fumaric acid であり succinic acid も大体に於て無効であつた。

3. 上の結果より種々の有効な有機酸の作用は呼吸系を旺盛に活動せしめて energy 代謝を通じて酵素の合成をなすもののように考えられるが、一方, malonic acid が顕著な酵素生成促進を示す事もあり簡単に energy 的見地より結論は出来ず、尙今後の研究を要する。

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Summary

1. The influences upon amylase forming ability of *Asp. oryzae* by addition of several organic acids to the CZAPEK medium (surfur culture) were studied.

2. Aconitic acid, α -ketoglutaric acid, maleic acid, malic acid, oxalacetic acid and malonic acid were effective and pyruvic acid, citric acid, fumaric acid and succinic acid were ineffective upon amylase formation.

好気性中温性繊維素醱酵菌に関する研究 (第 5 報, 其の 2)

Pseudomonas 属の分類学的研究に就て

上田清基, 石川七郎, 伊丹ツル, 朝井勇宜 (東京大学農学部農芸化学教室)

昭和 26 年 5 月 3 日 受 理

Studies on the Aerobic Mesophilic Cellulose-decomposing Bacteria. Part 5-2.

Taxonomical Study on Genus *Pseudomonas*.

By Kiyomoto UEDA, Shichiro ISHIKAWA, Turu ITAMI and Toshinobu ASAI, (Dept. of Agr. Chem., Faculty of Agr., Univ. of Tokyo)

其の 1 に於いて報告せる如く、本州東半部及び北海道の主として森林土壌を種々の条件で集積した際に、中温性好気性繊維素醱酵菌として現われたものは、*Cytophaga* 型が圧倒的に多く、一方 *Pseudomonas-Vibrio* 型は 264 strains 中僅かに 18 strains のみであつた。これらは濾紙培地で振盪により分解部分の濾紙が四散するという特徴ある現象を来す事、及び生体検鏡により判定したのである。*Pseudomonas* 型は Opt. pH が alkali 側にある事より、採取土壌の pH が一般に酸性に傾いているという事が推定出来る。

吾々はこれらの *Pseudomonas-vibrio* 型の菌より 4 種を純粋分離し、形態学的、生理学的研究を行つた結果、何れも *Pseudomonas* 属に属する新菌として、分類学的地位を決定した。

各菌の形態学的、生理学的性質は次の如くである。

Pseudomonas rubeolifaciens Culture No. : No. 10 (Isolated in 1949).

Etymology : From Latin *rubeo*, red or ruddy and *faciens*, make.

Vegetative morphology : Cells are straight rods with rounded ends, 0.5~0.7 by 1.2~1.8 microns*1. Size of majority is 1.5 microns long. Occurring singly, in pairs and in short chains. No capsules and endospores observed. Show straight or round-motion with one or two undulate flagella with single poles. Easy staining with basic aniline dyes. Gram-stain and acid-fast are negative. Tiny granules observed in rods.

Irregular forms : In old culture, 0.4~0.5 microns cocci or rarely 1 micron rods observed.

On cellulose agar, rounded margin, flat to concave, smooth, homogeneous, whitish-transparent and moist colonies are formed. On cellulose-dextrin agar^{*2}, rounded margin, flat to concave with echinulate center, smooth, homogeneous, milky white, shiny, translucent and viscid colonies are formed. The medium turns to slightly iridescent. On starch agar, similar growth is shown, except for flat to convex with raised center. On bouillon agar, rounded margin, flat to convex with raised center, smooth, homogeneous, pale yellowish brown, shiny and viscid colonies are formed. On these plate cultures, deep colonies are less to 1 mm. circle or fusiform, except for cellulose agar. On cellulose-dextrin agar slant culture, filiform, convex, smooth, milky white, lustrous, translucent, viscid and abundant growth are observed. The medium turns to iridescent, and condensed water is turbid. On bouillon agar slant culture, filiform, convex, smooth, pale yellowish brown, lustrous, translucent, moist and abundant growth are observed. The medium turns to brown and the condensed water is turbid. On potato, irregular margin, convex, smooth, light ochraceous salmon, lustrous, viscid and abundant growth are observed. Later potato shows pallid purplish gray coloration. On bouillon gelatin stab culture, saccate liquefaction becoming stratiform with pellicle and sediments, is observed.

On filter-paper medium, paper above the surface of liquid shows slightly yellow coloration, and partially translucent. The filter-paper in this part is eventually completely disintegrated and dispersed into fibril by shaking. If organic compounds are used as N-source, the medium becomes cloudy. In nutrient broth, turbidity in 2 days, sediments and milky white, granular and brittle pellicle in 4 days, are observed. In litmus milk, reduction of litmus begins from bottom to top of the tube, in 5 days, then, soft, fine and snow-like ppt. formed, with clear zone on this ppt. Litmus all reduced, shows alkaline reaction.

Indole not formed.

Hydrogen sulphide not formed.

Nitrates reduced to nitrites.

Ammonia produced from peptone, but not from nitrates.

Starch hydrolyzed.

Catalase is positive.

Grown up in 3% NaCl solution^{*4}.

Glucose, mannose, fructose, galactose, cellobiose, maltose, sucrose, lactose, xylose, arabinose, dextrin, glycerin, sorbit, mannit, starch, cellulose-dextrin and cellulose are utilized. Acid produced from glucose, mannose, galactose, cellobiose, maltose, sucrose, lactose, xylose, arabinose and dextrin. Gas not produced from these carbohydrates^{*5}.

NaNO_3 , $(\text{NH}_4)_2\text{SO}_4$, NH_4NO_3 , Urea, Asparagine, Peptone, Bouillon, Yeast-extract and nitrites are utilized as nitrogen source.

Facultative aerobic.

Opt. temp. for growth^{*3}: 25~30°C. Limits of temp. 17~37°C, Death point: 45~50°C. for 10 minutes.

Opt. pH for growth^{*3}: 6.5~7.5 Limits of pH: 5.5~8.5 Source: Dung from an ass of Ueno Zoological Garden, Tokyo.

Pseudomonas viscosissima Culture No.: No. 306 (Isolated in 1948).

Etymology: From Latin *viscum*, bird-lime.

Vegetative morphology: Cells are straight rods with rounded ends, 0.4~0.6 by 0.7~1.4 microns. Size of majority is 1.0 micron long. Occurring singly, in pairs and in short chains. No capsules and endospores observed. Show straight- or round-motion with single polar flagellum. Easy staining with basic aniline dyes. Gram-stain and acid-fast are negative. Tiny granules observed in rods.

Irregular forms: In old culture, 0.4~0.5 microns cocci observed.

On cellulose agar, rounded margin, flat to concave, smooth, homogeneous, whitish-transparent and moist colonies are formed. On cellulose-dextrin agar, rounded margin, flat to concave with echinulate center, smooth, homogeneous, milky white, shiny, translucent and viscid colonies are formed. On starch agar, similar growth is formed, except for flat to convex with raised center. On bouillon agar, rounded margin, flat to convex with raised center, smooth annular ring like, pale yellowish-buff, shiny and viscid colonies are formed. On these plate cultures, deep colonies are less to 1 mm. circle or fusiform, except for cellulose agar. On cellulose-dextrin agar slant culture, filiform, very convex, smooth, pale chalcedony yellow, shiny translucent, very viscid and abundant growth are observed. Condensed water is turbid. On bouillon agar slant culture, filiform, convex, smooth, pale yellowish-

buff, shiny, translucent, viscid and abundant growth are observed. Condensed water is turbid. On potato, filiform, very convex, smooth, pale ochraceous salmon, glistening, viscid and abundant growth are observed. Later potato turns to gray. On bouillon gelatin stab culture, infundibuliform liquefaction becoming stratiform with pellicle and sediments, are observed.

On filter-paper medium, paper above the surface of liquid shows slightly yellow coloration, and partially translucent. The filter-paper in this part is eventually completely disintegrated and dispersed into fibril by shaking. If organic compounds used as N-source, the medium becomes cloudy. In nutrient broth, turbidity in 24 hrs., sediments and milky white, homogeneous and hard pellicle in 3 days, are observed. In litmus milk, reduction of litmus begins from bottom to top of the tube, in 4 days, then, soft, fine and snow-like ppt. formed, with clear zone on this ppt. Litmus almost reduced, shows alkaline reaction.

Indole not formed.

Hydrogen sulphide not formed.

Nitrates reduced to nitrites.

Ammonia produced from peptone, but not from nitrates.

Starch hydrolyzed.

Catalase is positive.

Grown up in 3% NaCl solution.

Glucose, mannose, fructose, galactose, cellobiose, maltose, sucrose, lactose, xylose, arabinose, dextrin, glycerin, sorbit, mannit, starch, cellulose-dextrin and cellulose are utilized. Acid not produced from these carbohydrates. Gas produced from glucose, mannose, fructose, galactose, maltose, sucrose, lactose, xylose, arabinose, dextrin, glycerin, sorbit and mannit.

NaNO_3 , $(\text{NH}_4)_2\text{SO}_4$, NH_4NO_3 , Urea, Asparagin, Peptone, Bouillon, Yeast-extract and nitrites are utilized as nitrogen source.

Facultative aerobic.

Opt. temp. for growth : $25\sim 28^\circ\text{C}$. Limits of temp. : $10\sim 30^\circ\text{C}$. Death point : $45\sim 50^\circ\text{C}$. for 10 minutes.

Opt. pH for growth : $6.0\sim 7.0$ Limits of temp. : $-5.0\sim 9.0$

Habitat : Soil.

Source : Forest soil from Inogashira Park, Tokyo.

Pseudomonas alboflava Culture No. : No. 233-A. (isolated in 1949).

Etymology : From Latin *albus*, white and *flavus*, yellow.

Vegetative morphology : Cells are straight rods with rounded ends, $0.3\sim 0.5$ by $1.8\sim 3.0$ microns. Size of majority is $2.0\sim 2.5$ microns long. Occurring singly, in pairs and in short chains. No capsules and endospores observed. Show straight- or round-motion with single polar flagellum. Easy staining with basic aniline dyes. Gram-stain and acid-fast are negative. Tiny granules observed in rods.

Irregular forms : In old culture, $0.3\sim 0.5$ by $1.5\sim 1.3$ microns rods observed.

On cellulose agar, rounded margin, flat to concave, smooth, homogeneous, whitish-transparent and moist colonies are formed. On cellulose-dextrin agar, rounded margin, flat to concave with echinulate center, smooth, homogeneous, yellowish white, shiny, translucent and viscid colonies are formed. On starch agar, similar growth is shown, except for flat to convex with raised center. On bouillon agar, rounded margin, flat to convex with raised center, smooth, homogeneous pale yellowish-buff, shiny, and viscid colonies are formed. On these plate cultures, deep colonies are less to 1 mm. circles or fusiform, except for cellulose agar. On cellulose-dextrin agar slant culture, filiform, very convex, smooth, pale dull green yellow, lustrous, translucent, viscid and abundant growth are observed. Condensed water is turbid. On bouillon agar slant culture, filiform, convex, smooth, pale yellow-buff, lustrous, translucent, viscid and abundant growth are observed. Condensed water is turbid. On potato, filiform, very convex, smooth, cinnamon buff to pinkish buff, glistening, viscid and abundant growth are observed. Later potato turns to gray. On bouillon gelatin stab culture, stratiform with pellicle and sediments, is observed.

On filter-paper medium, paper above the surface of liquid shows pinhead yellow coloration, and partially translucent. The filter-paper in this part is eventually completely disintegrated and dispersed into fibril by shaking. If organic compounds are used as N-source, the medium becomes cloudy. In nutrient broth, turbidity in 24 hrs., sediments and milky white, homogeneous and hard pellicle in 3 days, are observed. In litmus milk, reduction of litmus begins from bottom to top of the tube, in 3 days, then,

soft, fine, and snow-like ppt. formed, with clear zone on this ppt. Litmus almost reduced, shows alkaline reaction.

Indole not formed.

Hydrogen sulphide not formed.

Nitrates reduced to nitrites.

Ammonia produced from peptone, but not from nitrates.

Starch hydrolyzed.

Catalase is positive.

Grown up in 3% NaCl solution.

Glucose, mannose, fructose, galactose, cellobiose, maltose, sucrose, lactose, xylose, arabinose, dextrin, glycerin, sorbit, mannit, starch, cellulose-dextrin and cellulose are utilized. Acid and gas not produced from these carbohydrates.

NaNO_3 , $(\text{NH}_4)_2\text{SO}_4$, NH_4NO_3 , Urea, Asparagine Poptone, Bouillon, Yeast-extract and nitrites are utilized as nitrogen source.

Facultative aerobic.

Opt. temp. for growth. : $22\sim 28^\circ\text{C}$. Limits of temp. : $10\sim 30^\circ\text{C}$. Death point : $45\sim 50^\circ\text{C}$. for 10 minutes.

Opt. pH for growth : $7.0\sim 8.0$ Limits of pH : $5.5\sim 8.0$

Habitat : Soil.

Source : Mud from ditch of Toyama.

Pseudomonas fluorescens var. *cellulosa*.

Culture No. : No. 107 (Isolated in 1948).

Ethymology : From chemical term, cellulose.

Vegetative morphology : Cells are straight rods with rounded ends, $0.5\sim 0.7$ by $1.2\sim 3.0$ microns. Size of majority is $1.5\sim 1.8$ microns long. Occurring singly, in pairs and in short chains. No capsules and endospores observed. Show straight- or round-motion with single polar flagellum. Easy staining with basic aniline dyes. Gram-stain and acid-fast are negative. Tiny granules observed in rods.

Irregular froms : In old culture, $0.3\sim 0.5$ by $0.8\sim 1.2$ microns rods observed.

On cellulose agar, rounded margin flat to concave, smooth, homogeneous, whitish-transparent and moist colonies are formed. On cellulose-dextrin agar, rounded margin, flat to concave with raised center, smooth, homogeneous, milky white, translucent and moist colonies are formed. The medium, concentric circles of colonies, shows fluorescent bluish green to yellowish green coloration. On starch agar, similar growth is shown, except for flat to convex with raised center. On bouillon agar, rounded margin, flat to convex with raised center, smooth, homogeneous, pale yellowish-brown, shiny and viscid colonies are formed. On these plate cultures, deep colonies are less to 1 mm. circle or fusiform, except for cellulose agar. On cellulose-dextrin agar slant culture, filiform, convex, smooth, milky white, lustrous, transparent, viscid and abundant growth is observed. The medium and turbid condensed water, show very beautiful fluorescent bluish green to yellowish green coloration. On bouillon agar slant culture, filiform, convex, smooth, pale yellowish-brown, lustrous, translucent, moist and abundant growth is observed. Condensed water is turbid and the medium turns to brown. On potato, filiform, convex, smooth, pinkish cinnamon to cinnamon buff, lustrous, viscid and abundant growth is observed. Later potato turns to gray. On bouillon gelatin stab culture, crateriform liquefaction becoming stratiform with pellicle and sediments, is observed.

On filter-paper medium, paper above the surface of liquid shows slightly yellowish green coloration, and partially translucent. The filter-paper in this part is eventually completely disintegrated and dispersed into fibril by shaking. Liquid shows yellowish green coloration, and if organic compounds are used as N-source, becomes cloudy. In nutrient broth, turbidity in 2 days, sediments and milky white, rugose and brittle pellicle in 3 days, are observed. In litmus milk, reduction of litmus begins from bottom to top of the tube, in 5 days, then, soft, fine and snow-like ppt. formed, with clear zone on this ppt. Litmus all reduced, shows alkaline reaction.

Indole not formed.

Hydrogen sulphide not formed.

Nitrates reduced to nitrites.

Ammonia produced from peptone, but not from nitrates.

Starch hydrolyzed.

Catalase is positive.

Grown up in 3% NaCl solution.

Glucose, mannose, fructose, galactose, cellobiose, maltose, sucrose, lactose, xylose, arabinose, dextrin, glycerin, sorbit, mannit, starch, cellulose-dextrin and cellulose are utilized. Acid not produced from these carbohydrates. Gas produced from fructose, galactose, arabinose and dextrin.

NaNO₃, (NH₄)₂SO₄, NH₄NO₃, Urea, Asparagine, Peptone, Bouillon, Yeast-extract and nitrites are utilized as nitrogen source.

Facultative aerobic.

Opt. temp. for growth: 37°C. Limits of temp.: 17~47°C. Death point: 50~55°C for 10 minutes.

Opt. pH for growth: 7.0~9.0 Limits of pH: 6.0~9.0-

Habitat: Soil.

Source: Field soil from Saitama-ken.

〔註〕 *1...*6 は其の 1 に同じ.

Pseudomonas の分類について

上記実験結果より, BERGEY'S Manual 6 th Ed. 中に分類されている近似菌と比較した結果, (107), (233-A), (10), (306) は cellulose 分解能力, その他種々の差異により, 明らかに新菌と認められる.

即ち, No. 107 は, cellulose を分解し, 青緑色の water soluble fluorescent pigment を出す事より, *Pseudomonas fluorescens* の変種と認め, *Pseudomonas fluorescens* var. *Cellulosa* と命名, No. 233-A は cellulose を分解し, 黄白色の菌体を有する事より, *Pseudomonas alboflava* nov. sp. 又, No. 10 は cellulose を分解し培地に淡紅色の water soluble pigment を生産する事より *Pseudomonas rubeorifaciens* nov. sp. No. 306 は cellulose を分解し, 且つ著しく粘質な菌苔を形成する事より *Pseudomonas viscosissima* nov. sp. と命名した.

此の 4 種の菌を BERGEY'S Manual 6th ed. に従つて分類を行うとき, 従来の儘では, 何れも適当と思われる場所に位置させる事が出来ない. 吾々はこれ等の菌につき検討した結果, 6 th ed. の分類に項目を次のように挿入するのが適当ではないかと考える.

Addition to the Bergey's Manual 6 th. ed. Genus *Pseudomonas*.

I. Soil and flesh water forms.

1. Green fluorescent pigment produced.

a. Gelatin liquefied.

b. Polar flagellate.

c. Grow readily at 30°. Usually bluish-green.

Pseudomonas fluorescens var. *cellulosa*

2. Green fluorescent pigment not produced.

a. Gelatin liquefied.

b. Polar flagellate.

c. Grow poorly or not at all at 37°

dd. Slow reduction of litmus and becomes alkaline.

e. Not known to digest cellulose.

Ps. mephitica, *Ps. geniculata*.

ee. Digests cellulose.

Pseudomonas alboflava

Pseudomonas viscosissima

Pseudomonas rubeorifaciens

この分類表中 *Ps. viscosissima* 及び *Ps. rubeorifaciens* は糖に対する態度, 生育温度範囲等により *Ps. alboflava* と同位置に並べる事は出来ず, 適当なる位置を作り得なかつたが, 大体に於いて, この附近に位置すべきものと思われる.

好気性中温性繊維素分解菌の中 6 th. ed で Family *Pseudomonadaceae* に属するものは, Genus *Pseudomonas*, Genus *Vibrio*, Genus *Cellulivibrio*, Genus *Cellulifalcula*, Genus *Xanthomonas* として分類されている. 6 th ed. では一応明瞭なる差異を認めて分類されているが, *Vibrio* (KALNINS⁽⁹⁾) *Cellulivibrio* (WINOGRADSKY)

ky⁽⁶⁾) の差異については、なお異論がある処である。又同 Manual 5th ed. (1939) が 10 年後に 6th ed. (1948) として改版される際に、*Bacteriaceae* に属する Genus *Cellulomonas* は大きく改変され、polar flagella なるものを *Pseudomonas* に Gram variable 且 non-motile のものは、*Corynebacterium* に移され、又 peritricous flagella を有するものを其のまま Family *Bacteriaceae* の Subgenus *Cellulomonas* として残されている。

これ等の歴史的変動は、cellulose 分解能力を如何に重要視するか程度の差に基づくものであろう。Cellulose 分解能力が無いという事は、cellulase の欠除を意味するものである。一方、吾々の研究によれば、*Pseudomonas* に属する繊維素分解菌を一旦 bouillon agar に接種すると、繁殖は旺盛であるが、cellulose 分解能力を喪失するという事実は、逆に考えると、今まで cellulose 分解については、何等報告されていない数多の細菌中には、本来は cellulose 分解能力を有していたものがあるのではないかと、そして分離過程で喪失したのではないかと、いう疑問も生ずる。Cellulose 分解を惹起する enzyme が adaptive なものであるなら、cellulose 分解能力を有する菌に還元し得るという可能性も有る。Cellulose 分解能力が本質的なものであるか、或いは適応性を有するものであるかの問題は、今後の研究に待つ他はないが、現在の処、吾々は cellulose 分解能力を重要視して、4 種の菌を新種と認めたのである。

要 約

1. 北海道及び本州東半部より、主として森林土壌を採集、種々の培養条件で集積した処、何れの条件に於いても圧倒的に多く *Cytophaga* 型の菌が集積された。
2. 分離した *Sporocytophaga* に属する 13 Strains を比較検討せる結果、*Sporocytophaga myxococcoides* として分類されるべき菌に 4 種の異つた strains を見出した。即ち、

Sporocytophaga ochracea nov. sp. (No. 2-1)

Sporocytophaga myxococcoides α (No. 289)

" β (No. 141)

" γ (No. 247)

3. 此の結果より、BERGEY'S Manual 6th ed. の Genus *Sporocytophaga* に対する分類法を更に細分する事を提唱する。
4. 電子顕微鏡による観察の結果 *Sporocytophaga* の vegetative cell の両端は明らかに丸い事を確認し、且つ myxocyst の発芽過程につき、2~3 の資料を得た。
5. 4 種の *Pseudomonas* 属に属する好気性中温性繊維素分解菌を分離し、何れも新種と認めて、次の如く命名した。

Pseudomonas alboflava nov. sp. (No. 233-A)

Pseudomonas rubeorifaciens nov. sp. (No. 10)

Pseudomonas viscosissima nov. sp. (No. 306)

Pseudomonas fluorescens var. *cellulosa* (No. 107).

6. 上記 4 種の新菌を適当な地位に位置させる為、BERGEY'S Manual 6th ed. Genus *Pseudomonas* に対する分類法に繊維素分解に関する項目を附加する事を提唱する。

終りに臨み、御協力を得た山田助教授に厚く感謝の意を表する。又電子顕微鏡写真の撮影に当り、種々御助力を得た慈恵医大寺田教授に厚く感謝の意を表する。

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Summary

1. To enrich the various aerobic cellulolytic bacteria, 68 soils, from Hokkaido and the east of the mainland of Japan, were inoculated in filter-paper medium under various conditions. *Sporocytophaga* was dominant bacteria in the forest soils.

2. 13 stains of *Sporocytophaga* were isolated and studied taxonomically, and 4 new different stains were found out of these 13 strains.

They are : *Sporocytophaga ochracea* nov. sp.

Sporocytophaga myxococcoides α

" " β

" " γ

3. Based on these 4 strains, we propose that the key to the species of genus *Sporocytophaga* in Bergey's Manual 6 th, ed. must be subdivided.

4. Several interesting pictures of *Sporocytophaga* were obtained by electron-microscopic studies.

5. Another 4 new species of aerobic mesophilic cellulose-decomposing bacteria were isolated, which should be placed in genus *Pseudomonas*.

They are : *Pseudomonas alboflava* nov. sp.

" *rubeorifaciens* nov. sp.

" *viscosissima* nov. sp.

" *fluorescens* var. *cellulosa*.

6. Based on these 4 species, we propose that the key to the species of genus *Pseudomonas* in Bergey's Manual 6 th ed. must be subdivided.

高周波による化学反応の追跡 (第 2 報)

滴定曲線の解釈及び Glycine の滴定曲線

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昭和 26 年 8 月 14 日 受理

Studies on Chemical Reaction by a High Frequency Oscillator. Part 2.

By Tetsuro KONO (Faculty of Agriculture, Univ. of Tokyo)

第 1 報⁽¹⁾に於て述べた如く、水中に電解質の稀薄溶液を加えると、その電気伝導度が大きい場合程メーターの針が大きく振れる。これは反応容器中の媒質により高周波のエネルギーの吸収される度合が異なり、そのために発振真空管のグリッド電圧が変化するためである。電気伝導度 λ_1 及び λ_2 、透電恒数 ϵ_1 及び ϵ_2 なる 2 種の媒質によつて吸収される高周波のエネルギー P_1 及び P_2 の比は $P_1/P_2 = \lambda_1/\lambda_2 (\epsilon_2/\epsilon_1)^2$ なる式であらわされる⁽²⁾。電気伝導度は低周波の場合と高周波の場合異つた値を示すことが知られているが、今第一近似として普通の当量伝導度をとると⁽³⁾ Table 1 に示す如く強酸は非常に大であり、強塩基がこれに次ぎ、塩類は一般に中程度で弱酸