

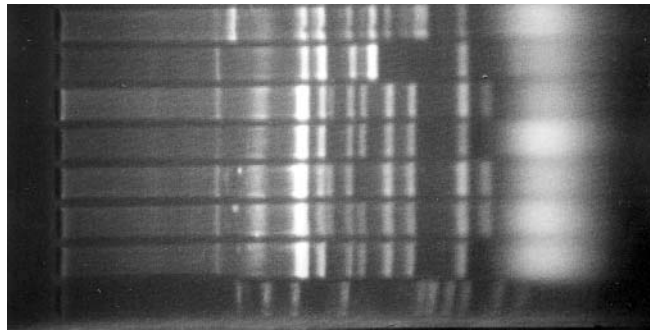
# Analysis of Norwegian Kveik blend yeast sample and isolation of individual strains for accession into the NCYC.

The liquid sample of the Kveik blend yeast sample, received from Håken Hveem was diluted and streaked on a YM agar plate to provide separate colonies for analysis. After 3 days growth at 25°C colonies on the plates were of sufficient size for analysis.

The initial analysis was carried out on four individual colonies, chosen at random, plus the confluent (mixed) growth as an initial check that the culture was indeed composed of a mixture and to see if the patterns produced were unusual when compared to brewing yeast already held at the NCYC.

No bacterial contamination was detected in the sample. Two UK microbrewery yeast were included for comparison.

The five tracks seen on the photograph of the initial gel are as follows:



1. Single Colony from UK microbrewery yeast 'IL'
2. Single Colony from UK microbrewery yeast 'ID'
3. Confluent growth from agar plate of Kveik blend yeast sample.
4. Single colony 1 from agar plate of Kveik blend yeast sample.
5. Single colony 2 from agar plate of Kveik blend yeast sample.
6. Single colony 3 from agar plate of Kveik blend yeast sample.
7. Single colony 4 from agar plate of Kveik blend yeast sample.
8. Standard Marker

## **Results:**

Three different pattern types were produced by the individual colonies:

Colonies 1 and 4 produced a pattern which matched the confluent growth type except for the lack of a band at the approx. 150bp position and a single band at the approx. 680bp position where the confluent growth has a doublet. **Designated Type 1.**

Colony 2 produced a pattern which matched the confluent growth type except for the lack of a band at the approx. 580bp position. **Designated Type 2.**

Colony 3 produced a pattern which matched the confluent growth type. **Designated Type 3.**

The patterns produced by the two comparison strains were different to the Kveik yeast patterns but similar enough to indicate that the Kveik yeast is not highly unusual in the patterns produced.

## **Conclusions:**

1) The four individual colonies produced three different pattern types, therefore confirming that the Kveik yeast is composed of a mixture of strains.

2) The patterns produced by Kveik yeast single colonies were different to the two UK comparison strains. However, the patterns produced were not markedly different to those produced by other brewing yeast.

**Actions:**

The single colonies for Types 1, 2 & 3 plus the original Kveik Mixed strain brewing yeast were given NCYC accession numbers as follows:

Confluent Mixed culture = NCYC 3545

Type 1 = NCYC 3546

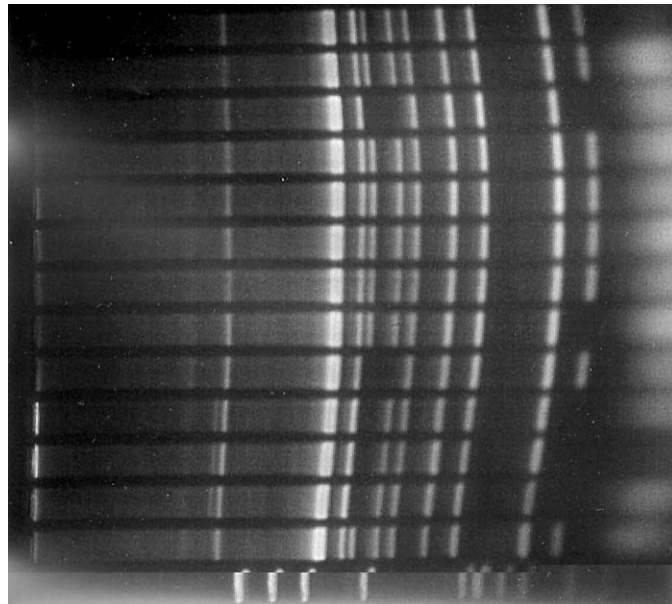
Type 2 = NCYC 3547

Type 3 = NCYC 3548

Since it is clear that the original sample was composed of a mixture of strains further work was carried out in order to find further strain/pattern types and to try to estimate the ratio of strains in the mixture.

A further streak plate was made from the original mixture to provide separate colonies. After 3 days growth at 25°C colonies on the plates were of sufficient size for analysis.

The fourteen tracks seen on the photograph of the second gel are as follows:



1. Confluent growth from agar plate of Kveik blend yeast sample.
2. Single colony 1 from agar plate of Kveik blend yeast sample.
3. Single colony 2 from agar plate of Kveik blend yeast sample.
4. Single colony 3 from agar plate of Kveik blend yeast sample.
5. Single colony 4 from agar plate of Kveik blend yeast sample.
6. Single colony 5 from agar plate of Kveik blend yeast sample.
7. Single colony 6 from agar plate of Kveik blend yeast sample.
8. Single colony 7 from agar plate of Kveik blend yeast sample.
9. Single colony 8 from agar plate of Kveik blend yeast sample.
10. Single colony 9 from agar plate of Kveik blend yeast sample.
11. Single colony 10 from agar plate of Kveik blend yeast sample.
12. Single colony 11 from agar plate of Kveik blend yeast sample.
13. Single colony 12 from agar plate of Kveik blend yeast sample.
14. Standard Marker

## **Results:**

Five different pattern types were produced by the individual colonies:

Colonies 1, 3, 4, 5, 6, and 12 produced a pattern which matched the confluent growth type. This is the same pattern as was seen on the first gel: **Designated Type 3.**

Colonies 9, 10 and 11 produced a pattern which matched Type 1 from the first gel except that it has an extra band at the approx. 1400bp position where Type 1 has a single band. **Designated Type 5.**

Single colony 2 produced a pattern similar to the confluent growth pattern but lacking bands at the approx. 150bp position, the 603bp position and the approx. 680bp position. **Designated Type 6.**

Single colony 8 produced a pattern similar to Type 6 but with the addition of a band at the approx. 150bp position. **Designated Type 7.**

Single colony 7 produced a pattern which matched the confluent growth pattern except for the lack of a band at the approx. 150bp position. **Designated Type 8.**

Note: No strain was designated as Type 4.

## **Conclusions:**

1) A further 4 pattern types were found, again confirming that the Kveik yeast is composed of a mixture of strains.

2) The patterns produced all appear to be variations on a single type. This strongly suggests that they are all closely related strains most likely having a single common ancestor.

3) Pattern Type 3 appears to be the major type in the mixture:

The overall strain ratios were as follows:

Type 1 2 Colonies

Type 2 1 Colony

Type 3 7 Colonies

Type 5 3 Colonies

Type 6 1 Colony

Type 7 1 Colony

Type 8 1 Colony

## **Actions:**

The single colonies for Types 5, 6, 7 & 8 were given NCYC accession numbers as follows:

Type 5 = NCYC 3549

Type 6 = NCYC 3550

Type 7 = NCYC 3551

Type 8 = NCYC 3552

## **Overall Conclusions:**

**The Kveik yeast is composed of a mixture of closely related strains with one type being the dominant/major type in the mixture.**

**Due to the historical nature of the mixture and the unusual source the NCYC has requested and been given permission to accession the mixture and the individual strain type isolates listed above into the open collection where they will be stored by freeze-drying and in liquid nitrogen.**