CORYNEBACTERIUM IRANICUM SP. NOV. ON WHEAT (TRITICUM VULGARE L.) IN IRAN, AND A COMPARATIVE STUDY OF IT WITH C. TRITICI AND C. RATHAYI

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(With plates 1 to 9)

INTRODUCTION

Diseased wheat ears, collected by the Agricultural Station of Azarbayedjan on September 25, 1956 at the village of Eedeloo in Sarajoo of Maragheh, were studied in the Bacteriology Laboratory of the Imperial College at London. At first sight the ears looked almost normal, but a close examination showed that the spikelets, instead of having a normal yellow colour, had an olivaceous tinge. All parts of the spikelets were stuck together by a honey-yellow, slimy secretion. Dissection of a spikelet revealed that, instead of normal grains, there were small abortive ovaries filled with the same type of slime. The cause of the trouble seemed to be a bacterium, as a slide prepared from the slimy substance by a negative staining with nigrosine showed large number of rod-shaped bacterial cells.

To isolate the bacterium a few diseased seeds were surface-sterilized by immersion for one to two minutes in a 1/1000 mercuric chloride solution, washed with sterile water and placed in plates provided with medium. From each seed grew a lemon yellow colony of a bacterium, sometimes in pure cultures and occasionally associated with a species of *Helminthosporium*. It showed all the morphological and biological characteristics of the genus *Corynebacterium* Lehmann & Neumann, but also showed many divergences with *C. agropyri* (O' Gara) Burkholder, *C. rathayi* (Erwin F. Smith) Dowson and *C. tritici* (Hutchinson) Burkholder, all of which produce the same type of symptoms on some grasses and cereals. The present bacterium must therefore be considered as a new species of *Corynebacterium* producing similar symptoms as *C. tritici* .

Cultures of *C. rathayi* and *C. tritici* were obtained from the National Collections of Plant Parasitic Cultures. They were studied at the Imperial College Bacteriology Laboratory together with the new *Corynebacterium* .

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OBSERVATIONS OF CULTURAL REACTIONS.

I. Media used

A. Solid media

1. Nutrient agar (Nut. agar): Lab_Lemco 1g.› bacteriological yeast extract 2g., bacteriological peptone 5g.› sodium chloride 5 g. › agar 15 g., water 1000 cc.

2. Potato _ dextrose agar (PDA): peeled potato 200 g. › dextrose 15 g. › agar 20 g. › water 1000 cc.

3. Glucose peptone sodium chloride agar (GPSA) : glucose 10 g. › peptone 20 g. › sodium chloride 5g. › agar 15 g. › water 1000 cc .

4. Wheat flour agar (WFA) : flour 40 g. › water 1000 cc.

5. Langeron glucose peptone agar (Lang. GPA) : glucose 20 g. › peptone 10 g. › agar 20 g. › water 1000 cc .

6. Sabouraud glucose peptone agar (Sab. GPA) : glucose 60 g. › peptone 10 g. › agar 20 g. › water 1000 cc .

7. Yeast glucose peptone agar (yeast GPA): yeast extract 5 g.› glucose 20 g.› peptone 10 g. › agar 20 g. › water 1000 cc .

8. Iron sulphite agar : tryptone 10 g. › sodium sulphite anhyd . 1 g. › iron citrate 0.5 g. › agar 20 g. › water 1000 cc.

9. Kligler iron agar : Lab _Lemco 3 g. › yeast extract 3 g. › bacteriological peptone 20 g. › sodium chloride 5 g. › lactose 10 g. › dextrose 1 g. › ferric citrate 0. 3 gr. › sodium thiosulphate (5H2O) 0.3 g. › agar 12 g.› phenol red 0.05g. › water 1000 cc.

10. Starch agar : Nut. agar + 0.2°/. soluble starch .

11. Nutrient gelatine : Lab _Lemco 5 g. › peptone 10 g. › sodium chloride 5 g. › leaf gelatine 120 g.› tap water 880 cc.

B. Liquid media

1. Nutrient broth: Lab_Lemco 5g.› peptone 10g. › sodium chloride 5g.› water 1000cc.

2. Nitrate broth : peptone water (peptone 10 g. › sodium chloride 5 g. › tap water 1000 cc.) + 1 °/. KN O 3.

3. Bacto _ tryptone broth : 1°/. soluton of bacto - tryptone in distilled water.

4. Litmus milk : milk without cream + litmus as indicator .

5. Meat infusion dextrose broth : meat infusion + 1°/. dextrose.

6. Cohn's nutrient solution: magnesium sulphate 5g. › potassium phosphate (K H2 PO4) 5 g. › ammonium tartrate (neutral) 10 g. › potassium chloride 0.5g. › distilled water 1000 cc .

7. "Sugar » broths : peptone water + 1°/. sugar + brom cresol purple as indicator. Sugars used were : dextrose› sucrose› lactose› maltose› fructose› levulose› rhamnose› meli_biose› also pectin .

For the preparation of the solid and liquid media mentioned, the proper technique suitable for each one has been used.

II. Macroscopic Characters

For this purpose 8 different agar media were used, namely : Nut. agar, PDA, GPSA, WFA, Lang. GPA, Sab. GPA, iron sulphite agar and yeast GPA.

A. Agar plates

On agar plates all three organisms grow convex, circular, smooth-surfaced, glistening colonies with an entire edge and an amorphous internal structure, but the colours of the colonies are different. Moreover, in each of these organisms the colour varies to some extent, depending on the media used (Table 1).

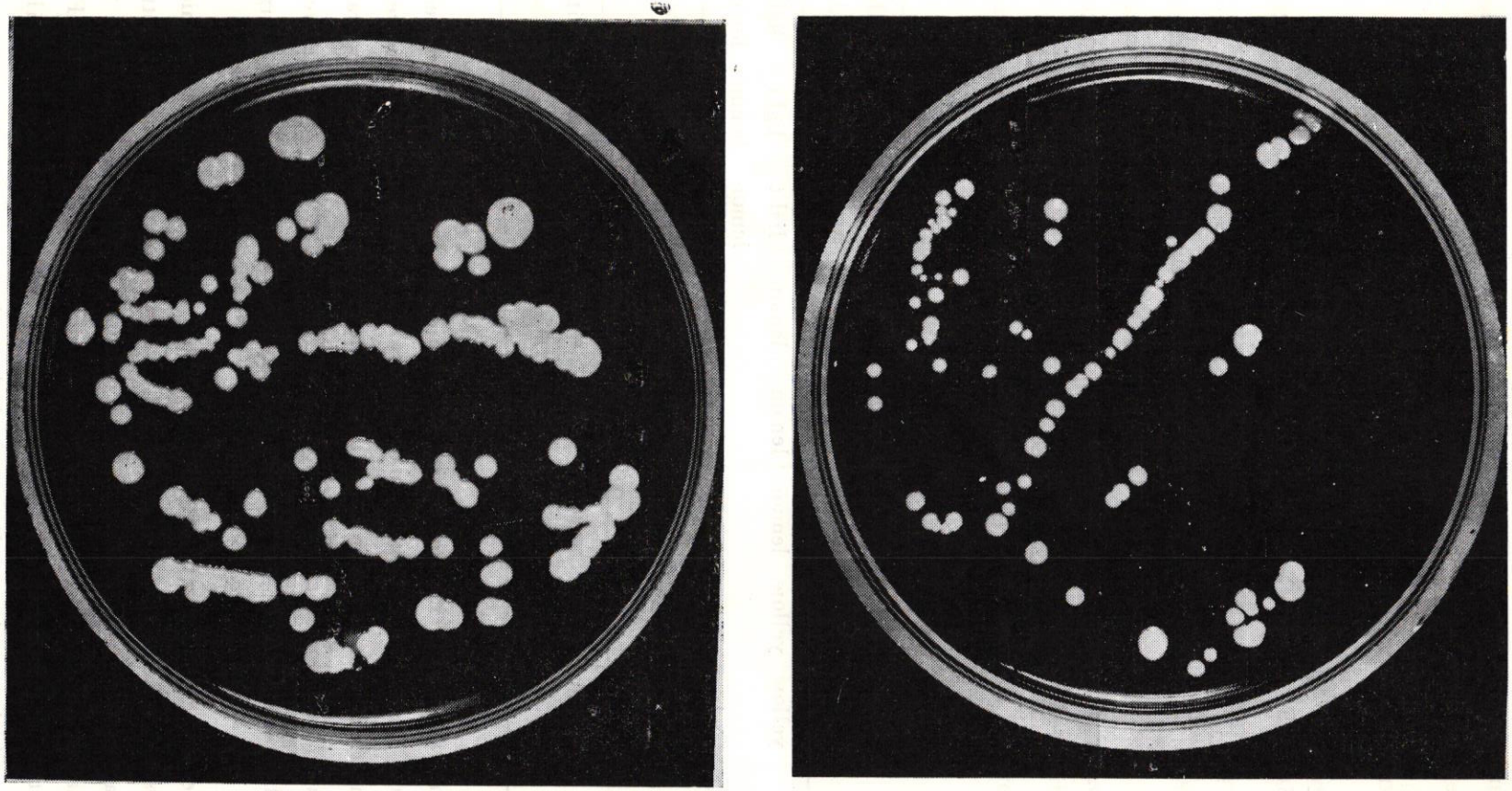
Table 1. Colour of colonies in *C. iranicum*, *C. rathayi* and *C. tritici* on different agar media .

	PDA	Yeast GPA	WFA	GPSA	Iron sulphite agar	Nut. agar	Lang. GPA	Sab. GPA
C. iranicum	yellow	yellow	lemon	lemon	lemon	light lemon	light lemon	light lemon
C. rathayi	light yellow	light yellow	light lemon	cream	cream	light lemon	cream	cream
C. tritici	cream	cream	cream	cream	white	white	white	white

The rate of growth and the maximum size of colonies also vary on different media for the three organisms. They grow better and more rapidly on PDA, Langeron GPA, yeast GPA, and GPSA. On nutrient agar and iron sulphite agar the growth is Less. On Sabouraud GPA the growth usually fails unless a heavy inoculum is used. The difference between Langeron GPA and Sabouraud GPA is simply that the latter is more acid, due to a larger amount of glucose (6 %) .

However once the growth started it may continue normally and the colonies, though sparse, may assume a large rize (4mm.) . In *C. tritici* the growth is usually better and the colonies are larger than in *C. iranicum* and *C. rathayi*. (Plate 1.A & B; Plate 2).

On agar media at 25° C. growth is very slow in the three *Corynebacterium*. For example in *C. iranicum* on nutrient agar, the first single colonies are usually visible within 4 or 5 days, and the maximum diameter of the colony is ordinarily not more



B A

Plate 1. *Corynebacterium iranicum*. Cultures 23/3/57, 17 days old : A) on potato-dextrose agar. B) on yeast glucose peptone agar.

than 1mm. On potato - dextrose agar, where the growth is good, the first single colonies are not seen sooner than 3 or 4 days, and the maximum size of the colonies is 3.5mm. The maximum diameter of the colonies is 4.5 mm. on Langeron GPA, and 6 mm. on yeast GPA. The first growth in *C. iranicum* and *C. tritici* appears usually about 24 hours sooner than in *C. rathayi*.

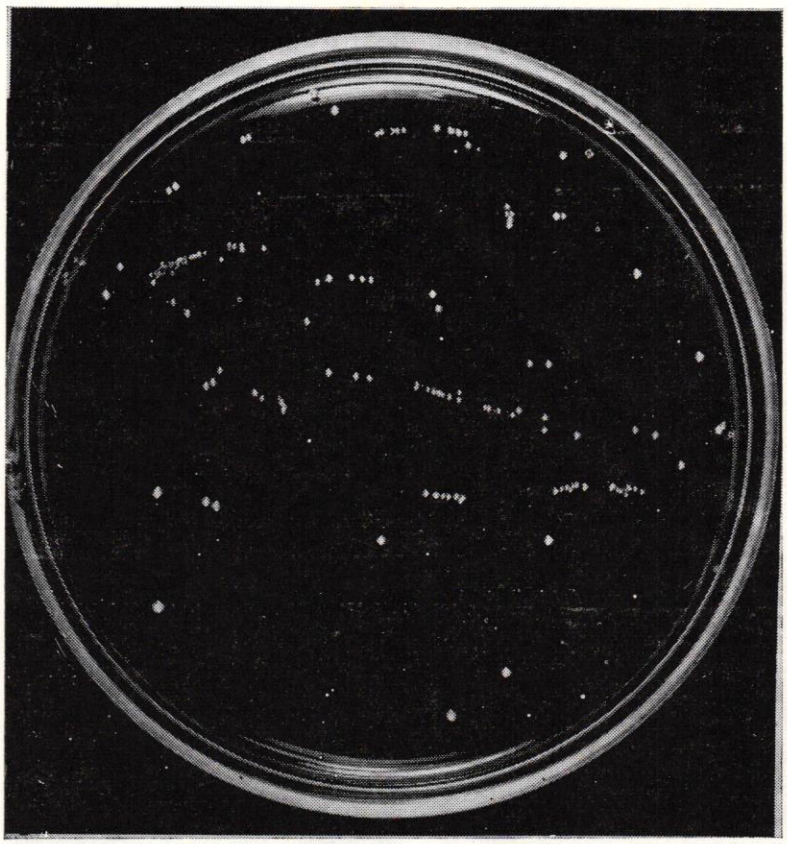


Plate 2. *Corynebacterium iranicum*. Culture 23/3/57, 17 days old on nutrient agar.

B. Agar slopes

On agar slopes a median streak showed a filiform, or sometimes partially beaded growth, glistening, slightly convex and of a viscid consistency. The differences between the three organisms as on agar plates, are in their colour and their rate of growth on different media (Table 2· plate 3) .

Table 2. Width of the streak in mm. on agar slopes 16 days old at 25° C.

	Nut. agar	PDA	Yeast. GPA	Lang.GPA	WFA	GPSA
C. iranicum	1.5	6	9	7	2	9
C. rathayi	1.5	5.5	5.5	3.5	2	4.5
C. tritici	1.5	5	3.5	3.5	2	6

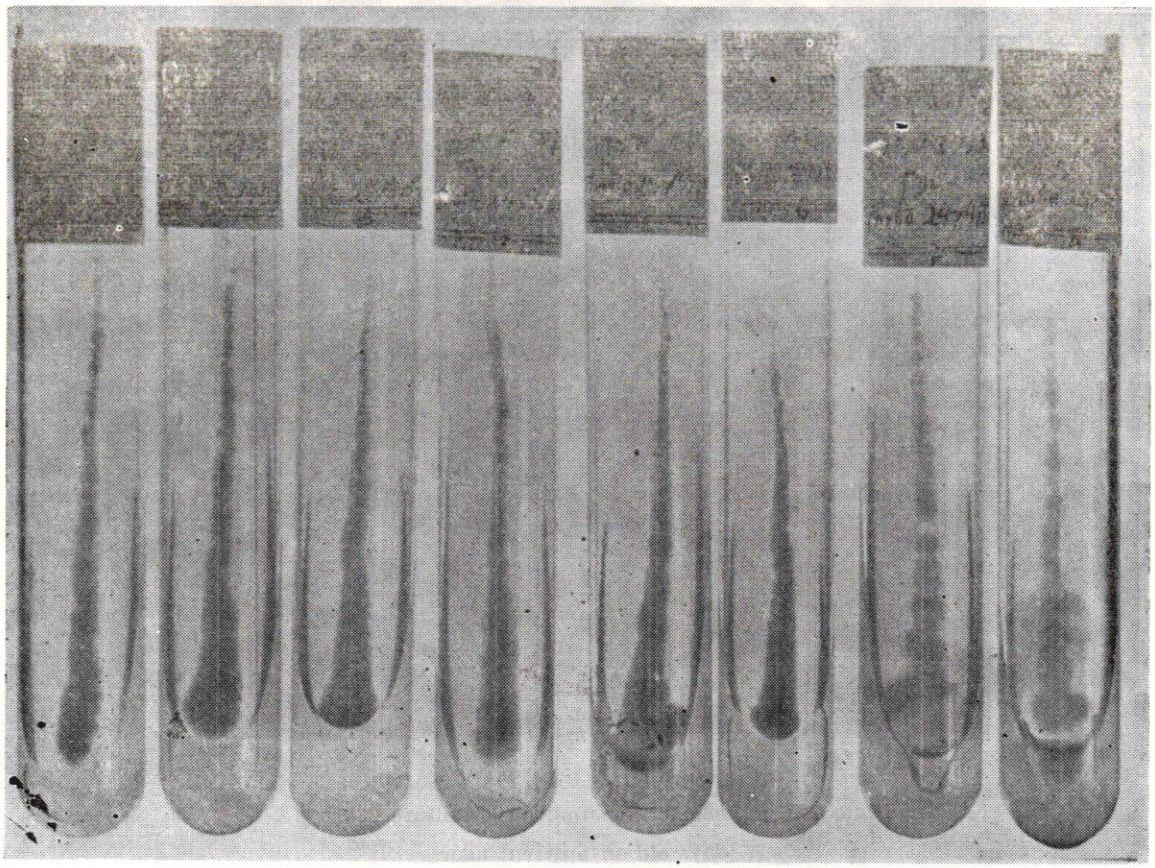


Plate 3. Streaks on slopes potato - dextrose agar. 1,2,3, *Corynebacterium iranicum*, which shows the darkest colour; 4, 5, 6, C. *rathayi*, which shows a medium colour; 7, 8, *C. tritici*, which shows the lightest colour.

C. Kligler iron agar

To examine the reaction of the three *Corynebacterium* on Kligler iron agar, slopes of this medium having a butt of about 2 to 3 cm. were used. Inoculations were made by smearing the surface of the slope with a loop and stabbing a needle into the

agar butt. *Escheria coli, Aerobacter aerogenes* and *Proteus vulgaris* were also used as controls for different reactions. When diluted inocula in distilled water were used, and the medium was also prepared by using distilled water, no growth was seen. Diluted inocula in nutrient broth gave no growth for *C. iranicum* and *C. tritici* on media prepared by distilled and tap water. Separate colonies and red reaction were seen for *C. rathayi* after 11 days when tap water was used in the medium and after 3 weeks when distilled water was used. Undiluted inocula from solid media produced growth and gave red alkali reaction on both types of media for *C. iranicum* and *C. rathayi* after 3 days and for *C.* tritici after 6 days. No blackening of the medium in the butt and therefore no H 2 S was produced .

D. Starch hydrolysis

Starch agar plates were inoculated by making a diametrical streak with a loop. Two or three weeks after inoculation the plates were flooded with saturated solution of iodine in 50% alcohol, left for a minute or two, and then drained. In all tests on *C. iranicum*

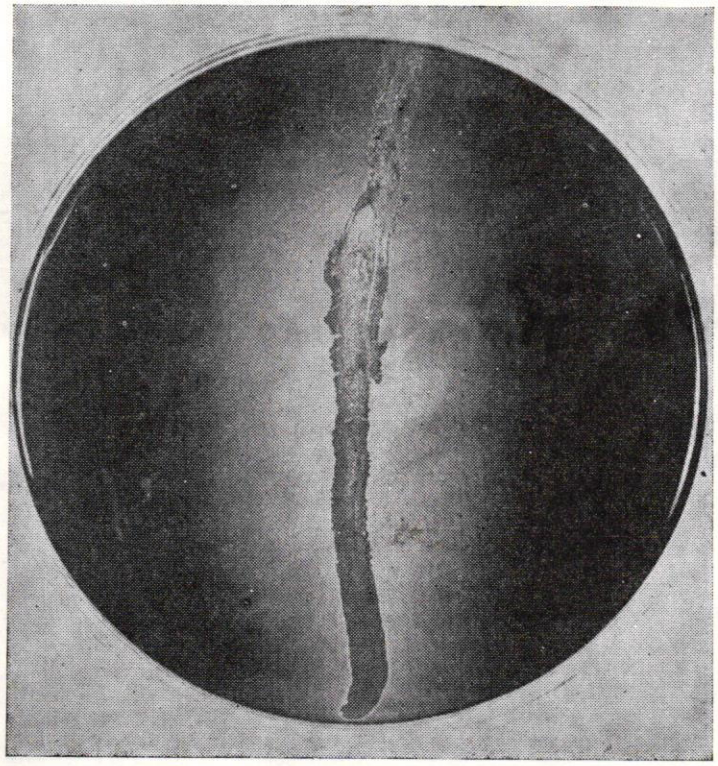


Plate 4. *Corynebacterium iranicum*. Streak on starch agar, after flooding the plate with saturated solutione of iodine in 50 % alcohol shows starch hydrolisis.

cultures, a clear zone consisiting of a line about 10 to 15 mm. on each side of the streak indicated destruction of the starch (Plate 4) , while on *C. rathayi* and *C. tritici* the

test was always negative .

E. Nutrient agar stab

Stabs made by an inoculating needle in a nutrient agar tube produced a filiform narrow cone of slight growth after 4 to 5 days, at 25° C.

After about 2 weeks, the *corynebacterium* cultures showed a denser growth at the widest part of the cone (3 to 5 m m . wide), and a beaded growth at the lower, tapering part .

There was some surface growth. No significant differences could be observed in the growth of the three organisms in nutrient agar stabs .

F. Gelatin liquifaction

The three organisms showed different aspects with regard to gelatin liquifaction. More than fifteen tests were carried out to study and to establish this feature of these bacteria. Stabs made in tubes of nutrient gelatin agar showed the results as stated in Table 3 (Plale 5).

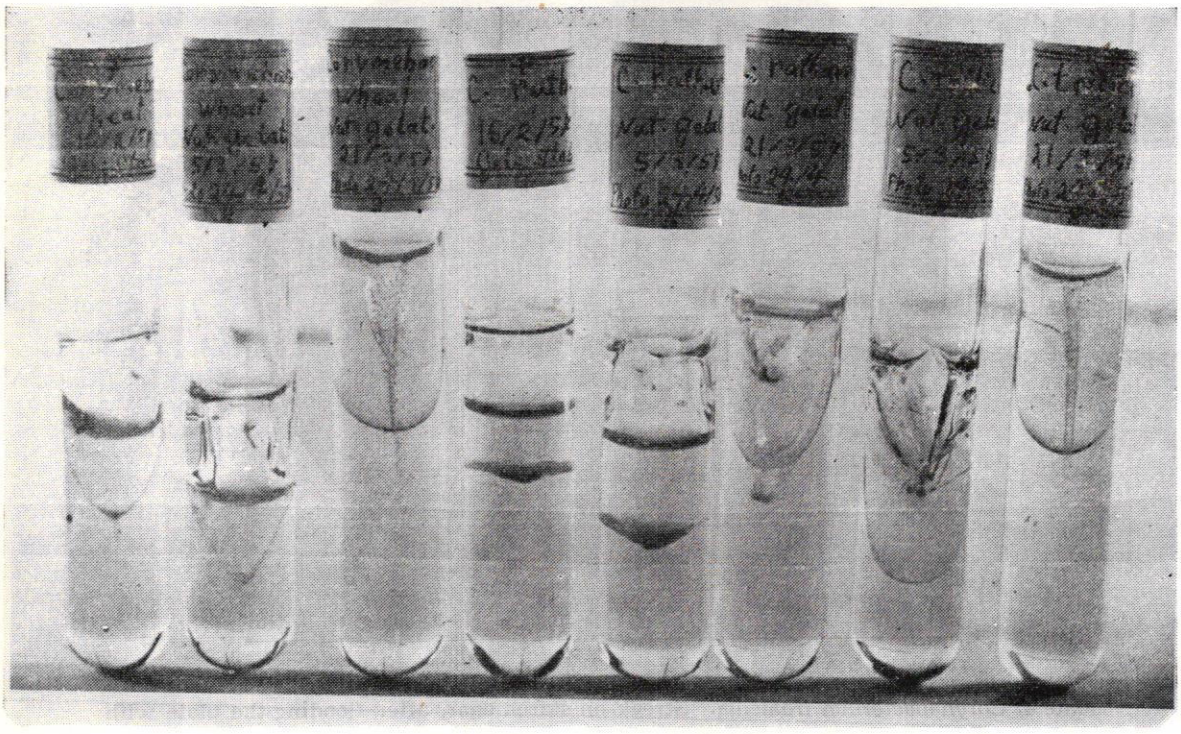


Plate 5. Gelatin liquifaction. 1, 2, 3 *Corynebacterium iranicum*, stabs 68,49 and 33 days old; 4,5,6 *C. rathayi*, stabs 68, 49 and 33 days old; 7, 8 *C. tritici*, stabs 49 and 33 days old.

Table 3. Gelatin liquifaction in the three *corynebacteria*, at 20° C.

Culture of	*C. iranicum*	*C. rathay*	*C. tritici*
7 days	Separate colonies on a narrow cone with some surface growth.	The same as in *C. iranicum* + some slight depression at the surface.	The same as in *C. iranicum*
11 days	Surface growth in a slight depression	Liquifaction just starting, but usually on small napiform sac without liquid (evaporation).	No change
15 days	Empty napiform sac with surface growth.	Still empty napiform sac, but larger than *C. iranicum*	No change
21 days	A little thick liquid at the bottom of the sac with colonies mixed in it.	A thin liquid four times more than in *C. iranicum* with sediments of the colonies at the bottom.	Only colonies along the stab show melted peripheries without mixing.
28 days	Liquifaction slowly improving with thick liquid containing parts of the growth.	Liquifaction improving better, napiform sac, clear thin liquid, the whole sediments at the bottom.	No change
35 days	Upper part 10 to 15mm. dried and evaporated; lower part is a napiform sac with thick liquid; the border has not reached the tube wall.	Upper part about 20mm. evaporated; lower part is funnel shaped having its border attached to the tube wall.	No change
45 days	The same type of thick liquid mixed with parts of growth in a funnel the border of which may not reach the tube wall. Sometimes evaporation is more rapid and the funnel remains dry and empty.	The clear thin liquid with all sediments at the bottom has a cylindrical shape, its wall being the tube wall.	No visible liquifaction. Only the medium starting from the stab dries up and may form an empty funnel with many colonies of the bacterium attached to its wall.

III. The Three Species of Corynebacteria in Liquid Media.

A. Nutrient broth

Examination of 6_ day – old cultures at 25° C. :

C. iranicum : Showed a yellow viscid sediment which, when the tube was shaken, rose up in a narrow cone_shaped column in the medium. No surface growth, no clouding.

C. rathayi: showed a flocculent yellow sediment which remained compactly attached to the bottom of the tube. No surface growth, no clouding.

C. tritici : showed a flocculent sediment which occupies almost half of the height of the nutrient broth (about 2 cm.) at the bottom of the tube. No surface growth, upper part of the broth clear, lower part occupied by sediments was cloudy. Sediments lighter in colour and growth better than for the other two organisms .

In nutrient broth growth is slow for the three *Corynebacteria*, and is not visible before three days .

B. Cohn's solution

No growth .

C. Milk and litmus milk.

The growth of the three *Corynebacteria* in milk and litmus milk was very slow and did not cause considerable change in the milk. No coagulation or peptonization for milk or litmus milk and no reduction or any change in litmus milk .

However, *C. iranicum* showed a yellow sediment after 2 to 3 weeks and sometimes a slight yellow surface in very old cultures. In *C. rathayi* a sediment may appear, but very late after about two months. In *C. tririci* the sediment is lighter in colour.

D. Nitrate broth

The three organisms produced a flocculent sediment. No turbidity or surface growth. In all tests carried out for nitrites the result was negative .

E. Bacto _ tryptone broth

C. iranicum produced a viscid yellow sediment and no turbidity. Indol test was negative for all *Corynebacteria*.

F. Sugar « broths »

To indicate the reactions of the three organisms in sugar «broths» many tests have been carried out (Tables 4 and 5) .

Table 4. Reactions of *C. iranicum*, *C. rathayi* and *C. tritici* in dextrose, sucrose and lactose broths (in one series of cultures at 25° C.).

Cultures examined after :			Dextrose	Sucrose	Lactose
	15 days	C.i.	Sediment	Sediment	Sediment
		C.r.	Sediment, surface growth	Sediment	Sediment
		C.t.	Sediment	Sediment	Sediment
	20 days	C.i.	Sediment, surface growth	Sediment, suface growth	Sediment
		C.r.	Sed. , surface gr . acid	Sediment, surface growth	Sediment
		C.t.	Sed. , surface gr., acid.	Sediment, slight acid	Sediment
	45 days	C.i.	No more change	No more change	Sediment, surface growth
		C.r.	No more change	Sed.,surface gr., acid	Sediment, surface growth
		C.t.	No more change	Sed., surface gr., acid	Sediment, surface growth

Growth is the most luxuriant in dextrose broth, and the least in lactose broth. In dextrose and sucrose broth acid reaction is sooner for *C. tritici* than for *C. rathayi* and nil for *C. iranicum*.

Table 5. Reactions of *C. iranicum*, *C. rathayi* and *C. tritici* in some sugar « broths» also in pectin, at 25° C. (the cultures have been observed for 17 days; the sugar « broths » have been brought exactly to the neutral point).

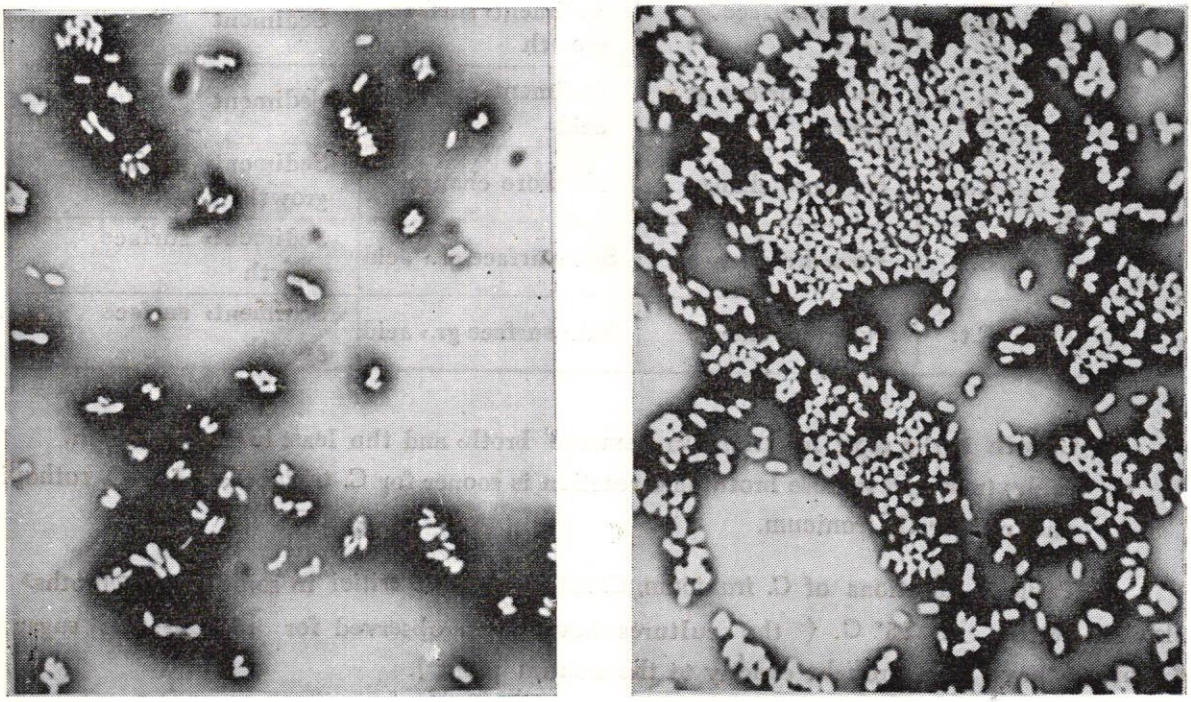
	Dextrose	Sucrose	Lactose	Maltose	Fructose	Rhamnose	Pectin
C. iranicum	no change	no change	no change	no change	no change	no change	no change
C. rathayi	acid after 8 days	acid after 8 days	no change	acid after 17 days	light colour, acid	no change	acid after 8 days
C. tritici	acid after 8 days	acid after 8 days	acid after 10 days	acid after 14 days	no change	no change	acid after8 days

MORPHOLOGY

I. C. IRANICUM

A. In nature

In the slimy substance secreted from diseased seeds, cells are mostly single cylindrical rods, slightly thinner than in artificial media, sometimes oval, ellepsoidal or short and club-shaped, straight or slightly curved. 1 to 5% are wedge-shaped and there are occasionally slightly larger cylindrical or club-shaped cells. Ordinary rods measure 9 to 3.8 by 0.5 to 0.9 μ. and large club-shaped rods up to 1.2 μ. wide. Cells wider than 0.7 μ. are exceptional.



A B

Plate 6. *Corynebacterium iranicum* A) a week old culture on PDA, to compare with a week old culture of *C. rathayi* on PDA, Plate 9 B. B) 27 day old culture on Sabouraud G P A.

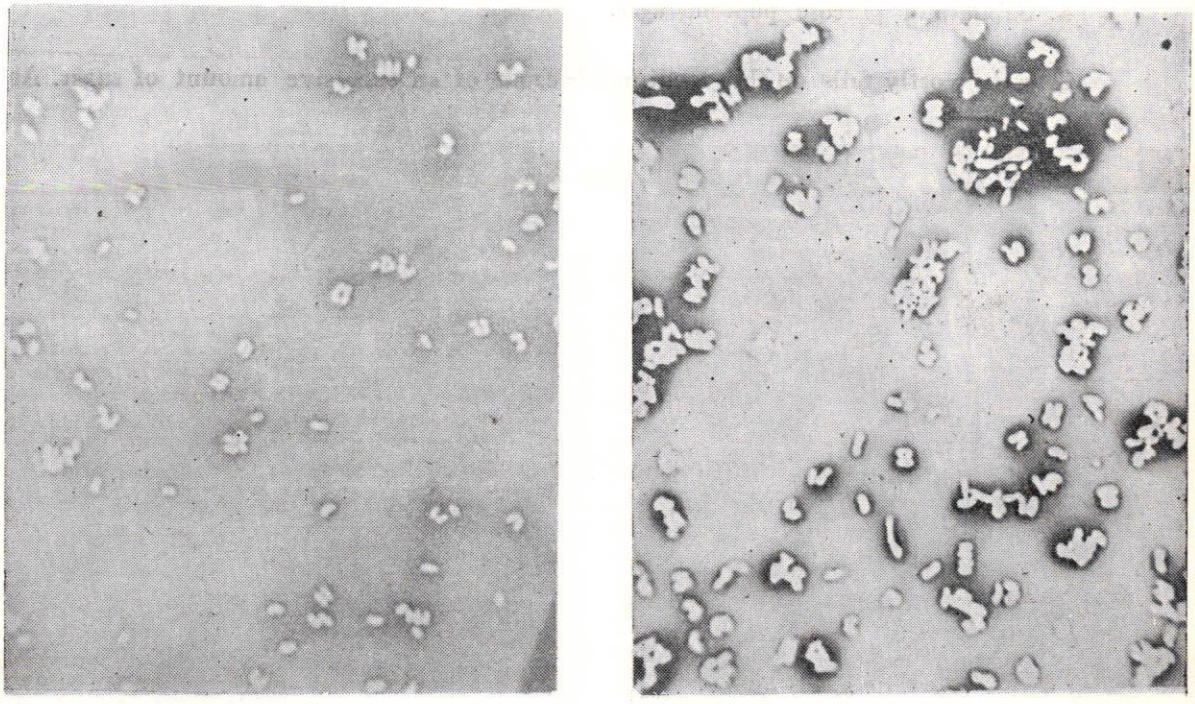
B. In cultures 7 to 30 days old

1. Wheat flour agar

This medium is the best for growing a culture close to the natural state. Colonies usually have a lemon yellow colour near to the colour of the slimy substance secreted from

diseased seeds, and cells have almost the same appearance as in nature. About 40% of individuals are false wedges (wedges showing depression at the angle) usually consisting of two short rods. There are a few palissades, usually in two and occasionally three or four (Plate 8 A).

No agglomeration of individuals as with Sabouraud GPA, so that the cells separate easily when diluted in water. Cells measure 1 to 3 by 0.7 to 0.9 μ, usually 99 % of cells no longer than 2.3 μ and very few up to 5 μ long and 1.2 μ wide. Some other compositions of cells may occur, but not often.



A B

Plate 7. *Corynebacterium iranicum* on Langeron's GPA, A, 27 day, B, 30 day-old culture, which show many club-shaped cells.

2. Potato-dextrose agar

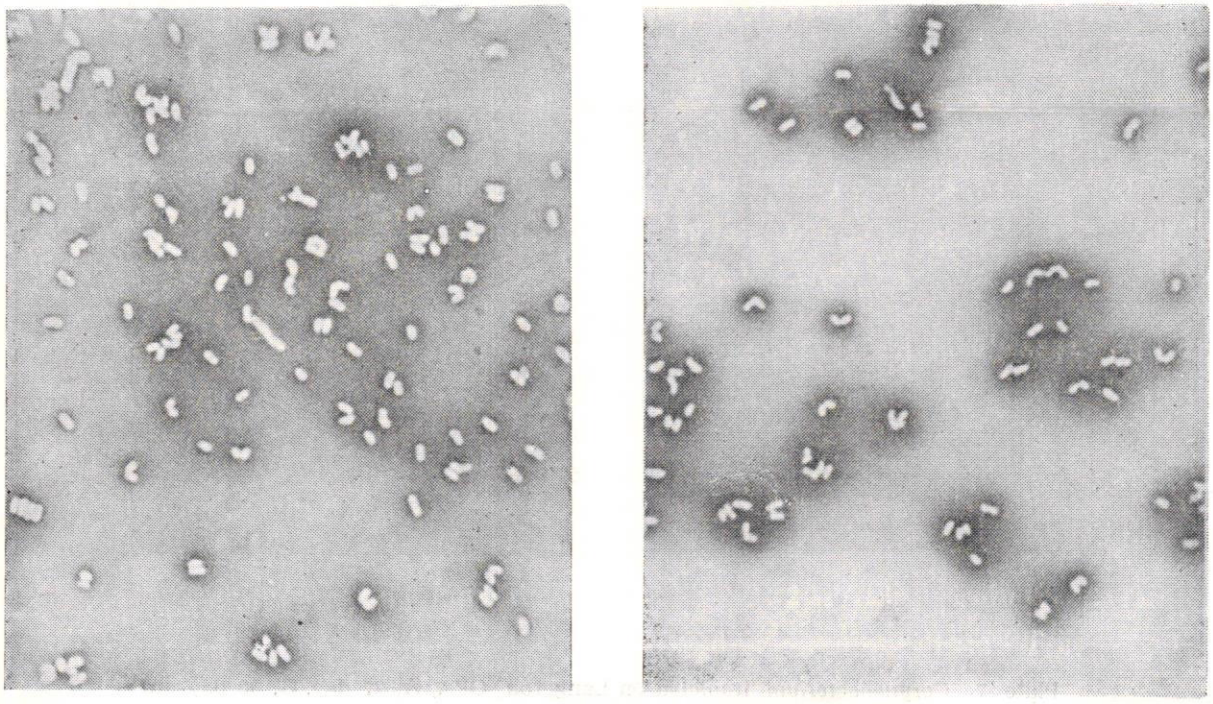
Growth is luxusiant on this medium. Cells are wider than on other media. About 70 % of individuals are single cylindrical, oval or short clubs. There are a number of short-branched wedge shaped, occasionally true wedges or large club-shaped individuals. Cells measure 1.3 to 3 by 0.8 to 1 μ, a very few up to 6 μ long and 1.2 μ wide(Plate 6 A).

3. Langeron glucose peptone agar

Growth is also luxuriant on this medium. Large club-shaped rods are more numerous than on the other media (Plate 7). There are about 10 to 20 °/₀ cylindrical rods, 10 to 20 °/₀ oval and about 60 °/₀ small or large club shapes, which are continue or barred, being wide at one or both ends. There are only a few wedge-shaped (up to 10 °/₀), and once in a while some branched or Y shaped cells. Cells measure 1.5 to 4. 5 by 0. 6 to 1μ; club-shaped cells up to 5, 3 μ long (exceptionally 9 μ) by 0.9 to 2.3 μ. Club shaped cells are more numerous in old cultures.

4. Sabouraud glucose peptone agar.

Growth mostly fails on this medium because of an excessive amount of sugar. As



A B

Plate 8. Slopes 13 day old cultures on WFA. A) *Corynebacterium iranicum*, B) *C. rathayi*.

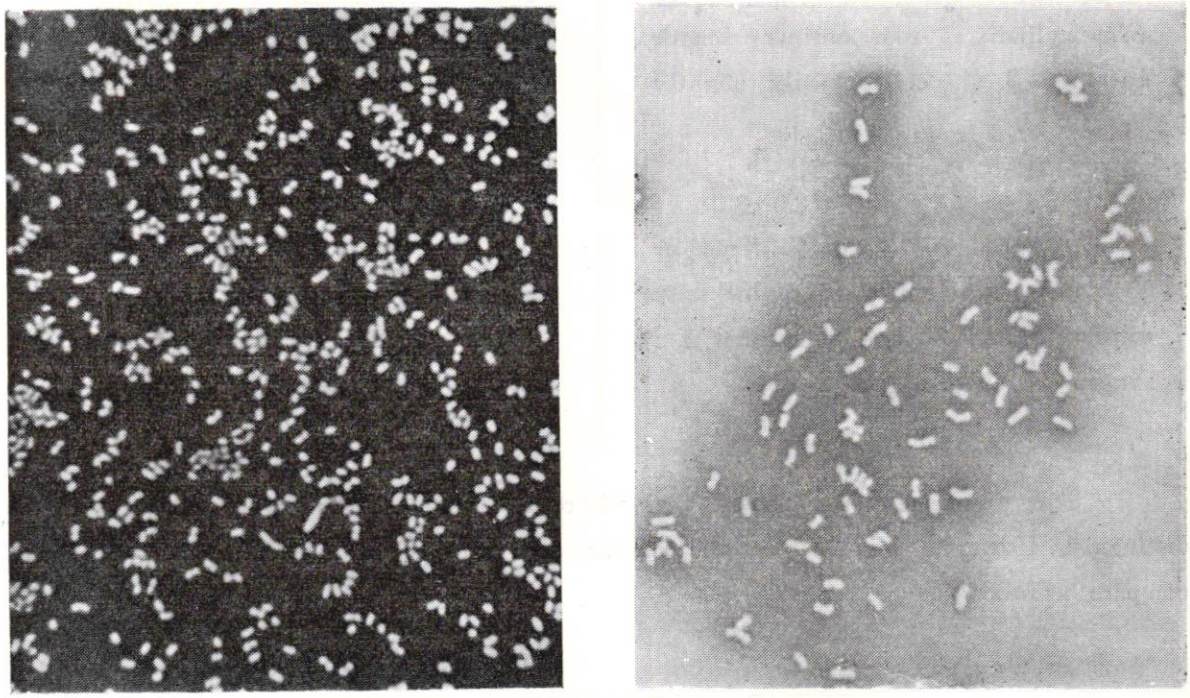
on Langeron medium, there is sometimes a large number of club-shaped cells, with most clubs wide at both ends. These clubs sometimes are dividing in the middle to form two separate wide oval or round cells (Plate 6 B).

5. Glucose peptone sodium chloride agar

Wedges are more numerous than single rods, usually having short angular branches. Sometimes there are quadruples where two wedges having a common origin are closely attached together. Cells measure 1. 7 to 2. 3 by 0. 7 to 0. 9 μ, and at times up to 3. 8 μ long. Larger club shapes are less than 1/1000 and up to 4. 5 μ long by 1. 4 μ wide.

6. Nutrient agar

Growth is less on this medium than on other media. Cells are also smaller, and



A B

Plate 9. A) *Corynebacterium tritici*, 13 day old culture on W F A. To compare with Plate 7 which are cultures of the same date and on the same medium.
B) C. *rathayi*, a week old culture on P D A. To compare with C. *iranicum*, Plate 6 A. which is the same date culture. Here shows longer cells and different combinations of cells.

are about 15 % wedge-shaped. On this medium cells are clumped together, forming agglomerations which are almost impossible to be separated in a drop of water.

II. Comparative morphology of C. iranicum, C. rathayi and C. tritici

All of these three organisms show the basic characteristics of the genus

Corynebacterium, with no very great morphological variance; however, *C. tritici* is some - what different from the other two.

For example, in a 13 - day - old culture on WFA it shows shorter cells, mostly short oval and sometimes round.

On the other hand *C. iranicum* and *C. rathayi* are somewhat distinguishable in a week old cultures on potato - dextrose solope at 25° C. (Plates 6 A & 9 B) :

C. rathayi	*C. iranicum*
Single ordinary rods: distinctly one or both ends wider than the middle, a few cylindrical rods, ordinary length up to 3. 8 μ, cells usually slightly wider.	Single ordinary rods : mostly oval or cylindrical, a few having one or both ends wider or if so at least not so distinctly as in *C. rathayi* , ordinary length of cells up to 2. 5 μ, cells usually slightly narrower.
About 10 °/₀ true V-shaped or Y, W, X, or U- shaped ; a few false wegdes; Y and W and sometimes X - shaped are common (Plate 9. B).	True V with no depression at the angle are exceptional, mostly false- V with shorter branches; Y and other forms not present or very few (Plate 6. A).

The difference on nutrient agar, where the growth is not luxuriant is harder to distinguish. However, in a 7 - day - old culture on a nurient agar slope at 25° C. they compare as follows :

C. rathayi	*C. iranicum*
Single rods more numerous than V - shaped. Cells slightly longer than in *C. iranicum*. True V and Y are some- times seen. Single rods show slight depression near the middle.	20 °/₀ single cells, 80 °/₀ false wedges and a few palissade. True wedges are the exception.

MICELLANEOUS TESTS AND EXAMINATIONS

I. Metachromatic granules

When examining a hanging drop of cells suspension of *C. iranicum*, *C. rathayi* or *C. tritici* from liquid med ia suchas nut. broth, and meat - infusion - dextrose broth, dark

refracting granules are easily distinguishable in some cells, especially in the longer ones.

Cells grown on solid or liquid media when stained by different spore method staining or by other bacteriological stains show granules which are darker coloured than other parts of cells.

For example, when using the Schaeffer and Fulton method of spore staining, the whole cavity of the cell becomes red (safranin) except that the poles accept more colour (dark safranin). Stained with Loeffler's methylene blue, darker blue granules on a lighter blue background are distinguishable; and with carbol fuchsin, dark and light red.

It is worth mentioning that, in a very short cell, almost the whole cavity of the cell absorbs the darker shade of the stain, while in a cell of a medium length only the poles become darker, and the middle part of the cell is lighter.

Later this second type may divide and form two cells of the first type. Longer cells usually have more than two metachromatic granules.

II. Catalase

For this test five-day-old nutrient agar slopes were used. A few drops of Hydrogen peroxide (10 volumes) were added to the cultres. All three *Corynebacteriums* produced bubbles (usually more from *C. rathayi* and *C. iranicum* than from *C. tritici*), so the test results for catalase are positive.

III. Capsule staining

This test was also carried out only for *C. iranicum*, and the result was positive.

IV. Gram staining

This test was also carried out only for *C. iranicum*, using cultures of from 3 to 24 days old, and cells from the slimy secretion on the the host. The results were positive in all cases.

V. Acid fast

This test was carried out several times times for *C. iranicum*, and the result was always negative.

VI. Motility

For this test, hanging drops from one or more day - old cultures in nutrient

and meat - infusion – dextrose broth and from water of condensation in potato – dextrose slopes were examined by microscope. None of the three *Corynebacteriums* showed true motility.

TEMPERATURE RANGE IN GROWTH AND VITALITY OF THE THREE CORYNEBACTERIA

Minimum, optimum and maximum temperatures of the growth of the three organisms and their death point were studied.

I. Minimum

To find the minimum temperature several experiments were carried out.

First, inoculated slopes and plates of different agar media were put in places of various low temperatures, and the minimum temperature of each organism was roughly indicated.

For different low temperatures different parts of a refrigerator were used.

The temperature of each part was controlled by a thermometer and registered several times each day. For final experiments, inoculated slopes were placed in glass jars containing water and a thermometer, and each jar was put in its proper place in the refrigerator.

Agar plates inoculated by each *Corynebacterium* and placed for a month in the cooling department of the refrigerator did not show any growth. The temperature was always below zero and the medium frozen. After a month in this temperature the plates were removed and placed in 25° C. incubator where growth appeared after a few days. Hence it is known that these *Corynebacteriums* can resist frost for at least a month.

The minimum temperatures of growth, determined from different experiments, are as follows :

C. iranicum : 1° C.

C. rathayi : 3° C.

C. tritici : $1\frac{1}{2}$° C.

Traces of growth were seen after 35 days on WFA, Lang. GPA and Yeast GPA for the *Corynebacteriums* in the above mentioned temperatures. No growth was seen below these temperatures.

II. Optimum

To find the optimum temperature for the growth of these organisms, slopes of WFA, PDA, Yeast GPA and Lang. GPA were used. Inocula were diluted in sterile water and with a dull needle a streak was made on each slope. Great care was taken not to make a deep streak, but just to smear the flat end of the needle on the medium. Many replications were used, 4 to 7 tubes for each temperature. Constant bath-temperatures, and sometimes incubators were used. Inoculated tubes were placed in water-filled jars, each equiped with a thermometer. Temperatures were controlled and registered several times a day.

After a series of experiments it was found that the approximate optimum temperature for C. *iranicum* was between 24° to 26° C.; for C. *rathayi* 23° to 24° C.; and for C. *tritici* 26° to 27° C. A. final experiment using 8 different constant temperatures and 4 or more replications showed the results as indicated in Table 6.

Table 6. Cultures at 8 different temperatures showing the optimum temperature for *C. iranicum*, *C. rathayi* and *C. tritici*.

	Cultures	20° C.	$23\frac{2°}{3}$ C.	$24\frac{1°}{8}$ C.	25 °C.	$26\frac{1°}{8}$ C.	$26\frac{2°}{3}$ C.	$26\frac{3°}{4}$ C.	28° C.
C. iranicum	2 days	4b	1a 3b	1a 3b	5a 1b	4b	3b 1c	3b 1c	1b 3c
	4 ,,	4c	3b 1c	4b	1a 3b	2b 2c	1b 3c	4c	4d
C. rathayi	2 ,,	4b	4a	4a	3a 2b	4c	4c	2c 2no gr.	4no gr.
	4 ,,	4b	4a 24° C.	4a	2a 2b	4c	4c	1d 3no gr.	4no gr.
C. tritici	2 ,,	3c 1no gr.	4c	2b 2c	3b 1c	4b	4b	2b 2c	4c
	4 ,,	4c	3b 1c	4b	2a 2b	4a 26.5° C.	4a	2a 2c	2a 2c

a = growth no. 1 > b = growth no. 2 > c = growth no. 3 > d = growth no. 4, considered in each horizontal line. no gr. = no growth. The numbers before the

letters show the number of tubes.

So results of this experiment indicate the optimum temperatures :

C. iranicum — 25° C.

C. rathayi — 24° C.

C. tritici — 26. 5° C.

III. Maximum

Many experiments were carried out to determine maximum temperatures, and the same procedures as for optimum were followed. Since the media were soon losing a great part of their moisture in high temperatures, slopes having some water of condensation were used, so that the media could retain their normal moisture throughout the experiment (20 to 30 days) .

The maximum temperatures indicated by these experiments were :

C. iranicum- 31° C. Above this temperature there is no growth even after a month. At 31° C. growth appears after about 6 to 8 days.

C. rathayi — 29° C. The first growth appears after about 6 to 8 days.

C. tritici — 34° C. There is a slight growth after about 2 weeks at 34° C.

Inoculated slopes placed in a maximum temperature usually do not grow regularly; mostly some separate colonies appear. When these slopes are placed for about 1 to 2 weeks at a temperature slightly above the maximum ($\frac{1°}{2}$ to 1° C.) and then placed in a lower temperature, no growth or only some separate colonies may appear.

IV. Death point

In a series of experiments it was found that the death points of the three organisms are between 49° to 56° C. Further experiments were carried out to determine the actual death point of each *Corynebacterium*.

For each organism and each temperature from 49° to 56°C., two tubes of nutrient broth were used. Inocula were first taken from slopes or plates and diluted in tubes of nutrient broth, of which a loopful was used for each experimental inoculation. Tubes of nutrient broth before being inoculated were placed for several minutes in the same constant temperature bath in order to get the determined temperature before inoculation. After

being inoculated, tubes were kept for exactly ten minutes in the constant temperature and then removed and placed in an incubator at 25° C. The results of the last four experiments are summarized in Table 7.

Table 7. Results of death point in four series of experiments. For each degree from 49° to 56° C. 2 tubes of nutrient broth were used. Tubes which showed no growth are indicated.

	Inoculum: slopes PDA, a week old.	Inoculum: slopes WFA, 25 days old.	Inoculum: slopes PDA, 35 days old.	Inoculum: plates Yeast GPA, 20 days old.
C. iranicum	all tubes from 51 to 56	all tubes from 51 to 56	1 tube 53 and all tubes from 54 to 56	all tubes from 52 to 56
C. rathayi	all tubes from 50 to 56	all tubes from 51 to 56	all tubes from 51 to 56	1 tube 49, 1 tube 51 and all tubes from 52 to 56
C. tritici	—	all tubes from 50 to 56	1 tube 49 and all tubes from 50 to 56	all tubes from 49 to 56

As shown in the table, *C. iranicum* has a death point between 51° to 54° and most probably 53° C.; *C. rathayi*, 50° to 52° and most probably 51° C.; and *C. tritici*, 49° to 50° C.

DESCRIPTION

CORYNEBACTERIUM IRANICUM SP. NOV.

Rods : single or sometimes occuring in a large proportion in false wedges (wedges having a depression at the angle-up to 90 °/°); very seldom true V (having no depression at the angle) or Y,W,U-shaped or with various short branching; or, in some old cultures, club-shaped with one or both ends wider; frequently beaded with metachromotic granales; ordinary rods 0.9 to 3.8 by 0.5 to 0.9 μ; large club shapes up to 6(9) μ long and 1.2 (2.3) μ wide; gram positive, not acid fast, not motile, capsule.

Agar colonies : round; edge entire; convex; smooth; glistening; lemon to yellow; viscid; non-transparent.

Meat_infusion_dextrose broth and nutrient broth: no clouding; no surface growth; only yellow viscid sediment.

Gelatin : very slowly and slightly liquified.

Nutrient agar stab : filiform; beaded toward the bottom.

Milk and litmus milk : not changed; yellow sediment and sometimes slightly yellow surface found only in old cultures.

Nitrates: not reduced.

Bacto-tryptone broth : no indol.

Kligler Iron Agar : with much inoculum becomes red; no hydrogen sulphide.

Cohn's solution: no growth.

Starch : hydrolyzed.

Catalase.

No acid & no gas from dextrose, sucrose, lactose, maltose, fructose, rhamnose or pectin.

Minimum temperature for growth 1° C.; optimum 25° C.; maximum 31° C.; death point about 53° C.

Symptoms: secretion of yellow bacterial slime from ovaries of wheat and abortion of the grains.

Host: *Triticum vulgare.*

Geographical distribution : Iran : Azarbayejan.

GORYNEBACTERIUM RATHAYI (ERWIN F. SMITH)DOWSON

The description is from results of the studies in this paper, „except where".

Rods : single or sometimes occuring wedge_shaped; about 10% of individuals may be true V_shaped (wedges having no depression at the angle) or Y, W, X, or U_shaped; ordinary rods 1 to 3.8 by 0.7 to 1.1 μ, a few up to 5.5 μ long and 1.3 μ wide.

" Gram positive, not acid fast" , non_motile, "capsule".

Agar colonies : round; edge entire; convex; smooth; glistening; viscid; cream or lemon to light yellow; non_transparent.

Nutrient broth: no clouding; not pellicle; flocculent yellow sediment.

Gelatin: slowly liquified.

Nutrient stab: filiform; bottom beaded.

Kligler Iron Agar: with much inoculum, becomes red; no hydrogen sulphide.

Nitrates : not reduced.

Milk and litmus milk : not changed; only very slow growth with yellow sediment.

Cohn's solution : no growth.

Bacto_tryptone broth :no indol.

Starch : not hydrolized.

Catalase.

No gas, but acid from dextrose, sucrose , maltose, pectin and probably from fructose; no acid from lactose and rhamnose.

Minimum temperature 3° C.; optimum 24° C.; maximum 29° C.; death point about 51° C.

CORYEBACTERIUM TRITICI (HUTCHINSON) BURKHOLDER

The description is from the studies in this paper.

Rods: single or occuring in wedges; rarely W and X_shaped or palissades; ordinary rods 0·9 to 2.3 by 0.7 to 0.9 μ; longer up to 5 μ or wider up to 1 μ; non_motile.

Agar colonies: round; edge entire; convex; smooth; glistening; white to cream; non-transparent.

Nutrient broth: not clouded at the upper part; not pellicle; flocculent sediment or particles cause clouding at the lower part.

Gelatin: not liquified.

Nutrient agar stab: filiform and beaded toward the bottom.

Kligler Iron Agar : with much inoculum, becomes red; not hydrogen sulphide.

Nitrates: not reduced.

Milk and litmus milk : no change except for sediment.

Cohn's solution: no growth.

Bacto–tryptone broth: no indol.

Starch: not hydrolyzed.

Catalase.

No gas , but acid from dextrose, sucrose, lactose (?) , maltose, pectin; no acid from fructose and rhamnose.

Minimum temperature $1\frac{1°}{2}$ C. ; optimum $26\frac{1°}{2}$; maximum 34°; death point about 50° C.

DISCUSSION

Corynebacterium iranicum shows some similarities to *C. agropyri* on *Agropyron smithii* , *C. rathayi* on *Dactylis glomerata, Secale cereale* and *Cynodon dactylon* and

C. tritici on *Triticum vulgare* in the production of slimy substance from inflorescence.

It differs from *C. agropyri* in gram staining, gelatin liquifaction, reduction of nitrates, reactions in sugar broths, etc.; from *C. rathayi* in gelatin liquifaction, starch hydrolysis, reactions in sugar broths, etc.; and from *C. tritici* in gelatin liquifaction, reactions in sugar broths, starch hydrolysis, etc.

C. rathayi, as studied here, shows some small differences with the description of Dowson (1942) in reaction in milk and lactose, and with studies of Sabet (1954) in motility and reaction in milk and litmus milk.

C. tritici, as studied in this paper shows some divergences, with the descriptions of Burkholder (1948) in motility, colour of colonies, reaction in milk, reduction of nitrates, etc., and with studies of Sabet in milk reaction, motility, etc.

It seems that these authors have been dealing with different forms or strains of these bacteria, and probably with different species; however, a complete comparative study of all slime-producing *Corynebacterium* species on inflorescences of grasses and cereals should attract the attention of nvestigators.

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