q  L  N  V  A  F  S  R  E  Q  A      600

CACAAGGTCTATGTTCAGCACCTGCTCAAGAGGGACAAAGAGCACCTGTGGAAGCTGATCCACGAAGGTGGTGCCCACATCTATGTCTGC             1942
 H  K  V  Y  V  Q  H  L  L  K  R  D  K  E  H  L  W  K  L  I  H  E  G  G  A  H  I  Y  V  C      630

GGGGATGCTCGAAATATGGCCAAAGATGTGCAGAACACATTCTATGACATCGTGGCCGAGTTTGGGCCCATGGAGCACACCCAGGCTGTG             2032
 G  D  A  R  N  M  A  K  D  V  Q  N  T  F  Y  D  I  V  A  E  F  G  P  M  E  H  T  Q  A  V      660

GACTATGTTAAGAAGCTCATGACCAAGGGCCGCTACTCGCTGGATGTATGGAGCTAGGAGCTGCCGCCCCCCACCCCTCGCTCCCTGTAA             2122
 D  Y  V  K  K  L  M  T  K  G  R  Y  S  L  D  V  W  S                                          678

TCACGTCCTTAACTTCCTTCTGCCGACCTCCACCTCTGGTGGTTCCTGCCCTGCCTGGACACAGGGAGGCCCAGGGACTGACTCCTGGCC             2212
TGAGTGATGCCCTCCTGGGCCCTTAGGCAGAGCCTGGTCCATTGTACCAGGCAGCCTAGCCCAGCCCAGGGCACATGGCAAGAGGGACTG             2302
GACCCACCTTTGGGTGATGGGTGCCTTAGGTCCCCAGCAGCTGTACAGAAGGGGCTCTTCTCTCCACAGAGCTGGGGTGCAGCCCCAACA             2392
TGTGATTTTGAATGAGTGTAAATAATTTTAAATAACCTGGCCCTTGGAATAAAGTTGTTTTCTGT                                 2457
```

Fig. 1

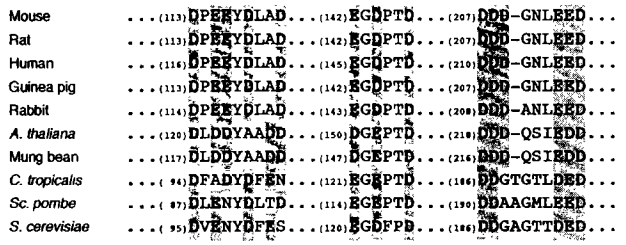

Fig. 2. Clusters of conserved acidic residues of P450 reductases from various species. Regions containing conserved acidic residues around the predicted FMN-binding domain are shown. Dashes indicate gaps inserted to optimize sequence alignment. Acidic residues are indicated by shading. The amino acid data of P450 reductases are quoted from the sources in square brackets; the rat [5], human [8], guinea-pig [16], rabbit [7], *Arabidopsis thaliana* [12], mung bean [12], *Candida tropicalis* [10], *Schizosaccharomyces pombe* [11], *Saccharomyces cerevisiae* [9].

gested with *Hind*III followed by insertion into pAAH5. The nucleotide sequence of the amplified DNA was determined and no base-substitution due to PCR was found. *Saccharomyces cerevisiae* YPH500 (a, *ura*3-52, *lys*2-801, *ade*2-101, *trp*1Δ63, *his*3Δ200, *leu*2Δ1) was transformed by the expression plasmids or the vector pAAH5. Yeast cultivation and preparation of yeast microsomes were carried out as described [19]. NADPH-cytochrome *c* reductase activity was determined by measurement of absorbance change at 550 nm at 25℃. The reaction mixture consisted of 0.35 M potassium phosphate buffer (pH 7.6), 50 μM cytochrome *c*, 1 mM KCN, 42 μM NADPH and approx. 20 μg protein of yeast microsomes.

The nucleotide and deduced amino acid sequences of the mouse P450 reductase share high identity with those of other mammalian P450 reductases (Table 1). In particular, the identity of the mouse P450 reductase with its rat counterpart is remarkably high (98.4 %). The strong conservation of the primary structures among P450 reductases implies the importance of the function of this unique enzyme.

It has been proposed that P450 reductase interacts with P450 by charge-pairing [20-22]. The sequence alignment of amino acids of P450 reductases from various species reveals that acidic residues of the enzymes are conserved better than basic and neutral residues. Three clusters of the acidic amino acids are found around a predicted FMN-binding segment [9] (Figs. 1 and 2). The first and third clusters overlap with the regions of the rat P450 reductase that have been identified by chemical modification studies as segments responsible for interaction with P450 [23-25]. Therefore the negatively

Table 1 Percent identity of nucleotide and amino acid sequences among mammalian P450 reductases

	Rat	Human	Guinea-pig	Rabbit
Amino acid	98.4	91.6	91.4	90.0
Nucleotide	94.2	82.7	81.1	84.4

Nucleotide and deduced amino acid sequences of dR25 are compared with those of P450 reductase cDNAs from the rat [5], human [8], guinea-pig [16] and rabbit [7]

Table 2 Cytochrome *c* reductase activity of microsomes prepared from transformed yeast

	Cytochrome *c* reductase activity (nmol cytochrome *c* reduction/ min per mg microsomal protein)
YPH500/none (control)	21.4± 3.5
YPH500/dR25Δpart	18.6± 4.1
YPH500/dR25Δwhole	279 ±44

Cytochrome *c* reductase activity was determined as described in the text. YPH500/none, YPH500/dR25Δpart and YPH500/dR25Δwhole represent yeast YPH500 transformed by pAAH5 carrying no insert, dR25 lacking noncoding regions in part and dR25 lacking whole noncoding regions, respectively. Values are means±S.D. for samples of microsomes prepared from three independent transformants.

charged residues in these clusters may play an important role in electrostatic association with positively charged residues of P450 molecules.

The vector pAAH5 carrying a constitutive alcohol dehydrogenase promoter and terminator was used for expression of the mouse P450 reductase in yeast. First we prepared a truncated dR25 cDNA containing 30 bp of the 5′ noncoding region and 305 bp of the 3′ noncoding region, but no increased activity of cytochrome *c* reductase was measured in microsomes of yeast transformed by the truncated dR25 (Table 2). Then the other modified dR25 cDNA that lacked whole noncoding regions was prepared by PCR-based mutagenesis. Microsomes from yeast transformed by the modified dR25 showed high cytochrome *c* reductase activity compared with the control yeast microsomes (Table 2). We have isolated a hamster P450 reductase cDNA clone (unpublished results). In contrast to the mouse P450 reductase, the hamster P450 reductase has been successfully expressed using the cDNA containing 55 bp of the 5′ noncoding region and 326 bp of the 3′ noncoding region. Thus, not only length but also nucleotide sequence of non coding regions of P450 reductase cDNAs might affect their expression efficiency in yeast cells.

P450 reductase is the essential electron carrier for P450s. Since the activity of endogenous

P450 reductase is generally low in yeast and cell lines, we are investigating stable and high expression of mammalian P450 reductase in these recipient cells [26]. Since highly expressed P450 reductase enhances the activity of P450 expressed simultaneously [8, 26-29], the recombinant cell expressing the mammalian P450 reductase will be useful for precise characterization of various P450 isozymes.

We wish to thank Drs. T. Sugiyama and N. Taniguchi of Osaka University for providing the rabbit P450 reductase cDNA.

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