UREA-HYDROLYZING BACILLI

I. A Physiological Approach to Identification

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Species of the genus *Bacillus* are classified primarily on the basis of the morphology of spores and sporangia and secondarily on biochemical properties (Smith *et al.*, 1952). The bacteriological criteria employed by Smith and co-workers (1946, 1952) have been endorsed generally by Knight and Proom (1950), who have surveyed the nutritional requirements of freshly isolated bacilli. The comprehensive studies by the above investigators are significant contributions to the systematics of the genus. However, urea-hydrolyzing species, the so-called "*B. pasteurii* group" of Gibson (1934), are generally inert at the diagnostic level and comprise a poorly defined and complex group.

Recent work (Larson and Kallio, 1954) has been concerned primarily with *Bacillus pasteurii*. The present investigation was undertaken to gain a broader understanding of ureolytic bacilli and the role of urease in the ecology of these organisms. The bacteriological criteria employed by Smith and co-workers for *classification* of the genus are not entirely suitable for *identification* of strains isolated from urea-enrichment cultures. This report proposes to examine a system allowing the identification of urea-hydrolyzing bacilli by evaluation of all-or-none growth responses over a range of pH values from 5 to 11.

MATERIALS AND METHODS

Preliminary studies. Bacteriological investigation of ureolytic bacilli was initiated with a collection consisting of *B. pasteurii* Gibson strain 22 and 25 isolates from various samples of soil. Only strains of the genus *Bacillus* have been isolated consistently from a 5 per cent solution of urea heavily inoculated with soil. When hydrolysis of urea was proceeding vigorously and ammonia fumes were being liberated, plates containing nutrient agar supplemented with 3 per cent urea were streaked from the primary enrichments. Single, isolated colonies were picked, and transferred onto 3 per cent urea-nutrient agar slants.

Macroscopic and microscopic examination of isolates, and tests for the hydrolysis of gelatin, starch, and casein were performed as described by Smith and co-workers (1952). Ability to hydrolyze urea was determined using urea-agar medium (Christensen, 1946) and reduction of nitrate to nitrite was tested in nitrate broth using sulfanilic acid and α-naphthylamine acetate. The growth response at different temperatures and pH values was investigated using a broth of the following composition: 0.5 per cent polypeptone (BBL), 0.2 per cent yeast extract (BBL), tap water to 1 liter; adjusted to pH 7. The inoculum consisted of a large loopful of a suspension of cells prepared by adding 1 ml of sterile water to a 24-hr slant culture. Unless otherwise specified, incubation was at 25 C.

Modified techniques. Twenty-seven known strains of bacilli from the collection of Dr. N. R. Smith were kindly furnished by Dr. Ruth E. Gordon of Rutgers University. Several species and strains of bacteria from other sources served as biological controls. All organisms were maintained as slants on a basal medium of the following composition (in percentages): enzymatic casein hydrolyzate, 0.4; polypeptone (BBL), 0.4; yeast extract (BBL), 0.2; agar, 1.5; adjusted to pH 8. Strains of *B. pasteurii* were cultured on the above medium amended with urea (final concentration, 1.5 per cent).

The basal medium, adjusted to pH 7.0 and containing thymol blue, indicated activity of urease if a blue color developed at the site of growth and diffused throughout the medium. Thymol blue provides for a slower indication of urease than is possible with phenol red as an indicator in urea media. Thus the ability to hydrolyze urea could be correlated with the

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TABLE 1

Properties of spores of bacilli isolated from 5 per cent urea enrichments

Designation Strain	Swollen Sporangium	Shape of Spore		Morphological* Group
		Oval	Round	
12	+	+		2
T-16	+	+		2
T-2	+	+		2
S-91	+	+		2
S-9	+	+		2
R-3	+	+		2
R-28	−	•†	•	
R-83	+	+	+	2, 3
R-18	+	+	+	2, 3
R-17	+	+	+	2, 3
R-19	+	+		2
R-20	+	+		2
Q-27	+	+		2
92	•	•	•	
81	+	+	+	2, 3
13	+	•	•	
82	+	+	+	2, 3
U/A-37	•		+	3
85	−	+	+	1
U/B-7	+		+	3
U-22	+		+	3
U/A-24	•	•	•	
89	•	•	•	
U/A-15	+		+	3
93	•		+	3
Gibson 22	•	•	•	

* Assignment based upon interpretation of criteria employed by Smith *et al.* (1952).

† Evidence insufficient for evaluation.

magnitude of surface growth after incubation of the culture for 24 to 48 hours.

To ascertain the ability of an organism to grow over a wide range of pH values on a medium lacking either added urea or NH_4^+, the basal medium was amended with buffers (final concentration, 0.01 M) as follows, and the pH adjusted to the appropriate value:

pH 5: KH_2PO_4; methyl red indicator (red at pH 4.2; yellow at pH 6.2)

pH 7: equimolar mixture of Na_2HPO_4 and KH_2PO_4; bromothymol blue indicator (yellow at pH 6.0; blue at pH 7.6)

pH 9: *tris*-(hydroxymethyl) aminomethane; metacresol purple indicator (yellow at pH 7.5; purple at pH 9.1)

pH 11: "tris" buffer; Nile blue A indicator (blue at pH 10; pink at pH 11)

Although each medium was adjusted to and buffered at the respective pH value, growth of any one strain at a particular pH may result in the accumulation of products altering the pH of the medium; consequently observation of both magnitude of growth response and acid-base change furnished information as to the ability of a particular strain to respond to and alter the *in vitro* environment.

Most species of the genus *Bacillus*, especially members of the morphological group 3, have been demonstrated to possess a nutritional requirement for amino acids (Knight and Proom, 1950). The ability of all strains to grow in the presence of only trace amounts of amino acids was assayed with a medium of the following composition in grams per liter: NH_4Cl, 10; glucose, 10; yeast extract, 1; Na_2HPO, 1.5; KH_2PO_4, 1.4; agar, 1.5; 250 ml double strength mineral solution (Hutner *et al.*, 1950); 750 ml distilled water. Bromothymol blue was the indicator in this medium.

RESULTS

Preliminary studies. Colonies on streak plates of 2 per cent urea-nutrient agar were either punctiform or circular with smooth surface and entire edge, and a diameter not greater than 2 mm. Colonies were either opaque or translucent; some cultures exhibited both characteristics. When growth occurred on ordinary nutrient agar without added urea, colonies were punctiform, circular (diameter not greater than 4 mm), or irregular. Colonies were either opaque or translucent with a smooth surface and entire edge. Growth on nutrient agar and in nutrient broth was abundant and of no determinative aid.

Microscopic observations of spores and vegetative cells over a period of two years indicated a general constancy of characteristics. These findings were of dubious value in classifying the collection according to the key advanced by Smith and co-workers (1952) for the identification of species of the genus *Bacillus*. According to this key, the first evaluation is determined by the degree to which the sporangium is swollen, the relative thickness of the spore wall, and the shape of the spore—cylindrical, oval, or spherical (round). After allocating a strain to a morphological group, it can be traced further on the basis of at least two physiological characters. Urea-hydrolyzing bacilli are generally inactive

TABLE 2

Physiological properties of ureolytic bacilli

Strain Designation	Initial pH of Nutrient Broth					Temperature*			Concentration of Urea*			
	5.2	6.6	7.4	8.1	8.8	45 C	37 C	25 C	0.2 M	0.4 M	0.8 M	1.6 M
Eury-responsive strains												
12	1	4	4	4	4	1	3	4	4	4	4	0
T-16	1	4	4	4	4	1	4	4	4	4	4	3
T-2	1	3	3	4	3	1	4	3	4	4	4	4
S-91	4	4	4	4	4	1	4	4	3	3	2	0
S-9	1	4	3	4	3	2	3	3	2	3	1	0
R-3	1	3	3	4	4	1	3	3	4	4	4	1
R-28	1	1	3	4	3	0	4	3	4	4	3	3
R-83	1	3	4	4	4	4	4	4	4	4	4	3
R-18	1	4	4	4	3	1	4	4	4	4	4	0
R-17	1	1	4	4	4	1	4	4	4	4	3	3
R-19	1	4	4	4	4	1	4	4	4	4	3	4
R-20	1	2	3	4	4	2	4	3	4	4	4	4
Q-27	1	2	3	4	4	1	3	3	4	4	4	4
92	1	1	2	3	3	0	2	2	4	4	4	4
81	0	1	1	1	2	3	1	1	3	3	3	3
13	0	2	1	2	2	0	4	1	1	0	0	0
82	0	0	1	2	3	0	0	1	4	4	4	4
Steno-responsive strains												
U/A-37	0	1	2	3	3	0	0	2	2	3	3	4
85	0	0	1	1	3	0	0	1	4	4	4	3
U/B-7	0	0	1	2	1	0	0	1	4	4	4	4
U-22	0	0	0	1	1	0	0	0	4	4	4	2
U/A-24	0	0	0	2	2	0	0	0	4	4	4	4
89	0	0	0	2	2	0	0	0	1	1	1	0
U/A-15	0	0	0	0	2	0	0	0	4	4	4	4
93	0	0	0	0	0	0	0	0	4	4	4	2
Gibson 22	0	0	0	0	0	0	0	0	2	2	2	0

Cultures were evaluated as follows after 4 days' incubation: 4, heavy growth; 3, moderate growth; 2, light growth; 1, scant growth; 0, no growth.

* The medium was nutrient broth at pH 7.

diagnostically—morphology and negative characteristics determine their identity. Gibson (1935*a*) recognized spore morphology and a preference for alkaline media as important criteria for the "*B. pasteurii* group," and proposed a graded rate response in the ability to hydrolyze urea. *B. pasteurii* was restricted to those species having spherical or slightly ovoid spores and hydrolyzing 2 per cent urea in broth within 48 hours. Although *B. loehnisii* possessed spherical or slightly ovoid spores and *B. freudenreichii* displayed ovoid to elliptical spores, both species brought about a slower decomposition of urea, and were able to grow in neutral media. Gibson found *B. sphaericus* to be closely related to the "*B. pasteurii* group," but it was unable to decompose urea and showed no preference for alkaline media containing urea.

Data concerning the microscopic characteristics of spores are summarized in table 1. Considerable difficulty was experienced because the distinction between swollen, slightly swollen and nonswollen sporangia, thick and thin spore walls, and spherical (round) and oval shaped spores is not very great. In addition, urea-hydrolyzing bacilli may become asporogenous on artificial media (Gibson, 1935*b*).

Survey of some physiological characteristics

TABLE 3

Biochemical characteristics of bacilli isolated from 5 per cent urea enrichments

Strain Designation	V-P reaction	Hydrolysis of			Reduction of Nitrate	Urease	Utilization of Citrate
		Starch	Casein	Gelatin			
Eury-responsive strains							
12	−*	−	+†	+	+	−	•‡
T-16	−	−	+	+	+	+	•
T-2	−	−	+	+	+	+	•
S-91	−	−	+	+	−	+	−
S-9	−	−	+	−	−	+	•
R-3	−	−	−	−	+	+	•
R-28	•	−	−	−	+	+	•
R-83	−	−	−	−	+	+	−
R-18	−	−	−	−	+	+	•
R-17	−	−	−	−	+	+	•
R-19	−	−	−	−	+	+	•
R-20	−	−	−	−	+	+	•
Q-27	−	−	−	−	+	+	•
92	−	−	−	+	−	+	−
81	−	−	−	−	−	+	−
13	−	−	+	−	+	+	•
82	−	+	−	−	−	+	−
Steno-responsive strains							
U/A-37	•	−	0§	0§	+§	+	•
85	−	−	0¶	+	+	+	−
U/B-7	•	−§	−§	−§	+§	+	•
U-22	•	•	•	−§	•	•	•
U/A-24	•	−§	+§	−§	+§	+	•
89	•	−	−	−	−	+	•
U/A-15	•	−§	+§	+§	+§	+	•
93	−	−	−	−	−	+	−
Gibson 22	•	−§	−§	−§	+§	+	−

* Negative reaction.
† Positive reaction.
‡ Not tested.
§ Urea added to medium.
¶ No growth.

of this collection of ureolytic soil isolates yielded the information presented in table 2. These data indicate that the 26 strains studied comprise two groups, termed *eury-responsive* and *steno-responsive*. Eury-responsive strains grew over a wide pH range, at temperatures from 25 to 45 C, and in nutrient broth containing various concentrations of urea up to 9.6 per cent (1.6 M). Steno-responsive strains exhibited growth over a narrow range of values, pH 8.8 being optimal. Growth occurred in nutrient broth supplemented with urea, but not in broth alone. The growth response at high concentrations of urea was not as great as at low concentrations. Eury-responsive strain no. 13 was inhibited by 0.4 M urea, and grew poorly in 0.2 M urea at 25 C.

The biochemical characteristics are recorded in table 3. Citrate-utilization and acetylmethylcarbinol-production were negative. Casein was hydrolyzed by 8 strains, and gelatin by 7 strains. Eighteen strains produced nitrite from nitrate. Urease activity was exhibited by all cultures except strain 12. Of the 26 strains of bacilli studied, only strain 82 displayed amylytic activity. This isolate was identified as a strain of *B. lentus* because it exhibited amylytic activity, hydrolyzed urea, hydrolyzed neither casein nor gelatin, and did not reduce nitrate.

On the basis of physiological characteristics, however, the steno-responsive strains were all tentatively considered to be strains of *B. pasteurii*.

Application of modified techniques to the investigation of known bacilli. Analysis of known organisms according to steno- and eury-responsive categorization is summarized in table 4. Since this collection is well represented by nonureolytic organisms, it was appropriate to subdivide the eury-responsive category into ureolytic and nonureolytic subgroups. The 4 strains of *B. pasteurii* belonged to the steno-responsive group. These strains were unable to grow well at any initial pH value, but grew well when urea was present in the medium.

Although all 12 known strains of *B. sphaericus* were eury-responsive, 4 of the 5 ureolytic strains had previously been considered (Smith *et al.*, 1946) as strains of *B. sphaericus* var. *fusiformis*. The only currently available type strains of *B. freudenreichii* and *B. loehnisii* were eury-responsive, as were strains of *B. lentus*. The remaining strains of known organisms, other than members of the genus *Bacillus*, were eury-responsive, ureolytic bacteria. Had they not been purposely included in this collection as controls, these would normally not have been subjected to this type of determinative approach because of their morphological and physiological characteristics.

Seven strains of *B. sphaericus* did not hydrolyze urea, and exhibited a similar eury-responsive pattern. Both cultures of strain 967 displayed good growth on a medium limited in amino acids.

TABLE 4

Physiological properties of known organisms

Organism		Urease		pH 5	pH 7		pH 9		pH 11	No Amino Acids
		pH 9.6	Growth	Growth	Growth	Reaction	Growth	Acid produced	Growth	Growth
Steno-responsive										
B. pasteurii	NRS 929	+*	4	0	0	•†	1	−‡	1	0
	NRS 673	+	4	0	0	•	0	•	1	1
	NRS 674	+	4	0	0	•	0	•	0	1
	NRS 675	+	3	0	0	•	0	•	0	1
Eury-responsive, ureolytic										
B. sphaericus	NRS T-156	+	1	4	4	B§	4	−	3	1
	NRS 339	+	3	4	4	B	4	−	4	0
	NRS 350	+	3	4	3	B	4	−	4	1
	NRS 1023	+	4	4	4	B	4	−	4	1
	NRS 866	+	4	4	4	B	4	−	4	1
B. freudenreichii	NRS 671	+	3	3	4	B	4	−	1	1
B. loehnisii	NRS 672	+	3	2	4	B	3	−	3	1
B. lentus	NRS 670	+	3	0	3	B	1	−	1	1
	NRS 1262	+	3	0	4	B	1	−	1	1
	NRS 883	+	3	0	3	B	1	−	1	0
Sarcina ureae	KRAL	+	3	1	3	B	1	−	4	1
	ATCC 6473	+	3	0	3	B	3	−	3	1
Bact. ammoniagenes	ATCC 6871	+	3	4	4	B	4	+	4	3, A
	ATCC 6872	+	3	4	4	B	4	+	4	3, A
Proteus vulgaris	ATCC 9920	+	3	4	4	A¶	4	+	0	4, A
Proteus morganii		+	3	4	4	B	4	−	0	4, A
Eury-responsive, nonureolytic										
B. sphaericus	NRS 344	−	3	4	4	B	1	−	3	1
	NRS 967	−	4	4	4	B	4	−	4	3
	967	−	4	4	4	B	4	−	4	3
	NRS 719	−	4	4	4	B	4	−	4	1
	NRS 348	−	3	4	4	B	4	−	4	1
	NRS 966	−	4	4	4	B	4	−	4	1
	NRS 810	−	4	4	4	B	4	−	4	1
B. pantothenticus	NRS 1317	−	1	0	1	•	3	−	3	1
	NRS 1318	−	1	0	4	A	4	+	3	0
	NRS 1319	−	1	0	4	A	3	+	4	0
	NRS 1320	−	1	0	3	A	4	+	4	0
	NRS 1321	−	1	0	1	•	3	+	3	1
	NRS 1322	−	1	0	3	A	3	+	3	1
B. rotans	NRS 633	−	3	0	4	B	3	−	3	1

Cultures were evaluated as follows after 2 days' incubation at room temperature (25 to 30 C): 4, heavy growth; 3, moderate growth; 2, light growth; 1, scant growth; 0, no growth.

* Positive reaction.

† No change.

‡ Negative reaction.

§ Alkaline reaction (pH $\geq$ 7.6).

¶ Acid reaction (pH $\leq$ 6.0).

TABLE 5

Physiological properties of isolates obtained from 5 per cent urea enrichments

Strain Designation	Urease		pH 5	pH 7		pH 9		pH 11	No Amino Acids
	pH 9.6	Growth	Growth	Growth	Reaction	Growth	Acid produced	Growth	Growth
Steno-responsive									
85	+*	4	1	1	•†	4	−‡	3	0
93	+	1	0	0	•	0	•	0	1
U/A-15	+	4	0	0	•	1	−	1	1
U/A-37	+	4	1	0	•	4	−	3	0
89	+	1	0	0	•	1	−	1	0
U/A-24	+	4	0	0	•	0	•	1	1
U-22	+	3	0	0	•	3	−	1	0
U/B-7	+	3	1	0	•	1	−	0	1
B. pasteurii Gibson 22	−	1	0	0	•	0	•	0	0
Eury-responsive, ureolytic									
T-2	+	4	4	4	B§	4	−	3	0
R-20	+	4	0	4	B	4	−	4	1
13	+	1	1	4	B	3	−	3	0
R-83	+	3	3	4	B	4	−	4	1
R-18	+	4	3	4	B	3	−	4	0
Q-27	+	4	3	4	B	4	−	4	1
T-16	+	4	4	3	B	4	−	4	0
S-91	+	2	4	4	B	4	−	4	1
S-9	+	2	4	4	B	4	−	3	3
R-3	+	3	0	4	B	4	−	4	1
R-19	+	4	4	4	B	4	−	4	0
81	+	4	1	4	B	4	−	3	1
R-28	+	4	1	4	B	4	−	4	1
R-17	+	4	0	4	B	4	−	4	1
92	+	4	0	4	B	4	−	4	0
B. lentus 82	+	4	1	4	B	3	−	3	0
Eury-responsive, nonureolytic									
12	−	1	4	4	B	4	−	4	0

Cultures were evaluated as follows after two days' incubation at room temperature (25 to 30 C): 4, heavy growth; 3, moderate growth; 2, light growth; 1, scant growth; 0, no growth.

* Positive reaction.

† No change.

‡ Negative reaction.

§ Alkaline reaction (pH ≥ 7.6).

B. rotans exhibited a response profile similar to that of nonureolytic strains of *B. sphaericus*. The 6 strains of *B. pantothenticus* were all characterized as eury-responsive, nonureolytic bacilli. In the presence of added urea, growth of *B. pantothenticus* strains was either inhibited or not appreciably different from that on the same basal medium without urea; growth on the basal medium occurred over a range of pH values from 7 to 9. Those cultures exhibiting only scant growth at pH 7 grew abundantly on media at higher pH values. Growth of *B. pantothenticus* strains 1318 to 1322 was accompanied by the production of acid. The characteristics of this species (Proom and Knight, 1950; Smith *et al.*, 1952) excludes *B. pantothenticus* from those

species of the genus exhibiting a negative response to classical laboratory determinative techniques.

Application of modified techniques to the investigation of isolates from 5 per cent urea enrichments. The collection initially studied was reinvestigated for comparison of results and possible identification (table 5). The isolates were urease-positive with the exception of strain 12. The negative urease reaction and poor growth exhibited by *B. pasteurii* Gibson strain 22 is believed to be due to a degeneration of this particular strain which exhibits urea-hydrolyzing ability (table 3) and which has been the source of a purified bacterial urease closely related to jack bean urease (Larson and Kallio, 1954). Therefore, under the rigid conditions of the present test, the negative urease reaction is neither typical nor valid. However, all ureolytic bacilli freshly isolated from 5 per cent urea enrichments could be clearly separated into either the steno- or eury-responsive category.

DISCUSSION

The results of this comparative study indicate that the physiological characterization of urea-hydrolyzing bacilli affords a presumptive identification of strains of *B. pasteurii*. Eury-responsive, ureolytic species comprise known strains of *B. freudenreichii*, *B. loehnisii*, *B. lentus*, and some strains of *B. sphaericus*. The occurrence of both urease-positive and urease-negative strains of *B. sphaericus* confounds identification of this species. Smith and co-workers (1946) tentatively allocated *B. fusiformis* as a variety of *B. sphaericus*, separated from it only by the ability to hydrolyze urea. Recently, urease-positive *B. sphaericus* var. *fusiformis* was stated (Smith *et al.*, 1952) to represent a biotype of *B. sphaericus*. The formation of urease was considered a variable characteristic of the species. This change in classification was reported to be strengthened as a result of the studies of Knight and Proom (1950) on the nutrition of *B. sphaericus* and of *B. sphaericus* var. *fusiformis*. Six of 9 strains of *B. sphaericus* grew on a casein basal medium without added thiamin. The remaining 3 strains of *B. sphaericus* and 10 strains of *B. sphaericus* var. *fusiformis* required the addition of biotin. Neither biotin nor thiamin was effective singly. No correlation was found to exist between nutritional requirements and the production of urease.

Smith and co-workers (1952) have transposed the currently available type strain of *B. rotans* and of *B. loehnisii* to an unclassified status. Previously, they had tentatively considered these organisms to be varieties of *B. sphaericus*. Gibson (1935*a*), however, considered *B. loehnisii* to be very close to *B. pasteurii* which requires free ammonia for growth, but to differ from it by the ability to grow in ordinary neutral media.

The known strains of *B. pantothenticus* employed in the present study were all characterized as eury-responsive, nonureolytic bacilli. They form a homogeneous group and exhibit a growth response profile different from the several urease-negative strains of *B. sphaericus* and the single strain of *B. rotans*. Prior to the discovery of *B. pantothenticus* (Proom and Knight, 1950), morphological group 3 consisted of *B. pasteurii* and *B. sphaericus*, and provisionally of *B. sphaericus* var. *rotans*, *B. sphaericus* var. *fusiformis*, and *B. sphaericus* var. *loehnisii* (Smith *et al.*, 1946). Smith and co-workers (1952) report that *B. pantothenticus* was placed in morphological group 3 because of round spores "although it would seem to be intermediate between groups 2 and 3." *B. pantothenticus* does not possess urease, grows slowly at 28 C, and produces acid without gas from glucose and sucrose. While characteristics of spores of some bacilli may be useful taxonomically, basing a classification of species comprising morphological group 3 on characteristics of spores engenders confusion, since spore morphology is employed to *allocate* this species to group 3 and must be utilized to identify less versatile "residents" of group 3.

The ability to hydrolyze urea seems to stand out as a useful property among otherwise negative or variable characteristics, and would appear to be of significant diagnostic value for the genus *Bacillus*. The validity of eury- and steno-responsive categorization of ureolytic bacilli is strengthened by the nutritional observation that steno-responsive cultures require free ammonia (or urea which is equivalent to ammonia in these strains). Neither conventional bacteriological characterization (Smith *et al.*, 1952) nor physiological categorization allow the several ureolytic species comprising the eury-responsive group to be differentiated one from the other. Nutritional screening (Knight and Proom, 1950) may be the key to complete identification of

steno- and eury-responsive species of ureolytic bacilli.

SUMMARY

Investigation of 25 freshly isolated strains of mesophilic, urea-hydrolyzing members of the genus *Bacillus* indicated the existence of two distinct physiological categories—eury-responsive and steno-responsive. Eury-responsive strains are widely responsive to the pH of the growth medium, and grow well upon a casein hydrolyzate-polypeptone-yeast extract medium (without added urea) at pH values from 5 to 11. Steno-responsive strains are narrowly responsive to pH, and may exhibit a slight degree of growth, if any, only at pH 9 to 11 on media unsupplemented with urea.

Twenty-four of 25 strains, isolated from soil by urea-enrichment technique, hydrolyzed urea. Other biochemical activities aided in the identification of only one isolate (a strain of *Bacillus lentus*), and did not indicate relationships of diagnostic aid. Comparative studies with 26 known strains of *Bacillus* species indicated that physiological characterization of urea-hydrolyzing bacilli afforded a presumptive identification of strains of *Bacillus pasteurii*. The validity of both eury- and steno-responsive categories was strengthened by the nutritional observation that steno-responsive cultures require free ammonia in addition to amino acids. Eury-responsive, ureolytic organisms comprised *Bacillus freudenreichii*, *Bacillus loehnisii*, *Bacillus lentus*, and some strains of *Bacillus sphaericus*.

The production of urease seems to stand out as a useful property among otherwise negative or variable characteristics, and appears to be of significant diagnostic value for the genus *Bacillus*.

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