

# *Actinobacillus actinomycetem-comitans*: Fermentative Capabilities of 140 Strains

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Fermentative capabilities of 140 strains of *Actinobacillus actinomycetem-comitans* were studied. Findings correspond closely with those reported previously by Heinrich and Pulverer (12 strains), and by King and Tatum (33 strains). All strains ferment glucose, levulose, and maltose and reduce nitrate to nitrite. Reactions with glycogen and starch are exceedingly diverse. Eight different biotypes have been identified on the basis of their reactions with galactose, mannitol, and xylose.

*Actinobacillus actinomycetem-comitans* is a gram-negative, slender, nonsporulating, nonmotile, and microaerophilic bacillus, first described by Klinger in 1912 (6). In 1959, Heinrich and Pulverer (2, 3) reported on diagnostic aspects and pathogenic significance of *A. actinomycetem-comitans* and described studies with 12 strains. These 12 strains, 11 of them isolated from patients with actinomycosis, possessed identical fermentative and serological properties. In 1962, King and Tatum (5) presented data concerning strain differentiation on the basis of findings with 33 *A. actinomycetem-comitans* strains. These 33 strains were divided into four subgroups on fermentative and serological grounds. To our knowledge, no additional experimental studies have been published. Since available data do not suffice for complete characterization of *A. actinomycetem-comitans*, the following studies on 140 additional strains were undertaken.

## MATERIALS AND METHODS

Of the 140 *A. actinomycetem-comitans* strains examined in these studies, 136 were isolated in our laboratory between 1956 and 1970 from patients with human actinomycosis. Strain 3349, described in a previous publication (2), was included for comparative purposes. Three additional strains (52105, 52106, 52108) were kindly provided by A. R. Prévot, Pasteur Institute, Paris. All 140 *A. actinomycetem-comitans* strains were classified by morphological and growth characteristics (2).

Carbohydrate fermentation by *A. actinomycetem-comitans* strains was examined by using the following basal medium: Casiton, 10 g; Difco yeast extract, 10 g;  $\text{KH}_2\text{PO}_4$ , 1 g;  $\text{MgSO}_4$  (7  $\text{H}_2\text{O}$ ), 1 g; distilled water, 1,000 ml. The medium was subsequently sterilized in an autoclave. The respective test carbohydrate (Seitz-filtered) was then added to a final concentration of 1% (w/v). Water blue was incorporated

as a pH indicator (0.4 ml of a 1% solution/100 ml of medium). After the pH of the medium had been adjusted to 7.4, 2-ml samples of freshly prepared solutions were placed into small tubes and again sterilized by means of two 10-min cycles in an autoclave at 100 C. Tubes were stored at 4 C until used.

*A. actinomycetem-comitans* 72-hr cultures, grown on solid agar media (Casiton, 12 g; yeast extract, 12 g;  $\text{KH}_2\text{PO}_4$ , 4 g;  $\text{MgSO}_4$  (7  $\text{H}_2\text{O}$ ), 1 g; agar, 25 g; pH 7.2), were inoculated into tubes and incubated without vaseline seals. All fermentative reactions were assessed after 10 days of incubation at 37 C. Carbohydrate fermentation reactions were considered positive when color reversal of the water blue indicator became clearly detectable. The pH was further determined with phenol red. Color changes of the phenol red indicator not accompanied by changes in the water blue indicator were considered weakly positive reactions. Whenever gas was elaborated, very little accumulated and provided no additional basis for *A. actinomycetem-comitans* characterization.

Indole production was determined in the basal medium by means of Kovacs reagent. Elaboration of  $\text{H}_2\text{S}$  was assayed in the basal medium by means of lead-acetate paper. For the gelatin test, the basal medium was mixed with gelatin in a ratio of 1 to 4. Nitrate reduction (basal medium plus 0.02%  $\text{KNO}_3$ ) was determined by addition of a mixture of sulfanilic acid, acetic acid, and alpha-naphthylamine. Hydrolysis of urea and esculin 1% resp. (0.1% in basal medium) was determined by means of phenolphthalein and with a solution of 0.5% ferric citrate, respectively.

## RESULTS

Results of fermentative tests with 140 *A. actinomycetem-comitans* strains and results previously reported by Heinrich and Pulverer (2), and by King and Tatum (5) are summarized in Table 1.

For quantitative determination of acid formation, pH changes were determined (pH meter

TABLE 1. Fermentative capabilities of *Actinobacillus actinomycetem-comitans*

Determination	Strain reactions of groups <sup>a</sup>		
	I	II	III
Adonitol			0
Esculin	0	0	
Arabinose	0	0	0
Cellulose			0
Dextrin			V
Dulcitol	0	0	0
Erythritol			0
Galactose	X	0	X
Glucose	+	+	+
Glycerol	0	0	0
Glycogen	V	+	X
Inositol	0	0	0
Inulin	0	0	0
Lactose	0	0	0
Laevulose	+	+	+
Maltose	+	+	+
Mannose			+
Mannitol	X	+	X
Melibiose			0
Melicitose			0
Raffinose	0	0	0
Rhamnose	0	0	0
Salicin	0	0	0
Sorbitol	0	0	0
Sorbose	0	0	0
Starch	V	V	X
Sucrose	0	0	0
Trehalose			0
Xylose	X	0	X
Nitrate	+	+	+
Indole	0	0	0
Gelatin	0	0	0
Urea	0	0	0
H <sub>2</sub> S	0	0	W

<sup>a</sup> Group I, 140 strains tested by authors; group II, 12 strains (2, 3); group III, 33 strains (5). Symbols: + = positive reaction, W = weakly positive reaction, 0 = negative reaction, V = strain reaction inconstant, X = some strains react positively, others negatively.

WTW, type pH 54) for five selected cultures after 10 days of incubation at 37 C. These repeatedly performed investigations show that variations in acid formation are paralleled by differences in fermentative capabilities among individual strains. High acid production by *A. actinomycetem-comitans* strains reduces pH from 7.4 to less than 6.0, the range generally lying between 5.5 and 5.8. A pH less than 5.2 was not observed. A pH range between 6.0 and 6.3 may still be considered evidence of some acidification.

Several *A. actinomycetem-comitans* biotypes can be identified on the basis of their capabilities

TABLE 2. Biotypes of *Actinobacillus actinomycetem-comitans*

Biotypes	Reaction to <sup>a</sup>			No. studied by <sup>b</sup>			Total
	Man-nitol	Xylose	Galac-tose	Authors	Hein-rich and Pul-verer (2, 3)	King and Tatum (5)	
I	+	+	+	12		3	15
II	+	+	0	36		1	37
III	+	0	+	15		8	23
IV	+	0	0	75	12	9	96
V	0	0	0	1			1
VI	0	0	+			2	2
VII	0	+	0	1		2	3
VIII	0	+	+			8	8

<sup>a</sup> Symbols: +, acid production; 0, no acid production.

<sup>b</sup> Authors studied 140 strains; Heinrich and Pulverer, 12 strains; and King and Tatum, 33 strains.

to ferment mannitol, xylose, and galactose. Table 2 summarizes these studies and also lists those previously reported (2, 5).

The 185 *A. actinomycetem-comitans* strains examined by King and Tatum (5) and by ourselves can be assigned to eight clearly defined biotypes. Of the 185 strains, 175 fall into four of the eight biotypes; the remaining 14 strains were assigned to the other four biotypes.

## DISCUSSION

*A. actinomycetem-comitans* is one of the microorganisms normally present in the human oral cavity (3). As expressed by its name, *A. actinomycetem-comitans* may likewise be found in human actinomycosis as a typical companion of *Actinomyces israelii*. The authors isolated 465 *A. actinomycetem-comitans* strains in the past 20 years. Among these 465 strains, 448 were obtained from human cases of actinomycosis, together with *Actinomyces israelii*. The remaining 17 strains were isolated from infectious processes in patients in whom the evidence for infection by *A. israelii* is inconclusive. *A. actinomycetem-comitans* was found twice in pure culture. Despite repeated attempts in three other cases, it was isolated in company only with other anaerobes, but not with *A. israelii*. In recent years, *A. actinomycetem-comitans* have been found in endocarditis (1, 4, 5, 8-13) and in one instance of cerebral abscess (7). As the 17 cases mentioned above indicate, *A. actinomycetem-comitans* must be considered a pathogen per se.

In accord with previous findings described by Heinrich and Pulverer (2) in 12 strains and by

King and Tatum (5) in 33 strains, present studies with 140 *A. actinomycetem-comitans* strains show that this microorganism exhibits characteristic fermentative capabilities. It does not ferment adonitol, arabinose, cellibiose, dulcitol, erythritol, glycerol, inositol, inulin, lactose, melibiose, melicitose, raffinose, rhamnose, salicin, sorbitol, sorbose, sucrose, trehalose. This microorganism does not affect esculin, gelatin, and urea, nor does it form indole or H<sub>2</sub>S in these studies. Glucose-, laevulose-, maltose-, and mannose-containing media are acidified by all strains, and nitrate is reduced to nitrite. Reactions with glycogen, starch, and dextrin are variable and unreliable. Galactose, mannitol, and xylose are fermented by some *A. actinomycetem-comitans* strains and not by others. These findings also coincide with those reported by Kayser and Bircher (4), Mitchell and Gillespie (8), and by Underhill (13) in one strain, respectively, with the exception of an allegedly positive reaction to inulin (8).

Quantitative estimations of pH, performed on five *A. actinomycetem-comitans* strains, demonstrate that extent of acidification may vary from strain to strain and from substrate to substrate. From an original pH 7.4, pH fell to ranges between 5.2 and 6.0 (distinct reaction), and between 6.1 and 6.3 (less distinct reaction) after 10 days of incubation at 37 C.

As shown in Table 2, at least eight biotypes of *A. actinomycetem-comitans* may be identified on the basis of their fermentative reactions with galactose, mannitol, and xylose. In our experience, this differentiation of biotypes is reliable and reproducible and may, therefore, be employed for epidemiological studies.

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