Acta vet. scand. 1972, *13*, 403—434.

From the Department of Food Hygiene, Royal Veterinary College, and the Department of Food Hygiene, National Institute of Public Health, Stockholm. Sweden.

EXPERIMENTAL STUDIES ON THE FORMATION OF VOLATILE NITROGEN COMPOUNDS INDUCED BY PSEUDOMONAS FRAGI IN A SYNTHETIC MEDIUM WITH AMINO ACIDS AS SOURCE OF NITROGEN

By

S. O. Florin

FLORIN, S. O.: *Experimental studies on the formation of volatile nitrogen compounds induced by Pseudomonas fragi in a synthetic medium with amino acids as source of nitrogen.* Acta vet. scand. 1972, *13*, 403—434. — The influence of the growth of Pseudomonas fragi strain F 111 was studied in a synthetic medium. It was shown that volatile nitrogen compounds were rapidly liberated as a result of the decomposition at 5°C of arginine and histidine. A later and slower increase of TVN was observed in media, which contained lysine or urea. From the other 7 amino acids which were included in the test, no increase of TVN was observed to be caused by the strain F 111.

It was shown that within the range of 0.05 to 0.8 % histidine added to the basic salt solution the amount of TVN liberated was correlated to the amount of histidine included in the media. At the TVN maximum approx. 50 % of the amino acid nitrogen of the medium was recovered as TVN.

It was further shown that the liberation of TVN was correlated to the added amount of arginine and histidine included in the growth medium. The presence of lysine also influenced on the TVN maximum which was reached. In the synthetic medium to which arginine or histidine was added the growth of strain F 111 was stimulated by the presence of glucose. The acids produced by the oxidative breakdown of glucose were neutralized partly by the volatile bases produced in the decomposition of amino acids.

The ability of the Pseudomonas fragi to grow in anaerobic conditions and to produce enzymes which could decompose histidine was studied. It was concluded that the anaerobic breakdown of histidine was similar to the anaerobic breakdown of arginine reported by other authors.

volatile nitrogen compounds; Pseudomonas fragi; synthetic medium; amino-acid-breakdown.

The spoilage of food caused by microorganisms is of prime importance because it determines the end-point of the shelf life of different food products. In refrigerated foods the pseudomonads are often the cause of these changes. One of the most important microorganisms in connection with the spoilage of precooked foods is Pseudomonas fragi, and studies on its effect on cooked fish during storage have been presented (*Florin* 1971, 1972). In model experiments it was shown that Pseudomonas fragi caused an increase of volatile bases in fish extract. This increase was delayed but not inhibited, if the fish extract was layered with paraffin oil and the contact with the air broken some time after the inoculation. Although Pseudomonas fragi was known to produce a fruity ester-like smell in cultures and in foods such as raw fish and pasteurized milk, the trials to provoke this type of smell in cooked fish were unsuccessful. This was not in agreement with the reports of *Castell & Greenough* (1957, 1959). These authors studied the different odours produced in the raw fish muscle and they could reproduce many of them in media containing single amino acids. It was shown that this effect depended on the action of Pseudomonas fragi on amino acids and related compounds. These enzymatic reactions often ended in the liberation of ammonia (*Bramstedt & Auerbach* 1961).

The content of the different amino acids in fish muscles varies with the species (*Souci et al.* 1962). Variations are also depending on the environmental conditions, such as season and the fishing ground. During spoilage considerable changes within the amino-acid pool of raw fish meat were caused by microorganisms (*Shewan & Jones* 1957). In cod the content of lysine, leucine, valine, and aspartic acid was shown to increase during spoilage, whereas alanine disappeared. In the spoilage of the herring the histidine was broken down to histamine. Among the other main amino acids in the fish meat arginine was of particular interest, because Pseudomonas, e.g. Pseudomonas fragi, produced arginine dehydrolase (*Sherries et al.* 1957). The arginine was completely broken down under anaerobic conditions.

The object of the present investigations was to study the following questions:

Which of the amino acids generally found in fish meat could act as precursor of the volatile nitrogen compounds produced by Pseudomonas fragi in an extract of cooked halibut?

Does Pseudomonas fragi cause an increase of TVN in a me-

dium which contains urea, a substance present in the meat of some fishes and mentioned as one of the intermediary steps in the decarboxylation of arginine caused by microorganisms (*Möller* 1955)?

Does the presence of more than 1 amino acid influence the utilization of a single amino acid that may act as precursor of the volatile nitrogen compounds produced by Pseudomonas fragi?

Does the presence of an oxidizable carbohydrate influence the increase of TVN caused by Pseudomonas fragi in media which contain a precursor of volatile nitrogen compounds?

Can Pseudomonas fragi cause anaerobic breakdown of an amino acid?

MATERIALS AND METHODS

Ingredients in the culture media

The media used in these experiments were prepared from the following sterile solutions:

a) Solution A:

sodium sulphate, puriss ($Na_2SO_4 \cdot 10\ H_2O$)	56.7 g
sodium lactate, 50 %, Ph. Nord.	50.0 g
magnesium chloride, p.a. ($MgCl_2 \cdot 6\ H_2O$)	1.07 g
aq. dest.	1,000 ml

b) Solution B:

secondary potassium phosphate, p.a. (K_2HPO_4)	7.5 g
primary potassium phosphate, p.a. (KH_2PO_4)	2.5 g
aq. dest.	1,000 ml

c) Solutions of 10 amino acids in different concentrations. The differences in this respect are described separately in "Methods" under each section.

d) Solution of glucose:

glucose	5.0 g
aq. dest.	100 ml

The glucose solution was used only in the experiments reported under section 5, p. 19.

The amino acids were dissolved in glass-distilled water according to general recommendations in *Handbook of Chemistry and Physics* (1964—1965).

Generally, 50 ml of the complete media was prepared in Erlenmeyer flasks by mixing equal amounts of stock solutions A and B (10 ml of each) with an amino-acid solution (30 ml) with or without the addition of glucose.

The media were adjusted to pH 7.0 and sterilized at 120°C for 20 min. (10 min. when glucose was added). The flasks were kept at 5°C until inoculation.

The content of the various salts was the same in all media but the content of amino acid varied. The media, therefore, may be regarded as composed of a basal medium, to which amino acids and, in 1 experiment, glucose were added. In the following the basal medium will be referred to as B_{mod}.

The B_{mod}, then, contained (w/1,000 ml): sodium sulphate 5.0 g, magnesium chloride 0.1 g, secondary potassium phosphate 1.5 g, primary potassium phosphate 0.5 g, and sodium lactate 5.0 g. The content of the other ingredients will be described separately in each section calculated as percentage of the complete medium.

Preparation of inoculate

The previously described strain of Pseudomonas fragi, strain F 111, was used in the present experiments (*Florin* 1971). The strain was grown on milk-peptone-agar medium at 17°C for 24 hrs. The medium was composed of Tryptone Extract Agar (Oxoid) to which skim milk, 1 %, was added. Cells from 2 colonies were suspended in physiological saline solution and washed by repeated centrifugation and resuspension. A barely visible suspension was prepared in order to inoculate the medium in each Erlenmeyer flask.

Determination of total number of microorganisms

All incubations were performed at 5°C and samples were drawn at intervals according to a preset schedule. In order to determine the total number of live organisms in the inoculated media, 0.1 ml of appropriate dilutions was spread on the surface of poured plates of milk-peptone-agar. The plates were incubated at 17°C for 48 hrs. (*Florin* 1971).

Determination of total volatile nitrogen compounds

The amount of total volatile nitrogen compounds (TVN) was determined in duplicates by the Conway microdiffusion method of *Farber & Ferro* (1956) described previously (*Florin* 1972).

Determination of pH

The pH was measured with a Metrohm AG Battery pH-meter E 280 A, Herisau, Switzerland.

Discussion

The basal medium used was a modification of Brown's salt solution (see *Hallman* 1953). This solution contains ammonium chloride as the sole source of nitrogen together with the salts mentioned under a) and b) on page 405. In a preliminary experiment, the growth of strain F 111 in this medium was tested. The total number of organisms per ml immediately after the inoculation was 1.5×10^2. After 10 days of incubation at 5°C, a max-

imum level of 1.5×10^9 was reached (Fig. 1). The growth of the strain was also tested in a medium (salt solutions A and B), to which ammonium chloride was not added. Only a restricted growth was expected, because the medium did not contain any source of nitrogen and the culture should not be able to fix atmospheric nitrogen. In this case the number of microorganisms increased from 1.5×10^2 to the maximum level of 1×10^8 per ml after 7 days (Fig. 1). The surprisingly good growth of the strain in this medium indicated the occurrence of impurities of nitrogenous compounds.

The growth of strain F 111 was also tested in a salt solution including the ammonium chloride (0.5 %) but without the addition of sodium lactate. This medium, therefore, lacked a carbon source. The increase of microorganisms per ml at 4°C in this medium was from 1×10^4 to 2×10^6. A maximum level was reached after 12 days of incubation. The growth curve of the strain in the complete Brown's salt solution at the same time showed an increase from 1×10^4 to 3×10^9 organisms per ml. Similar observations on the growth of Pseudomonas fluorescens in a medium with no carbon source have been reported *(Garvie* 1955, *Rhodes* 1959).

The differences in the results obtained with Brown's salt medium with or without the addition of ammonium chloride or sodium lactate indicated impurities in the sodium lactate, which probably contained minute amounts of nitrogen. The description of the sodium lactate in the *Pharmacopea Nordica* (1964) and the lack of analysis for impurities indicated that it was not a completely pure product.

The lower growth maximum in the absence of ammonium chloride showed that a source of nitrogen available to the microorganisms should be included in the media. It was shown that when the ammonium chloride in Brown's solution was substituted by histidine or leucine, the growth of strain F 111 reached a maximum level per ml of approx. 1.5×10^9, which was of the same order as in the Brown's solution. The preliminary tests showed that it was possible to use the tested solution in which the ammonium salt was substituted by 1 or more amino acids as the source of nitrogen. It was further shown that strain F 111 caused a rapid increase of TVN in the Brown's solution; it was therefore necessary to exclude the ammonium chloride in the media and to use amino acids as the source of nitrogen.

1. Formation of TVN in media containing 1 amino acid as the source of nitrogen

This experiment was designed to study which of the amino acids present in fish meat could be split by strain F 111 with liberation of volatile nitrogen compounds. Tests were performed with media containing 1 of the following 10 amino acids*:

L arginine	L lysine**	DL tryptophan
L histidine**	L methionine	DL valine
LD isoleucine	DL phenylalanine	
L leucine	DL threonine	

These amino acids and cysteine and tyrosine are listed in the food tables of *Souci et al.* (1962) as present in the fish meat. Tests have been run with a medium containing cysteine, but as this amino acid caused the formation of crystals in the medium, the results obtained were excluded.

Methods

Because of a lower solubility, 0.5 g of phenylalanine was dissolved in 100 ml of distilled water. When added to the B_{mod} (see p. 3), the concentration of this amino acid in the complete medium was 0.3 %. To prepare the other media containing only 1 amino acid, 0.83 g of each amino acid was dissolved in 100 ml of distilled water and added to B_{mod} (see p. 3) in amounts that resulted in a concentration of 0.5 % amino acid in the complete medium. The media were adjusted to pH 7.0.

To each of the Erlenmeyer flasks containing 50 ml of medium 1 ml of the suspension of carefully washed cells was transferred. After shaking, the total number of organisms was determined as described above. The flasks were incubated at 5°C and samples were withdrawn for determination of the total number of organisms (Figs. 1—4) and the TVN (Figs. 5 and 6), as indicated in the figures.

Results

Generally, the total number of organisms per ml in the newly inoculated media was between 1×10^5 and 1×10^6. The growth of the strain was good in all the different media (Figs. 1—4). The maximum stationary phase at the level of 3×10^9 was generally

---

* All preparations of the quality 'bio. pur.'.

** The preparations used were histidine monohydrochloride and lysine monohydrochloride.

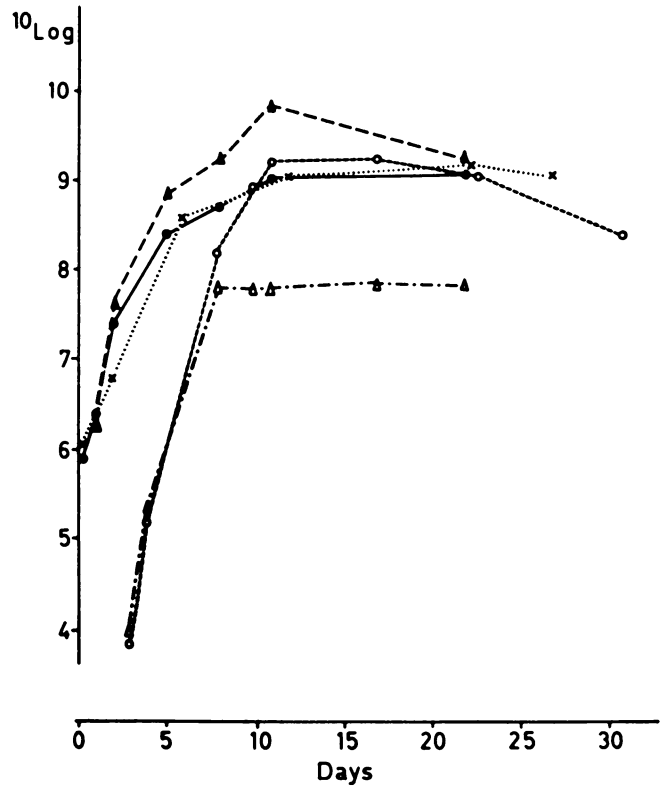

Figure 1. Growth curves in Brown's medium (------), in Brown's medium without ammonium chloride (—·—·—), B_{mod} with threonine (———), B_{mod} with valine (·····), and B_{mod} with urea (— — — —).

reached within 5 days. From then on there was generally a slight decrease in the total number during the following 3 weeks of the experiments. At the end of the tests the total number was still approx. 1×10^9 per ml.

The following results were obtained by the TVN tests (means of duplicates).

Histidine. The experiment with the histidine-containing medium was repeated twice. It was found that the TVN had a lag phase of 3 days (Fig. 5), followed by a very rapid increase up to 40 to 50 mg N per 100 ml of medium. In 1 of the experiments (circlets in Figs. 3 and 5) the number of inoculated microorganisms was extremely large (8×10^7). The increase of TVN was apparently slower than in the other 2 experiments.

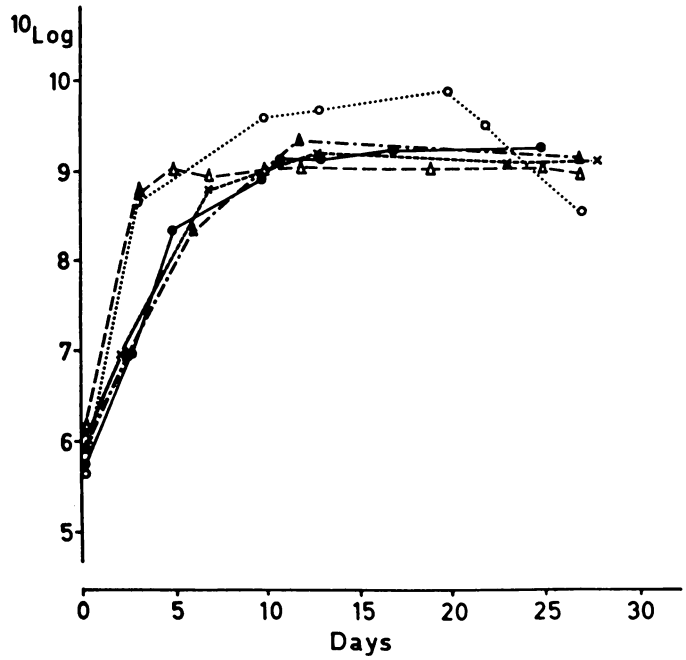

Figure 2. Growth curves in B_{mod} with isoleucine (—.—.—), with leucine (·····), with methionine (— — — —), with phenylalanine (------), and with tryptophan (———).

Arginine. In the medium containing arginine (0.5 %) the inoculation resulted in a total number of organisms of 1.5×10^5 per ml. The growth curve showed a 24-hr. lag phase (Fig. 4). The TVN present in the samples investigated after 6 to 15 days showed an increase comparable with the results obtained in the histidine-containing medium. In the arginine medium a maximum TVN level of approx. 40 to 45 mg per 100 ml was established (Fig. 6).

Lysine. In the 2 experiments with the medium containing lysine (0.5 %) the growth had a 24-hr. lag phase and reached its maximum level after 7 days. In 1 of the experiments a more pronounced reduction of the total number of organisms was observed (Fig. 4). In the lysine medium the TVN started to increase after approx. 15 days of incubation. The increase was slower than in the histidine or arginine media, and after 30 days, when the experiment was stopped, the TVN had reached the level of 20 to 25 mg per 100 ml (Fig. 6).

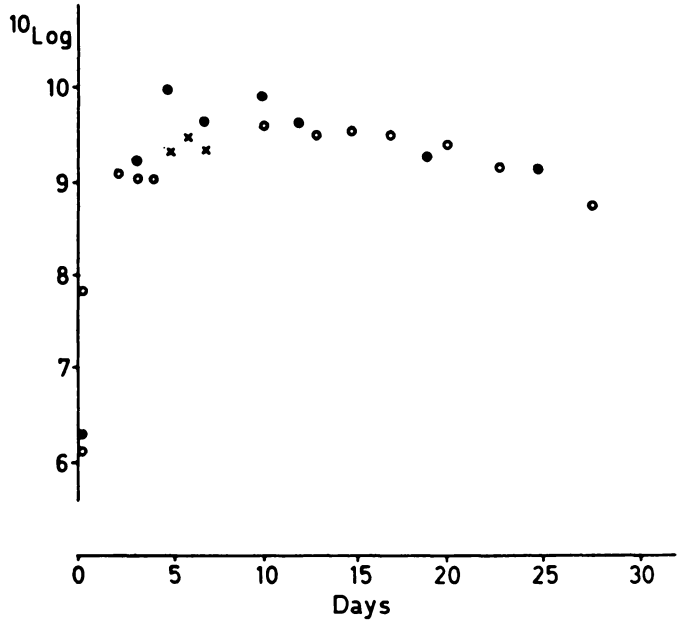

Figure 3. Growth in B_{mod} with histidine at 3 tests.

Isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, and valine. The growth curves are very similar and have maxima at or slightly above 1×10^9 organisms per ml (Figs. 1 and 2). No production of TVN was detected in media containing any one of these amino acids.

Discussion

The growth of strain F 111 caused an increase of TVN only when arginine, histidine, or lysine was present in the medium. There was, however, a distinct difference between the increase in the lysine-containing medium in comparison with the other 2 amino acids. In the case of arginine or histidine the TVN increase was observed at a time when the culture was entering or had entered the maximum stationary phase. The onset of the TVN increase when lysine was present occurred 10 days after the maximum stationary phase was reached. Two possible explanations of this late liberation of volatile nitrogen are that it is the result of a weak enzyme activity or that the ability to cause a complete breakdown of the lysine was originally lacking in strain

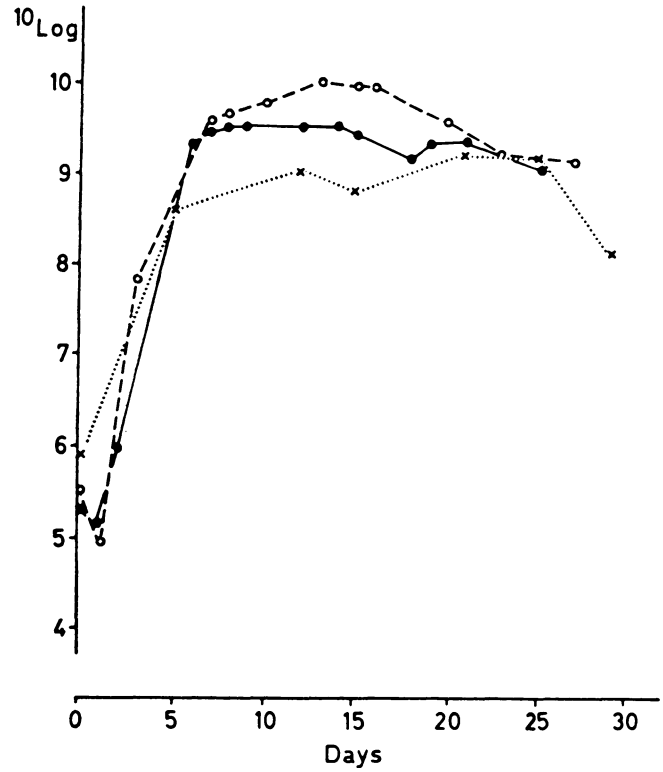

Figure 4. Growth curves in B_{mod} with arginine (——), and with lysine (·····), and (— — — —).

F 111 but was induced later. If the experiment had been repeated using cells transferred from this first test flask, the last-mentioned possibility could have been verified.

It would also have been of interest to include tests on the amino acid cysteine. It was impossible, however, to keep the amino acid dissolved in this medium. The results obtained were therefore considered not valid.

The results of the present experiments indicated that Pseudomonas fragi produced arginine- and histidine-splitting enzymes. Since the meat of halibut contains arginine but no histidine, the observed increase of TVN in the extract of halibut (*Florin* 1972) could be explained, at least partly, as being caused by arginine-splitting enzymes. In other fish species, however, the splitting of histidine as well as arginine might cause an increase of TVN.

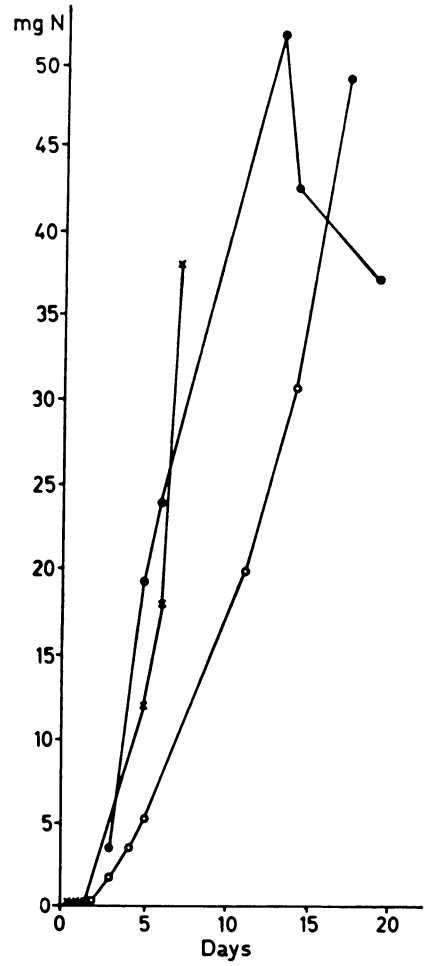

Figure 5. Total volatile nitrogen (TVN) in mg N per 100 ml of B_{inod} with histidine at 3 tests.

The reaction obtained by this strain of Pseudomonas fragi in the lysine-containing medium may not occur in a natural food which contains other amino acids more easily available to the microorganisms. It had also been shown in sea fish, which was stored on ice, that the content of lysine was steadily increasing during the first weeks of storage (*Shewan & Jones* 1957). This was in contrast to the behaviour of other amino acids.

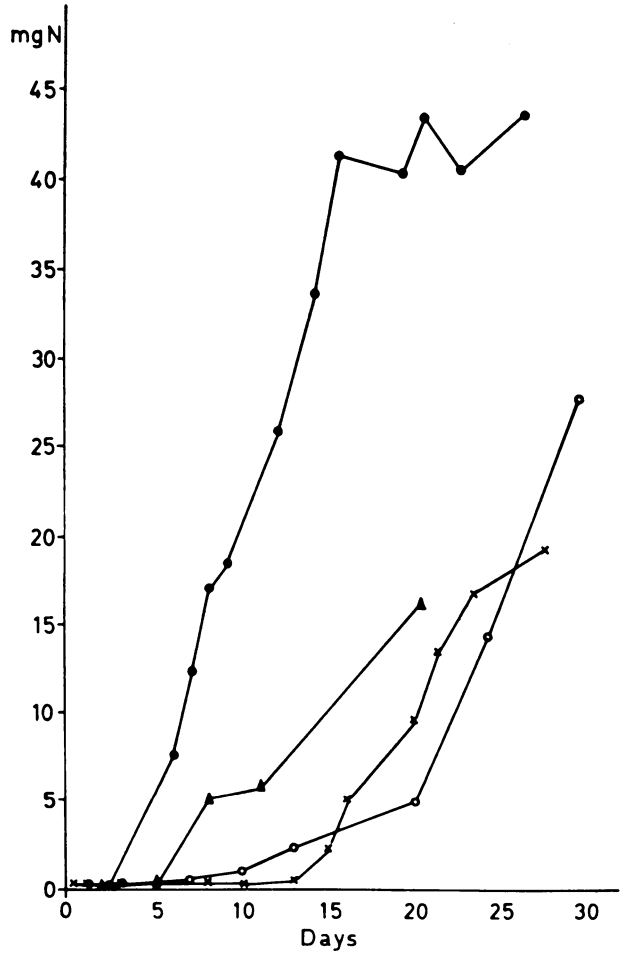

Figure 6. Total volatile nitrogen (TVN) in mg N per 100 ml of B_{mod} with arginine (●), with lysine (○) and (×) and B_{mod} with urea (▲).

2. *Formation of TVN in a medium containing urea*

The ability of strain F 111 to split urea had been observed in Christenson's urea medium *(Florin* 1971). As this substance is found in some species of fish and is also considered to be one of the intermediary steps in the decarboxylation of arginine (*Möller* 1955), it was considered necessary to study further the splitting of urea by strain F 111.

Methods

The test was performed in the medium B_{mod}, to which sterile-filtered urea, 0.5 %, was added. Fifty ml of this medium was inoculated with washed cells as described previously. The total number of microorganisms immediately after the inoculation was 5×10^5 organisms per ml. During the following 3 weeks of incubation at 5°C 6 samples were withdrawn and the number of organisms and the TVN were determined as indicated in Figs. 1 and 6.

Results and discussion

The growth of strain F 111 in the medium containing urea was similar to the growth in the media containing 1 amino acid as the source of nitrogen (Fig. 1). An increase of TVN was observed in the sample investigated on the 4th day after the inoculation (Fig. 6). During the following days the TVN increased and was 16 mg of nitrogen per 100 ml after 3 weeks of incubation.

The increase of TVN due to the splitting of urea was not as rapid as that of histidine and arginine but faster than that of lysine. After 3 weeks of incubation, only 7 % of the urea nitrogen included in the medium was recovered as volatile nitrogen in the TVN test. The experiment confirmed the findings that strain F 111 could split urea. The low recovery of urea nitrogen as volatile nitrogen in this test suggests that the splitting of urea, if this compound was formed as an intermediary step in a possible arginine decarboxylation, would probably be of minor importance.

3. Formation of TVN in media containing the amino acids histidine and lysine

The TVN had been shown to increase rapidly in media containing histidine or arginine. In a medium containing lysine as the source of nitrogen a late and slow breakdown of the amino acid had been observed. It was therefore considered possible that the presence of lysine in the medium could change the pattern of TVN formation in media containing 1 other amino acid which was rapidly split by the microorganisms with formation of volatile nitrogen. Strain F 111 had further been found to grow in an atmosphere of hydrogen (95 %) and carbon dioxide (5 %). After 48 hrs. of incubation at 17°C, however, the colonies were minute in comparison with the corresponding aerobic colonies (*Florin* 1971). An anaerobic enzyme system produced by Pseudomonas spp. had been shown to split arginine (*Thornley* 1960).

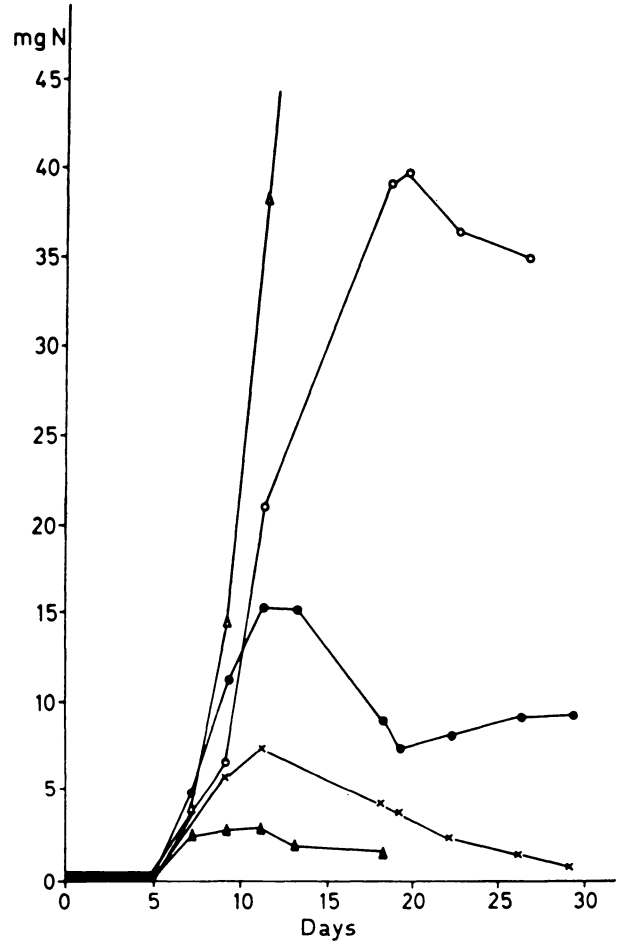

Figure 7. Total volatile nitrogen (TVN) in mg N per 100 ml of B_{mod} with histidine in concentrations 0.75 % (△), 0.40 % (○), 0.20 % (●), 0.10 % (×), and 0.05 % (▲).

The present experiment was designed to study the changes of TVN in aerobic as well as in anaerobic conditions in media which contained lysine, 0.1 %, and to which different amounts of histidine were added.

Methods

The media used in these tests were composed of B_{mod} with 0.1 % lysine to which was added histidine in the following 5 concentrations: 0.75 %, 0.40 %, 0.20 %, 0.10 %, and 0.05 %. As a reference to previous

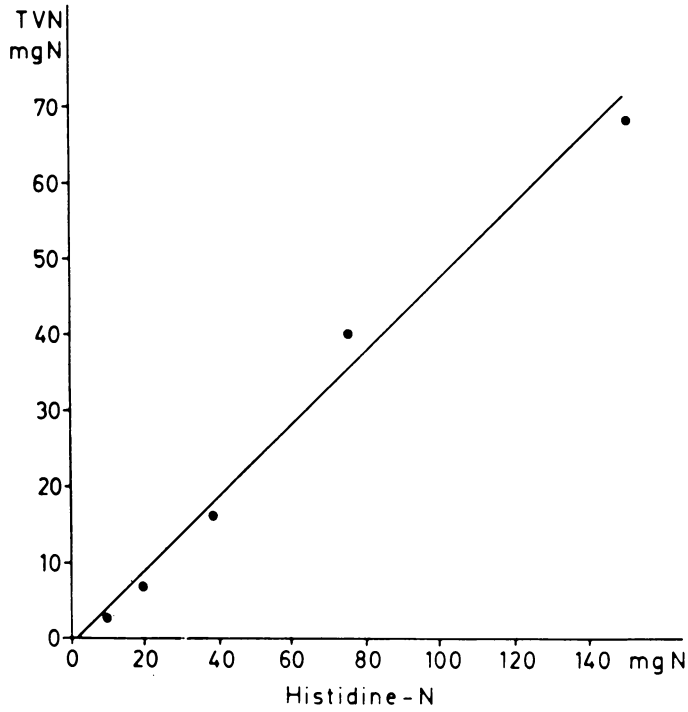

Figure 8. Total volatile nitrogen (TVN) in mg N per 100 ml of B_{mod} with histidine against the concentrations of histidine N in mg per 100 ml.
b = 0.48; y = 0.48 x — 0.83; t = 16.7**

tests was used a medium composed of B_{mod} with 0.5 % histidine. Two Erlenmeyer flasks — 1 for aerobic and 1 for anaerobic cultivation — with 50 ml of medium in each were prepared from each of the 6 media. The sterilized media were rapidly chilled. The content in 1 flask of each medium was covered with sterile paraffin oil in order to ensure anaerobic conditions. The thickness of the oil layer was approx. 15 mm.

The 12 flasks were inoculated with 0.5 ml of a suspension of washed cells of strain F 111. The inoculated flasks contained 2×10^4 cells per ml. The flasks were incubated at 5°C and samples for chemical and microbiological tests were withdrawn at intervals as indicated in Fig. 7.

Results and discussion

The growth of strain F 111 in the 5 media containing histidine and lysine was of the same order as in the salt medium to which histidine alone had been added. The increase in TVN started on

the 5th day after the inoculation and reached a maximum about 5 days later (Fig. 7). The attained level of TVN depended on the amount of histidine added to the media. It was shown by the linear regression method that the attained TVN maximum was directly proportional to the amount of histidine nitrogen present (Fig. 8). The results showed that almost 50 % of the histidine nitrogen could be found as volatile nitrogen. During the 30 days of incubation no effect on the TVN was observed that could be attributed to the addition of lysine in the media.

From the flasks with the media layered with paraffin oil samples were collected with a 5-ml pipette. Care was taken to avoid introduction of air into the medium. In these samples the anaerobic and the aerobic total numbers of organisms growing at 17°C were approximately the same and equalled the results of the aerobic culture. There was, however, no increase in the volatile nitrogen during the first 10 days of incubation. On the 11th day the TVN was 0.5 mg N per 100 ml of medium. During the following weeks a slow increase was observed and after 32 days the TVN was 5.7 mg N per ml. It might, however, be possible that introduction of minute amounts of air during the sampling had caused this small increase of TVN.

4. Formation of volatile nitrogen in media containing several amino acids as the source of nitrogen

The experiments reported in the foregoing showed that strain F 111 could split arginine and histine, with liberation of volatile nitrogen compounds. It was also shown that approx. 50 % of the histidine nitrogen could be found in the volatile nitrogen fraction. The purpose of the present model experiment was to study further these effects in media which have an amino-acid content more similar to the amino-acid pool in halibut meat or in other fish meat.

Methods

The composition of the amino-acid solutions to be added to B_{mod} was calculated from the composition of halibut meat according to the food tables of *Souci et al.* Cysteine and tryptophan were excluded because of the difficulties to obtain stable media when these compounds were added. The media used in this experiment had the following compositions:

Medium 1: Seven amino acids were added to B_{mod} which gave the following composition (g per 100 ml): sodium sulphate (Na_2SO_4), 0.5 g; magnesium chloride ($MgCl_2$), 0.01 g; secondary potassium phosphate (K_2HPO_4), 0.15 g; primary potassium phosphate (KH_2PO_4), 0.05 g; sodium lactate, 0.5 g; DL valine, 0.13 g; L isoleucine, 0.13 g; L leucine 0.20 g; L methionine, 0.07 g; DL phenylalanine, 0.09 g; thyrosine, 0.07 g; DL threonine, 0.10 g.

The following 7 media (media 2 to 8) are composed of medium 1, to which 1 or more of the amino acids lysine, arginine, and histidine are added.

Medium 2: medium 1 with 0.4 g lysine;
Medium 3: medium 1 with 0.2 g arginine;
Medium 4: medium 1 with 0.2 g histidine;
Medium 5: medium 1 with 0.4 g lysine and 0.2 g arginine;
Medium 6: medium 1 with 0.4 g lysine and 0.2 g histidine
Medium 7: medium 1 with 0.4 g lysine, 0.2 g arginine, and 0.2 g histidine;
Medium 8: medium 1 with 0.2 g arginine and 0.2 g histidine.

The inoculation of washed cells from a 48-hr. milk-peptone-agar culture resulted in a total number of approx. 10^4 organisms per ml of medium in each test flask. The flasks were incubated aerobically at 5°C and samples were withdrawn at intervals as indicated in Fig. 9.

Results

The growth of strain F 111 was almost the same in the tested media. The inoculation corresponded to 1×10^4 organisms per ml. The number of bacteria had increased to 4×10^8 in the samples investigated after 5 days of incubation and to 5×10^9 — 8×10^9 in those investigated on the 7th to 10th days after the inoculation.

Strain F 111 did not cause any increase of TVN in medium 1 containing the amino acids valine, isoleucine, leucine, methionine, phenylalanine, thyrosine, and threonine within 3 weeks of incubation at 5°C.

Lysine. In medium 2, containing lysine, 0.4 %, together with the 7 other amino acids, only a very small increase of TVN was observed. This increase was remarkably small compared with the results shown in the medium containing lysine as the sole source of nitrogen (Fig. 6).

Arginine. The TVN increase in media 3 and 5 containing arginine occurred rapidly between the 7th and the 10th days and reached the level of 20 ml N per 100 ml. During the next 10 days

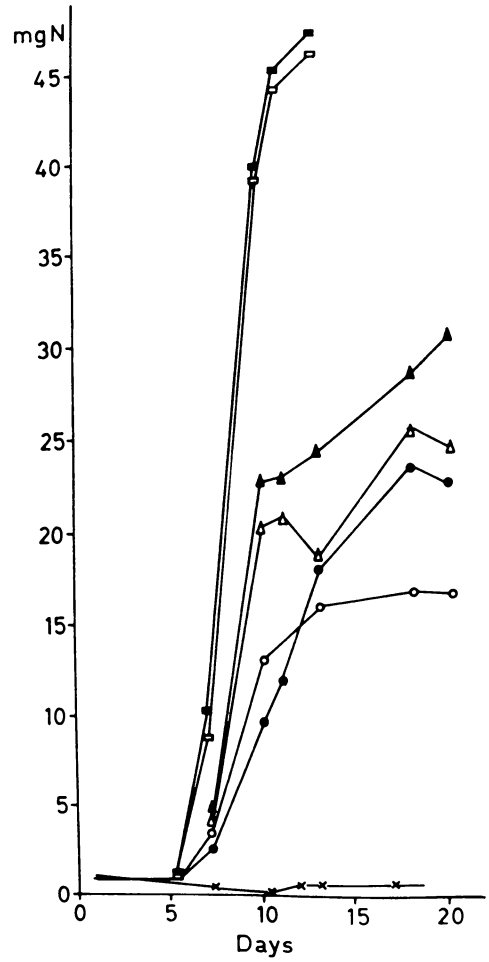

Figure 9. Total volatile nitrogen (TVN) in mg N per 100 ml of medium 1 (B_{mod} with 7 amino acids); with lysine (×), arginine (△), histidine (○), lysine and arginine (▲), lysine and histidine (●), lysine, arginine and histidine (■), and with arginine and histidine (□),

the increase in medium 3 was slower up to the level of 25 mg N per 100 ml. In the corresponding medium to which lysine, 0.4 %, was added, a similar TVN curve line was established up to 7 days after the inoculation, when the TVN level was 22.8 mg N per 100 ml. During the next 10 days the TVN reached 31 mg N per 100 ml.

Histidine. The increase of TVN in the media containing histidine (media 3 and 6) was slower than in the media containing arginine. The increase started on the 7th day, continued to 13.5

mg N per 100 ml on the 11th day, and reached a maximum of 16.2 mg N on the 18th day. When the histidine medium was enriched with lysine (medium 6) the curve line representing the TVN showed an increase between the 7th and the 18th days of incubation and the maximum of 23 mg N per 100 ml was reached.

Arginine and histidine. The TVN increase in the 2 media containing arginine and histidine or arginine, histidine, and lysine also started on the 7th day. A rapid increase during the next 3 days was followed by a levelling off which on the 13th day gave 46.9 mg N per 100 ml of medium 1 and 45.5 mg N per 100 ml of medium 8.

Discussion

It was shown in previous experiments that a late increase of TVN occurred in the medium containing lysine as the sole source of nitrogen (Fig. 6). In this experiment medium 2 contained lysine together with the 7 amino acids included in medium 1. The microbial growth in medium 2 liberated only small amounts of volatile nitrogen. On the other hand, when lysine was present together with arginine and histidine respectively (media 5 and 6), a higher level of TVN was found compared with that found in the corresponding media without lysine.

The linear regression of TVN on the concentration of histidine (Fig. 8) indicated that the 0.2 % histidine present in medium 4 should cause an increase of TVN to approx. 17 mg N per 100 ml. Strain F 111, however, caused an increase of TVN in medium 6 to 23 mg N. The presence of 0.1 % lysine in this medium increased the maximum level by 6 mg N (33 %) above the expected level.

Obviously the complex amino-acid pool containing 9 different amino acids did not inhibit the production of lysine-splitting enzyme. No explanation was found of the inhibited TVN increase in medium 2. As strain F 111 utilized lysine in the presence of arginine and histidine (media 5—8), it was improbable that the amino-acid pool of medium 2 should prevent the production of lysine-splitting enzymes.

It was concluded that the addition of lysine to the media containing the rapidly split arginine and/or histidine may be of importance to the enzyme reactions that cause an increase of TVN. The amount of TVN produced in media containing histidine and

arginine was also correlated to the amount of the amino acids present in the inoculated media. The 7 different amino acids included in medium 1 had no obvious influence on the TVN liberated in the media.

5. The effect of glucose on the formation of volatile nitrogen in media containing arginine or histidine as the source of nitrogen

The influence of an oxidizable sugar on the development of volatile nitrogen compounds in a medium containing histidine or arginine was studied with glucose added to the media. It has been shown that glycogen and carbohydrate breakdown products, such as glucose, are present in small amounts in the meat of the newly caught sea fish (*Shewan* 1961). The amount of carbohydrates, however, is so low that it is not included in the available food-composition tables. It was shown that the glucose in the fish meat disappears within 12 days (*Shewan* 1961).

Methods

The media used in the experiments had the following composition:
Medium 1: B_{mod} with arginine, 0.5 %;
Medium 2: B_{mod} with arginine, 0.5 %, and glucose, 1.0 %;
Medium 3: B_{mod} with histidine, 0.5 %;
Medium 4: B_{mod} with histidine, 0.5 %, and glucose, 1.0 %.

The media were adjusted to pH 7.0. Erlenmeyer flasks with 50 ml of medium were inoculated with 1 ml of a suspension of washed cells of strain F 111. The inoculate corresponded to 2×10^4 microorganisms per ml of medium. The flasks were incubated at 5°C and samples were withdrawn at intervals as indicated in Fig. 10.

Results

The total number of microorganisms per ml in medium 1, which contained arginine but no glucose, was after 4 days of incubation 4×10^9. The maximum level was reached 3 days later, and thereafter the total number slowly decreased to approx. 3×10^9. In medium 2, which contained arginine and glucose, the growth of strain F 111 was slower than in the glucose-free medium, but after 8 days of incubation the same maximum level (1×10^{10}) was reached.

The release of volatile nitrogen compounds from arginine was similar to the previous findings (Fig. 6). From the 4th day on,

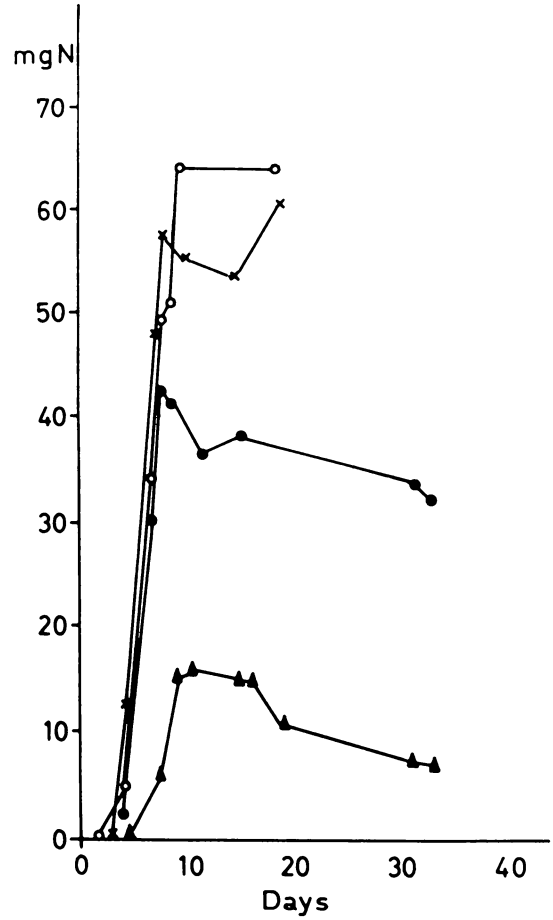

Figure 10. Total volatile nitrogen (TVN) in mg N per 100 ml of B_{mod} with arginine (○), arginine and glucose (●), histidine (×), and with histidine and glucose (▲).

the TVN rapidly increased to a maximum level of 64 mg N per 100 ml after 11 days of incubation. In medium 2, with glucose added, the TVN equalled the TVN of medium 1 during the first 8 days. After this time, the TVN curve line showed a turn at the maximum level of 43 mg N per 100 ml and then decreased slowly.

In the 2 media that contained histidine with or without glucose the events were similar. The growth in medium 3 followed the expected pattern and reached a maximum level of 6×10^9 microorganisms per ml after 4 days of incubation. The growth

in the presence of histidine and glucose was slower but the growth curve turned to a higher maximum level of 2.5×10^{10} organisms per ml after 7 days of incubation.

The TVN in medium 3, which contained histidine, was very low after 3 days but increased during the next 24 hrs. to 13 mg N per 100 ml. After 8 days, a level of 58 mg N was reached. With glucose present in medium 4 no increase of TVN was observed during the first 96 hrs. of incubation. After 7 days, an increase to 8 mg N was recorded and 3 days later the maximum of 16 mg N per 100 ml was reached.

Discussion

The growth curves of strain F 111 showed that glucose enhanced the growth of the microorganisms, especially in the presence of histidine. This medium showed a maximum total number 3 times higher than the level reached in the glucose-free medium. The level of TVN was also influenced by glucose. In the medium which contained arginine and glucose the maximum level of TVN was only ⅔ of the expected level. When histidine and glucose were present in the medium, the liberation of TVN caused by strain F 111 ceased at a maximum level which was only ⅓ of the expected level.

In this experiment, parallel flasks of media 2 and 4 had been inoculated with strain F 111 and incubated at 5°C. The content of these 2 flasks was not stirred during the first 2 weeks. The first sampling of these stay cultures was performed after 16 days. The total number of organisms, the TVN, and the pH representing these stay cultures were compared with the corresponding results obtained in the parallel flasks which were aerated by the repeated stirring and sampling (Table 1). The results showed that the TVN of the stay culture in the medium that contained histidine and glucose was considerably lower than the TVN of the aerated culture. The pH was in the former 6.0, as against 7.9 in the aerated culture. After further incubation at 5°C of the now aerated stay culture, the number of organisms per ml increased to 2×10^{10} and a TVN maximum of 25 mg N per 100 ml was reached 13 days later.

In the stay culture grown in the presence of arginine and glucose the pH was 8.1 after 16 days of incubation, being the same pH value as in the aerated parallel flask. The TVN, however, was only 6.3 mg N in the stay culture, as against 38.6 mg N in the

Table 1. Comparison after 16 days of incubation of total number of organisms, total volatile nitrogen (TVN), and pH of cultures which had been repeatedly shaken (A) or not shaken (B) during the period of incubation.

Medium (B_{mod}) with	A. Repeated sampling			B. First sampling after 16 days		
	total number	TVN	pH	total number	TVN	pH
arginine	3×10^8	62.2	8.7			
arginine and glucose	3×10^{10}	38.6	8.1	3×10^9	6.3	8.1
histidine	2×10^9	52.6	8.7			
histidine and glucose	8×10^9	14.6	7.9	8×10^9	1.4	6.0

aerated culture. After further incubation, the TVN in the first-mentioned culture increased to 38 mg N.

Observations on the utilization of glucose by a not defined psychrophilic pseudomonad in relatively vigorously aerated cultures have been presented (*Brown* 1957). A defined medium with ammonium sulphate as the source of nitrogen and containing 1 % glucose had been used. The organism was shown to produce acids from glucose, which at 0°C gave a final pH of 3.0—3.2. At this stage the growth ceased and no further acid production was observed. The growth of psychrophiles is enhanced by aeration, although the resulting growth depends on the medium used (*Jezeski & Olsen* 1962). The present experiments were performed on cultures which were shaken for a few min. at each sampling point indicated in Fig. 10. As a complement, the medium layer in the flask was thin and with a comparatively large surface area. The purpose of the experiment was to show a possible influence of the glucose metabolism of strain F 111 on the breakdown of nitrogenous compounds, as this was indicated by the liberation of volatile nitrogen compounds. The results of the experiments showed that the metabolism of glucose had such an influence on the enzymatic reactions involved in the decomposition of arginine and histidine.

The acidity produced at the oxidative breakdown of glucose counteracted the alkalinity produced by the splitting of arginine and histidine. The comparison made after 16 days of incubation (Table 1) showed that in the presence of glucose a smaller amount of volatile amines was produced and a lower pH was

recorded. A pH of 6.0 was measured in the stay culture of the medium containing histidine and glucose. This indicated that the acids produced during the glucose metabolism in the exponential growth phase inhibited or delayed the action of the enzymes that cause a breakdown of the amino acids. The growth in the aerated media that contained glucose gave low TVN maxima. This indicated that the acids produced during the glucolysis neutralized the basic compounds liberated from the amino acids or that perhaps both types of compounds were used as material for other enzymatic reactions. Further experiments, designed to show more directly how the metabolism of arginine and histidine is affected by the glucose metabolism, are necessary to explain these results. The growth of strain F 111 was apparently stimulated by the presence of glucose, since the maximum total number of microorganisms was equally high (at pH 6.0) or higher (at pH 7.9) than the maximum level reached in the glucose-free medium.

This experiment was performed with a high level of the carbohydrate compared with the level of the amino acid. The fish meat, however, contains very small amounts of carbohydrate. In cod muscles the amount of glucose varied between 9 mg and 35 mg per 100 g (*Shewan & Jones* 1957). This low level of glucose has, probably, no or very little influence on the amino-acid splitting reactions. It is possible that this lack of sources of energy easily available to the growing microorganisms necessitates to a larger extent a complete breakdown of amino acids. It is also possible that the development of bad odour or taste in the fish meat is enhanced because of this lack of oxidizable carbohydrates.

6. Formation of volatile nitrogen induced by Pseudomonas fragi under anaerobic conditions in a medium containing histidine as the source of nitrogen

It had been shown that the psychrophilic gram-negative microorganisms found in recontaminated precooked food often had the ability to grow under anaerobic conditions and that at 17°C the breakdown of nitrogenous compounds occurred in fish extract layered with paraffin oil (*Florin* 1972). Furthermore, the arginase produced by Pseudomonas spp. was splitting arginine under anaerobic conditions in cultures layered with paraffin oil (*Thornley*). The ability of strain F 111 to split histidine in anaerobic cultures was therefore studied.

Methods

Strain F 111 was grown in a fish extract which was covered with paraffin oil 24 hrs. after the inoculation. After 3 weeks of incubation at 5°C, subcultures were grown on milk-peptone-agar incubated at 17°C for 96 hrs. in a Tatlock Anaerobic Jar with 95 % hydrogen and 5 % carbon dioxide. This strain was called F 111_{anaer} to distinguish it from the original strain F 111.

Strain F 111_{anaer} grew in the presence of or without oxygen. The aerobic growth gave the same white butyrous colonies on milk-peptone-agar as strain F 111. The diameter of these colonies was 2—3 mm after 48 hrs. at 17°C. The anaerobic colonies were smaller and less opaque and had an approx. diameter of 0.5 mm after 96 hrs. at 17°C. Exposed to air, these colonies grew to the same size as the aerobic colonies. The aerobic and anaerobic colonies of strain F 111_{anaer}, however, did not produce the fruity sweet odour which was typical of strain F 111 on milk-peptone-agar cultures. On the contrary, a slight ammonia smell was observed in older cultures.

The medium used in this test was B_{mod}, to which 0.5 % histidine had been added. The medium was adjusted to pH 7.0 and 50 ml were transferred to an Erlenmeyer flask. The oxygen was removed by boiling the medium. While still hot, the medium in the Erlenmeyer flask was layered with newly sterilized paraffin oil and was stored at 5°C overnight.

The inoculation was performed with 0.1 ml of a dilution 1/10,000 of an anaerobic fish extract culture which gave a total number of 300 microorganisms per ml. The inoculated medium was incubated at 5°C.

Results and discussion

When strain F 111_{anaer} was grown in the histidine medium, the anaerobic total number equalled that of the aerobic total number of microorganisms. On the 6th day, the total number of microorganisms per ml was 1×10^9 and 4 days later it was 3×10^9.

Strain F 111 was apparently able to multiply and to develop visible minute colonies under anaerobic conditions. The anaerobic growth curve of F 111_{anaer} was similar to the aerobic growth of strain F 111. After 6 days of incubation, when the total number of organisms approached the maximum level, an increase of TVN was observed. From the 10th day on, this increase was rapid. In this experiment a very low inoculation dose was used, compared with that used in the aerobic experiments. The reason for this arrangement was simply that the microorganisms should undoubtedly have grown in the anaerobic conditions of the experiment. The increase of TVN was in this experiment observed

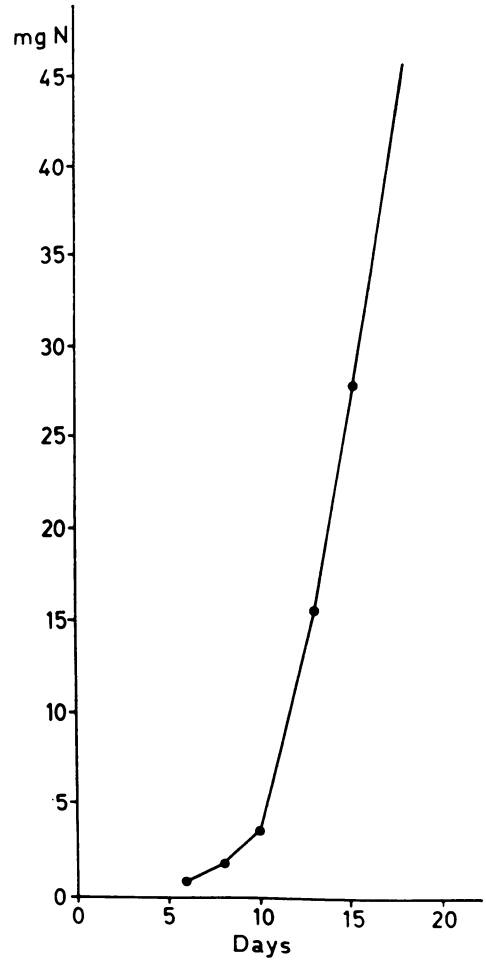

Figure 11. Total volatile nitrogen (TVN) in mg N per 100 ml B_{mod} with histidine caused by strain F 111_{anaer}

when the growth of the strain had reached a maximum level. In this respect, no difference between the aerobic and the anaerobic culture was observed (Figs. 5 and 11).

The results of this experiment indicated that Pseudomonas fragi could, partly, be adapted to anaerobic conditions and that the metabolism of histidine was performed with an enzyme system which was not depending on the presence of oxygen. The similarity to the enzyme system of Pseudomonas spp. that causes a breakdown of arginine was therefore apparent (*Thornley*). For

this reason, it is possible that the vacuum packing of precooked food does not always prevent unwanted changes of the quality of food stuffs caused by aerobic psychrophiles.

GENERAL DISCUSSION

There are many different problems in connection with the safety and sanity of foods. The spoilage of food and the changes of the taste or odour of the food is of prime interest for the storage of fresh food. The preceding papers (*Florin* 1971, 1972) were designed to study the action of Pseudomonas fragi on precooked fish during storage at 0°C to 4°C. Cooked fish was selected because Pseudomonas fragi was of importance especially to the shelf life of recontaminated moist precooked food with a mild flavour. Similar changes in pasteurized milk were caused by this microorganism (*Witter* 1961). *Castell & Greenough* (1959) showed that Pseudomonas fragi produced different odours, such as sweet, oniony, fruity, and vegetable-like odours due to its action on amino acids although they were not always able to show that fruity odours were produced in cooked fish meat. It has also been shown that Pseudomonas fragi produces extracellular proteolytic enzymes (*Juffs et al.* 1968). The proteolytic activity was measured as the amount of tyrosine liberated from peptone and casein. It was greater at 3°C than at 28°C and showed a peak in the early logarithmic phase. The proteolytic activity decreased after this peak and was low after 6 days at 3°C. The experiments of *Juffs et al.*, therefore, show a similar proteolytic activity as was shown in Pseudomonas fluorescens by *Peterson & Gunderson* (1960 a and b). The present investigation, however, deals only with the action on some of the amino acids typical of the fish meat. Most amino acids belong to the extractable parts of the nitrogenous compounds of foods. When herring was cooked at 116°C for a long time, a decrease of histidine was observed, but the contents of other amino acids were unchanged (*Hughes* 1961). Since halibut does not contain histidine, it was assumed that the cooking of the fish meat for 20 min. within plastic pouches(*Florin* 1971) liberated amino acids in the liquid part, but did not change significantly the total amount of the different amino acids.

In the present studies on the action of Pseudomonas fragi on amino acids it was shown that the rapid increase of TVN was

Table 2. Breakdown of arginine.

1. Decarboxylation

Arginine
↓ Arginine decarboxylase
Agmatine + CO_2
↓ Agmatinase
Putrescine + Urea
↓ Urease
$2NH_3 + CO_2$

$$\begin{matrix} NH_2 \\ NH \end{matrix} \!\!\diagdown\!\!\!\!\diagup C \cdot NH(CH_2)_3 \cdot CH(NH_2)COOH + 2H_2O \rightarrow$$
$$\rightarrow 2NH_3 + 2CO_2 + NH_2(CH_2)_4 \cdot NH_2$$

2. Dihydrolization

Arginine
↓ Arginine desimidase
Citrulline + NH_3
↓ Citrulline ureidase
Ornithin + CO_2 + HN_3

} Arginine dihydrolase

Overall arginine dihydrolase reaction:

$$\begin{matrix} NH_2 \\ NH \end{matrix} \!\!\diagdown\!\!\!\!\diagup C \cdot NH(CH_2)_3 CH(NH_2)COOH + 2H_2O \rightarrow$$
$$\rightarrow 2NH_3 + CO_2 + NH_2(CH_2)_3 CH(NH_2)COOH$$

(After *Möller* 1955 and *Thornley* 1960).

characteristic of media containing histidine or arginine. A prolonged incubation of strain F 111 in a lysine-containing medium also resulted in an increase of the volatile amines.

The results further indicated that between 40 and 50 % of the histidine nitrogen or arginine nitrogen could be found as volatile nitrogen compounds, owing to the action of strain F 111 in media with a low content of amino acids (< 1 %). The differences in TVN produced in the media containing arginine and/or histidine depended on the amount of amino-acid nitrogen present. It was also noted that the presence of lysine influenced the TVN maximum observed in the media containing arginine or histidine. The presence of the other tested amino acids had no influence on the liberation of TVN from these 2 amino acids.

Several species of Pseudomonas have been shown to cause a rapid decomposition of arginine (*Sherris et al.* 1959). In Pseudomonas, this depends on an anaerobic enzyme system which causes the rapid increase of the alkalinity observed in arginine medium. The pathways of this breakdown of arginine were described by *Möller* (1955) for the Enterobacteriaceae and have later been studied in Pseudomonas and other gram-negative rods (*Sherris et al.* 1959 and *Thornley* 1960). Two pathways have been described, namely the decarboxylase and the dihydrolase system (Table 2).

Regardless of which pathway is followed, 2 molecules of ammonia will be released from each molecule of arginine, viz. 50 % of the nitrogen in arginine will be found as volatile nitrogen. The arginine-dihydrolase system has been demonstrated in different Pseudomonas spp. or has been regarded as the cause of ammonia liberation from arginine by Pseudomonas. In the present experiments, no attempt was made to prove which enzyme system was used by strain F 111. The dihydrolization was probably the most likely pathway. It was, however, shown that ammonia was liberated from urea by strain F 111. This indicates the possible use of the decarboxylation pathway but presupposes the occurrence of the arginine-decarboxylase reaction and the breakdown of agmantine by agmantinase.

No attempt was made to analyze the amino-acid content of the fish extract used in previous experiments (*Florin* 1972). According to available food tables, the content of protein in halibut is 18.6 g and of amino acids 9.0 g per 100 g of fish meat. The content of 12 amino acids are recorded in the tables and among these the content of arginine in halibut is 1.05 g per 100 g of fish meat. With the dilution used in preparing the afore-mentioned fish extract and if almost all arginine is recovered in the fish extract, this amount of amino acid would give approx. 150 mg of arginine per 100 ml of extract, which corresponds to 50 mg of nitrogen per 100 ml. It had been shown in the pilot experiments with Pseudomonas fragi that only 40 to 50 % of the amino-acid nitrogen could be recovered as volatile nitrogen. Therefore the 150 mg of arginine per 100 ml of fish extract would give 20 to 25 mg of volatile nitrogen. It is interesting that TVN in the halibut extracts infected with strain F 111 repeatedly showed a maximum level at 20 to 23 mg of TVN per 100 ml. Therefore, the breakdown of arginine should be of prime importance in the increase of TVN

in cooked halibut and in the development of some of the changes in quality that occur in precooked fish during storage. To prove this relationship, however, further experiments, designed to show the decrease of the arginine content and the increase in TVN of the halibut extract and the halibut meat inoculated with Pseudomonas fragi, are necessary.

Other fish, e.g. cod and herring, contain both arginine and histidine. The experiments with media that contained arginine and histidine together with several other amino acids showed that the total volatile nitrogen was the sum of the amounts of volatile nitrogen compounds liberated from these 2 amino acids. It is therefore to be expected that the increase of TVN in fish will be the added effect of Pseudomonas fragi on the arginine and the histidine present.

REFERENCES

Bramstedt, F. & Margarethe Auerbach: The spoilage of freshwater fish. *In* Borgstrom, G.: Fish as Food. Vol. I. Academic Press, New York and London 1961, 613—637.

Brown, A. D.: Some general properties of a psychrophilic pseudomonad: the effects of temperature on some of these properties and the utilization of glucose by this organism and Pseudomonas aeruginosa. J. gen. Microbiol. 1957, *17*, 640—648.

Castell, C. H. & Maxine F. Greenough: The action of Pseudomonas on fish muscle: 1. Organisms responsible for odours produced during incipient spoilage of chilled fish muscle. J. Fish. Res. Bd Can. 1957, *14*, 617—625.

Castell, C. H. & Maxine F. Greenough: The action of Pseudomonas on fish muscle: 4. Relation between substrate composition and the development of odours by Pseudomonas fragi. J. Fish. Res. Bd Can. 1959, *16*, 21—31.

Farber, L. & M. Ferro: Volatile reducing substances (VRS) and volatile nitrogen compounds in relation to spoilage in canned fish. Food Technol. 1956, *10*, 303—304.

Florin, S. O.: Experimental studies on the growth of Pseudomonas fragi in precooked fish and its influence on the decomposition of the fish during storage at + 4°C. Nord. Vet.-Med. 1971, *23*, 484—498.

Florin, S. O.: Experimental studies on the volatile nitrogen compounds produced by Pseudomonas fragi in fish extracts. Acta vet. scand. 1972, *13*, 381—402.

Garvie, E. I.: The growth of Escherichia coli in buffer substrate and distilled water. J. Bact. 1955, *69*, 393.

Hallman, L.: Bakteriologische Nährböden. (Culture media for microorganisms). Stuttgart 1953.

Handbook of Chemistry and Physics. 45th Ed. The Chemical Rubber Co., Cleveland, Ohio 1964—1965.

Hughes, R. B.: Chemical studies on the herring (Glupea harengus). V. The effect of heat processing on the extractable nitrogen fraction. J. Sci. Food Agric. 1961, *12*, 475—483.

Jezeski, J. J. & R. H. Olsen: The activity of enzymes at low temperatures. Proc. Low Temperature Microbiology, Symposium 1961. Campbell Soup Co., Camden, New Jersey 1962, 139—154.

Juffs, H. S., A. C. Hayward & H. W. Doelle: Growth and proteinase production in Pseudomonas spp. cultivated under various conditions of temperature and nutrition. J. Dairy Res. 1968, *35*, 385—393.

Möller, Vagn: Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system. Acta path. microbiol. scand. 1955, *36*, 158—172.

Peterson, A. C. & M. F. Gunderson: Role of the psychrophilic bacteria in frozen food spoilage. Food Technol. 1960 a, *14*, 413—417.

Peterson, A. C. & M. F. Gunderson: Some characteristics of proteolytic enzymes from Pseudomonas fluorescens. Appl. Microbiol. 1960 b, *8*, 98—104.

Pharmacopea Nordica. Ed. Svecica. Vol. II, Kungl. Medicinalstyrelsen, Stockholm 1964.

Rhodes, M. E.: The characterization of Pseudomonas fluorescens. J. gen. Microbiol. 1959, *21*, 221—263.

Sherris, J. C., N. W. Preston & J. G. Shoesmith: The influence of oxygen and arginine on the motility of a strain of Pseudomonas sp. J. gen. Microbiol. 1957, *16*, 89—96.

Sherris, J. C., J. G. Shoesmith, M. T. Parker & D. Breckon: Tests for the rapid breakdown of arginine by bacteria: Their use in the identification of pseudomonads. J. gen. Microbiol. 1959, *21*, 389—396.

Shewan, J. M.: The microbiology of sea-water fish. *In* Borgstrom, G.: Fish as Food. Vol. I. Academic Press, New York and London 1961, 536—543.

Shewan, J. M. & N. R. Jones: Chemical changes occurring in cod muscle during chill storage and their possible use as objective indicies of quality. J. Sci. Food Agric. 1957, *8*, 491—498.

Souci, S. W., W. Fachmann & H. Kraut: Die Zusammensetzung der Lebensmittel I. (Composition of food I). Dtsch. Forschungsanstalt Lebensmittelchemie, München 1962.

Thornley, Margaret J.: The differentiation of Pseudomonas from other gram-negative bacteria on the basis of arginine metabolism. J. appl. Bact. 1960, *23*, 37—52.

Witter, L. D.: Psychrophilic bacteria — A review. J. Dairy Sci. 1961, *44*, 983—1015.

SAMMANFATTNING

Experimentella studier över bildningen av flyktiga kväveföreningar inducerad av Pseudomonas fragi i ett syntetiskt medium, som innehåller aminosyror som kvävekälla.

Inverkan av tillväxten av Pseudomonas fragi, stam F 111, på aminosyror studerades i ett syntetiskt medium. Det visades att flyktiga kvävebaser bildades snabbt vid nedbrytningen av arginin och histidin vid inkubering i 5°C. En senare och långsammare ökning av TVN observerades i media som innehöll lysin eller urinämne. Ingen ökning av TVN förorsakad av stam F 111 påvisades i media som innehöll någon av de sju övriga aminosyror som ingick i prövningen.

Inom gränserna 0,05 till 0,8 % histidin visades mängden frigjort TVN vara korrelerad till den mängd av aminosyran som tillfördes bassubstratet. Vid maximihalten av TVN kunde ungefär 50 % av aminosyrans kvävehalt återfinnas som flyktigt kväve.

Det visades också att halten bildat flyktigt kväve var korrelerad till summan av mängden histidin och arginin. Närvaron av lysin påverkade också TVN maximum. I det syntetiska mediet till vilket arginin eller histidin hade tillsatts stimulerades tillväxten av Pseudomonas fragi av närvaron av glykos. De syror som producerades vid den oxidativa nedbrytningen av glykos neutraliserades av flyktiga baser som producerades vid nedbrytningen av aminosyror.

Förmågan hos Pseudomonas fragi att växa under anaeroba förhållanden och att producera enzym som kan bryta ned histidin under dessa förhållanden studerades. Det påvisades att nedbrytningen av histidin liknade den anaeroba nedbrytningen av arginin som rapporterats av andra författare.

(Received September 6, 1971).

Reprints may be requested from: Stig-Olov Florin, Board of Health and Sick Care, Stockholm County Council, Långholmsgatan 34—36, Fack Stockholm 9, Sweden.