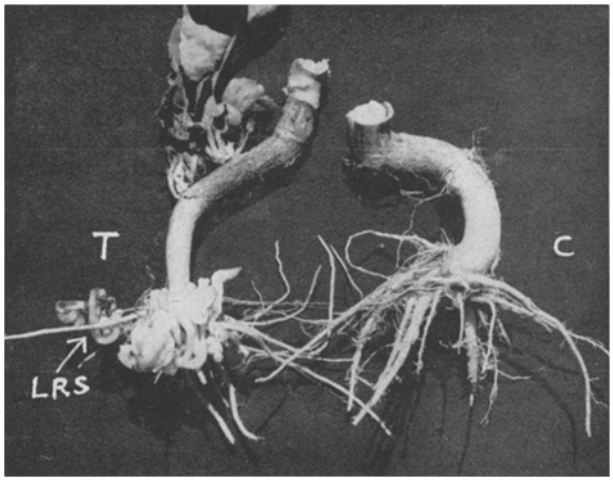
surfactant). For controls, one set was treated with surfactant only, whilst the other set remained untreated; 10 plants were used for each treatment.

Ten weeks after treatment, some of the morphactin treated plants showed shoots arising from the underground part of the plant. Three plants from each group were removed from the soil and the adhering soil washed off. It was observed that the morphactin treated plants at all concentrations bore numerous shoots on the underground part of the stem, tap root and lateral roots (Figure). There were no shoots/buds on the roots of control plants. 3 plants of each group were studied a week later and another 3 in the following week. The remaining one plant of each group was retained in the field for further observations.

These shoots first grew downward then upward making a U shaped base, and some of these bore roots. The shoots that had become exposed to sun were green, whilst those still underground had numerous crumpled leaves with thickened petiole and midrib and yellow lamina. As many as 35 underground shoots were observed on one of the plants treated with 1000 ppm morphactin solution and the lowest number for this group was 20 shoots. In one case as many as 16 shoots were seen arising from different loci on a lateral root. Plants treated with 500 ppm had 18–28 shoots, whilst those treated with 250 ppm had 13–20 and those with 100 ppm had 8–15 shoots. Shoots were rare on the lateral roots at lower concentrations.



Basal portion of cauliflower plants after 10 weeks of treatment. (C) control, (T) aerial portions treated with 1000 ppm morphactin solution, showing numerous shoots on tap root and one of the lateral roots. LRS, lateral root shoots.

Cytokinins are known to stimulate shoot bud initiation on explants in vitro, this holds good even for the triploid tissue viz. the mature endosperm in tissue culture[3]. In potato plants, treatment with cytokinin resulted in upward and erect growth of stoloniferous shoots. The presence of cytokinins in leaves of *Bryophyllum* and *Begonia* strengthens the view that cytokinin and auxins mediate environmental influence on regeneration capacity in these plants[4]. Morphactins can 'simulate' the effect of cytokinins[5], by increasing the number of regeneration loci on leaf discs of *Begonia*. Recent work[6] has shown that Morphactins are capable of inducing cytokinin-dependent tobacco callus tissue to become cytokinin-autonomous, i.e. to continue growth without cytokinin. This shows that fluorenes can induce a directed and heritable cellular change in tobacco tissue in culture in which a specific biosynthetic system, i.e. endogenous cytokinin system, is regularly and persistently activated.

The present findings can be explained on the assumption that morphactin absorbed via the aerial parts of the plant was transported to roots, and there it either simulated cytokinin's activity or it activated the cytokinin's biosynthesis, and manifested the consequences in the root zone. Such shoots would indeed be of use in propagation of important types of cauliflower. Work is in progress to elucidate the mechanism of action of morphactin on cauliflower.

Résumé. Des plantes de choux-fleurs traitées avec une solution de morphactine ont produit des bourgeons sur leurs racines.

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Photoreactivable Sectors and the Systematics of the Genus *Pichia*[1]

On the bases of biochemical, ecological and life-cycle considerations, Wickerham[2] has arranged the 25 species of yeasts belonging to the genus *Hansenula* according to a phylogenetic scheme consisting of 5 major and one subsidiary lines of descent. Spencer et al.[3,4] noted that variations in the structure of cell wall mannans of species of *Hansenula* correlate well with the relationships proposed by Wickerham. We recently reported[5] that the magnitudes of the photoreactivable (PR) sectors (i.e. fraction of the ultraviolet, inactivational cross-section of a cell subject to repair by exposure to visible light) of these species also are consonant with Wickerham's phylogenetic scheme; only very primitive species fail to photoreactivate, advanced species have PR sectors ranging from 0.3 to 0.7 and all species belonging to line 5 of the scheme have a distinctive PR sector of 0.1.

The genera *Hansenula* and *Pichia* are differentiated, categorically, only by the inability of species of *Pichia* to utilize nitrate. As yet, intrageneric evolutionary relationships among species of *Pichia* are moot. Wickerham and Burton[6] have adduced strong arguments to the effect that the genus *Pichia* arose from one or more primitive species of *Hansenula*. Spencer and Gorin[3] have shown that the cell wall mannans of many *Pichias* are struc-

PR sectors compared with features of the life cycles and fermentative capabilities of species of *Pichia* as given by KREGER-VAN RIJ[7]

Species and strain[a]	PR Sector	Ploidy[b]	Mating system[c]	Fermentation[d] Glucose	Galactose	Sucrose	Maltose
Pichia delftensis NRRL Y-7119	0.0	d	—	±	—	—	—
P. farinosa ATCC 2252	0.0	h	ho	+	+	—	—
P. fermentans NRRL Y-1619	0.0	d	—	+	—	—	—
P. fluxuum NRRL YB-4273	0.0	d	—	—	—	—	—
P. kudriavzevii NRRL Y-5396	0.0	d	—	+	—	—	—
P kluyveri NRRL YB-4277	0.0	d	—	+	—	—	—
P. media NRRL Y-7122	0.0	h	ho	—	—	—	—
P. membranaefaciens NRRL Y-2026	0.0	h or h/d	ho or he	±	—	—	—
P. pastoris NRRL Y-1603	0.0	h	ho	+	—	—	—
P. polymorpha NRRL Y-4279	0.0	h	ho	+	±	+	±
P. pseudopolymorpha NRRL- YB-4228	0.0	h	ho	+	±	+	±
P. stipidis NRRL Y-7124	0.0	h	ho	+	+	—	+
P. toletana NRRL YB-4494	0.0	h	ho	+	—	—	—
P. vini NRRL Y-1459	0.0	h	ho	—	—	—	—
P. angophorae NRRL Y-7118	0.1	h	ho	+	—	+	+
P. bovis NRRL YB-4184	0.1	h/d	ho	+	—	—	—
P. dispora NRRL Y-7120	0.1	h	ho	+	—	—	—
P. halophila NRRL YB-3647	0.1	h	ho	—	—	—	—
P. pinus NRRL Y-9555	0.1	h	ho	±	—	—	—
P. saitoi NRRL Y-6671	0.1	d	—	+	—	—	—
P. terricola NRRL YB-4310	0.1	d	—	+	—	—	—
P. trehalophila NRRL Y-6781	0.1	h	ho	+	—	—	—

turally comparable to those of primitive *Hansenulas*, but that other *Pichias* have mannans typical of the highly evolved *Hansenulas*. This would suggest either 1. that both *Hansenula* and *Pichia* originated from common progenitors and have undergone independent but closely parallel evolutions or 2. that new *Pichia* species often have arisen independently of each other as nitrate-negative variants of individual *Hansenula* species at various levels of evolutionary development. Either of these alternatives implies that, for *Pichia* as well as *Hansenula*, the PR sectors of individual species should be dependent on their evolutionary statuses. To explore this possibility, the PR sectors of each of the 35 species of *Pichia* currently accepted by KREGER-VAN RIJ[7] were determined by procedures described previously[5] and compared with certain fermentative and life cycle characteristics of the species. The data recorded in the Table show the following correlates to earlier findings with the *Hansenulas*.

1. Like the *Hansenulas*, species of *Pichia* can be grouped as those which do not photoreactivate, those with PR sector of 0.1 and those with PR sectors of 0.3 or higher. These groupings of the *Hansenulas* have been shown to reflect the evolutionary placement of species according to WICKERHAM's interpretation of the phylog-

[1] These studies were aided in part by a contract No. AT (11–1)-1772 with the U.S. Atomic Energy Commission.

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Species and strain	PR Sector	Ploidy	Mating system	Fermentation Glucose	Galactose	Sucrose	Maltose
P. acaciae NRRL Y-7117	0.4	h	ho	+	—	—	+
P. chambardii NRRL YB-4249	0.5	d	—	—	—	—	—
P. etchellsii NRRL Y-7121	0.5	h	ho	+	—	±	±
P. guilliermondii NRRL Y-2075	0.4	h/d	he	+	±	+	—
P. ohmeri NRRL Y-1922	0.5	h/d	he	+	+	+	+
P. pijperi NRRL YB-4309	0.7	d	—	+	—	—	—
P. quercuum NRRL YB-4281	0.6	h	ho	+	—	—	—
P. onychis NRRL Y-7123	0.3	h/d	ho	+	—	+	—
P. rhodanensis NRRL Y-2380	0.3	h/d	he	+	—	±	—
P. salictaria NRRL Y-6780	0.4	h	ho	—	—	—	—
P. scolytii NRRL Y-5512	0.3	h/d	he	+	+	±	±
P. strassburgensis NRRL Y-6730	0.7	h/d	he	+	±	+	—
P. wickerhamii NRRL Y-2436	0.4	h/d	he	+	—	—	—

[a] Source of strains: ATCC, American Type Culture Collection, Rockville, Maryland, USA; NRRL, Northern Regional Research Laboratory, Peoria, Illinois, USA. [b] ploidy of cells during vegetative growth; h, haploid; d, diploid. [c] ho, homothallic; he, heterothallic; —, mating system not known. [d] ±, occurrence of fermentation is variable.

eny of the genus. The possibility that such groupings also may be indicative of systematic relationships among *Pichias* is suggested by the fact that homothallic species of *Pichia* occur within each of the three sorts of groups whereas the known, purely heterothallic species exhibit only large PR sectors.

2. The non-photoreactivating, primitive species of *Hansenula* ferment few sugars and the majority of such species grow vegetatively only in haplophase; the strongly photoreactivating, advanced species are more versatile fermentatively and, typically, grow vegetatively either as haplophase-diplophase mixtures or in diplophase exclusively[2,5]. Analogous correlations are noted with the *Pichias*. Though species of *Pichia*, collectively, have a much more restricted range of fermentative capabilities than the *Hansenulas*, KREGER-VAN RIJ has compared all species of *Pichia* for abilities to ferment four common sugars[7]. The Table indicates that the nonphotoreactivating species of *Pichia* ferment an average of 1.4 of these sugars each, whereas species having large PR sectors ferment an average of 2.0 sugars each; furthermore, 64% of the non-photoreactivating species but only 30% of the species having large PR sectors grow vegetatively in haplophase, exclusively.

3. Proton magnetic resonance spectra of the cell wall mannans of the *Hansenulas* have established that the weakly photoreactivating species (PR sector, 0.1) belonging to line 5 of WICKERHAM's phylogeny and the non-photoreactivating progenitor of that line, *H. wickerhamii*, possess a unique mannan structure which distinguishes them from other members of the genus[3,4]. This same distinctive structure has been identified in the cell wall mannans of the following species of *Pichia*[3]: *P. angophorae, P. fermentans, P. fluxuum, P. kluyveri, P. membranaefaciens, P. pinus, P. terricola, P. trehalophila.* The Table reveals that these particular species of *Pichia*, like their *Hansenula* counterparts, either do not photoreactivate or have PR sectors of 0.1.

These observations suggest that PR sectors constitute an additional parameter of affinity between the genera *Hansenula* and *Pichia* and may serve as useful data in the eventual resolution of phylogenetic relationships among species of *Pichia*.

Zusammenfassung. Es wird der Beweis erbracht, dass photoreaktivierungsfähige Sektoren von Zellen einen zusätzlichen Affinitätsparameter zwischen Hefen der Gattungen *Hansenula* und *Pichia* bilden und vielleicht wertvolle Daten für die endgültige Erschliessung der intragenerischen Phylogenie der *Pichia* liefern.

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COGITATIONES

Flow in the Phloem and the Immobility of Calcium and Boron: A New Hypothesis in Support of an Old One

The sieve tubes of the phloem of plants are the conduits through which photosynthate and additional, quantitatively minor solutes are distributed from the leaves and other 'sources' to 'sinks' – regions where these nutrients are utilized, including the roots. The most widely held view of the mechanism of movement in the sieve tubes is the pressure flow hypothesis of MÜNCH; see CRAFTS and CRISP[1]. According to this view, a mass flow of water and dissolved solutes moves through the lumen of the sieve elements, and from one such element to the next through the connecting pores which are clustered in sieve areas of the cell walls between contiguous sieve elements abutting end to end.

For such a flow to occur the interior of the sieve elements and the pores connecting them must be relatively open; the numerous membrane-bound organelles present in other types of cells would tend to plug up the conduit. The vacuole, in particular, would represent an obstacle to any free flow. In mature cells not specialized for conduction it occupies the bulk of the volume of the cell, restricting the cytoplasm to a thin layer between the plasmalemma, the outer membrane which lies appressed against the cell wall, and the tonoplast – the membrane between the cytoplasm and the central vacuole.

In accord with expectations based on the pressure flow hypothesis, the interior of the mature sieve element is indeed devoid of much of the cytoplasmic apparatus which would impede a longitudinal flow of a solution. The tonoplast (and hence a true vacuole) is absent, and so is the nucleus. The relatively few membranous stuctures remaining (mitochondria, plastids, endoplasmic reticulum) usually lie near the plasmalemma, along the wall, leaving a relatively structureless lumen within, which appears to offer minimal resistance to the longitudinal flow envisioned by the MÜNCH hypothesis.

Through the sieve tubes move not only sugars but also inorganic nutrients including potassium, magnesium, phosphate, and others[1,2]. But curiosly, concentrations of calcium and boron in the sieve tubes are very low, and so, as a result, are the quantities of these nutrients moved in the phloem[1,2].

The relative immobility of calcium and boron in the phloem is disadvantageous or non-adaptive in that it may cause local deficiences resulting from their failure to be redistributed, via the phloem, from regions where their concentrations are high, as in older leaves, to young, actively growing regions[2]. I offer the following hypothesis to explain the paradox of an adaptation which is seemingly disadvantageous.

Calcium. This nutrient is essential for the functional and structural integrity of plant cell membranes[2,3]. In calcium deficient cells there is 'a general disintegration of the cell contents'[4], 'structureless areas' appear and extend progressively, and there ensues a 'disintegration of the various membranous structures'[5]. These observations on calcium deficient cells are quite parallel to those made by students of phloem structure concerning the contents of sieve tubes – cells known to contain very little calcium. I

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