

This communication is a continuation of earlier investigations<sup>8,9</sup> of the physiology of the alder root-nodule symbiotic association.

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### RADIOBIOLOGY

#### Assimilation and Fermentation Patterns of Osmophilic Yeasts in Sugar Broths at Two Concentrations

OSMOPHILIC yeasts commonly found in intermediate sugar refinery products are strains of *Saccharomyces rouxii* and *S. mellis* with *Torulopsis* spp. which normally grow more slowly under laboratory conditions.

Recent isolates of *S. rouxii*, *T. dattila*, *T. globosa* and *T. apicola* showed differences in reaction where: (1) The ability to ferment various sugars was compared at concentrations of 2 and 10 per cent w/v in yeast extract broth; sterilized in McCartney bottles with Durham tubes at 5 lb./in.<sup>2</sup> for 15 min. Results are summarized in Table 1. (2) The ability to assimilate various sugars was compared at concentrations of 1 and 10 per cent w/v in Difco 'YNB' broth (filter-sterilized and dispensed as 5 ml. vol. into pre-sterilized, capped tubes). Results are summarized in Table 2.

Inocula were taken from fresh slants on osmophilic agar<sup>1</sup>, incubated for 72 h at 30° C, washed and centrifuged in sterile Ringer's solution and, finally, re-suspended in 10 ml. sterile Ringer's solution. One drop of suspension was used per tube.

The variation in reaction does not appear to be due simply to osmotic pressure; as if 9 per cent of the sugar is replaced by an equivalent amount of lactose (negative in every case) the stimulation of the higher concentration was not demonstrated.

Anomalous fermentation patterns were reported last year by J. Santa Maria<sup>2</sup> for *S. rouxii* isolated from sugar cane molasses, but tested at standard sugar concentrations.

All the yeasts were isolated from situations of very high sucrose concentrations. They sub-cultured more readily

Table 1. FERMENTATION TESTS

Yeast % sugar	<i>S. rouxii</i>		<i>T. dattila</i>		<i>T. globosa</i>		<i>T. apicola</i>	
	2%	10%	2%	10%	2%	10%	2%	10%
Glucose	++	++	++	++	±	++	-	++
Sucrose	-	-	+	++	±	++	-	++
Maltose	-	++	-	-	-	-	-	-
Galactose	-	-	-	-	-	-	-	-
Raffinose	-	-	-	++	±	++	-	-
Lactose	-	-	-	-	-	-	-	-

Table 2. ASSIMILATION TESTS ON *S. rouxii* AND *T. dattila* ONLY (the other two species showed less obvious differences)

Yeast % sugar	<i>S. rouxii</i>		<i>T. dattila</i>	
	1%	10%	1%	10%
Glucose	++	++	++	++
Sucrose	-	-	++	++
Maltose	++	++	-	++
Galactose	-	+	-	+
Raffinose	-	-	++	++
Lactose	-	-	-	-

++ = positive; - = negative; + = weakly positive; ± = variable.

on an osmophilic or semi-osmophilic medium; growth on wort agar or in wort broth is usually very poor. The use of broths with 10 per cent sugar was adopted rather than a higher value because of the low solubility of some sugars such as lactose.

Similar tests on cultures from the Centraalbureau voor Schimmelcultures Delft do not show these variations. The problem arises, therefore, as to whether the incorporation of what are really ecological variations in a test is justified (a parallel case is the use of Koji extract by Onishi<sup>3</sup>). Yeast identification is essentially the 'building-up' of a picture by a number of tests, so ecological modifications must surely be considered. In the case of the osmophilic yeasts, this character may even be lost after growing in media of lower concentrations of sugar for a period<sup>4</sup>.

We consider, therefore, that this group of yeasts should: (a) always be stored in an osmophilic medium which retains these typical properties; (b) biochemical tests should, so far as possible, be adapted to the ecology of the organisms and be carried out in broths containing a minimum of 10 per cent sugar w/v as this produces more consistent results.

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### VIROLOGY

#### Resistance to Sigma Virus Infection in *Drosophila*

*Drosophila melanogaster* is subject to infection by the sigma virus, either by virus carried in the fly gametes or by extracts from infected flies. Infected flies somehow become 'CO<sub>2</sub>-sensitive' and thereafter are fatally poisoned if subjected to a dose of CO<sub>2</sub>, which is merely anaesthetic to virus-free flies. Plus<sup>1</sup> showed that the time lag (incubation period) between a sigma injection and the onset of CO<sub>2</sub>-sensitivity is a measure of the injected dose, and sigma extracts are now usually titred by using them as inocula and measuring the resultant incubation period in recipient adult flies.

Brun<sup>2</sup> recently reported, for a certain dose, that the incubation time increases in direct proportion to the age of the recipient fly at injection. Further information concerning this age effect is important to the standardization of the assay for sigma virus and to understanding the course of the infection. In addition, the age effect may be related to the poorly understood general phenomenon whereby virus grows slowly or is less pathogenic in aged host tissue, an effect reported for plant<sup>3</sup> and animal<sup>4</sup> viruses.

The influence of imaginal age on the incubation time was tested in the present study by injecting virus extracted from the *Lw Drosophila* strain into reference-strain flies according to procedures previously described<sup>5</sup>. Twenty-five to 30 flies of each age were injected and the average incubation times calculated. The results obtained were similar to Brun's except that, under these conditions, the age-dependence of the incubation time was not linear. As in Brun's study, females required longer incubation times than did males (Fig. 1).

When incubation period is used to estimate inoculum titre, age differences among recipient flies can lead to significant discrepancies. For example, a 17-h difference