Extracellular Polysaccharides and Classification of the Genus *Lipomyces*

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SUMMARY

Strains classified as *Lipomyces lipoferus* and *L. starkeyi* produce specific extracellular acidic heteropolysaccharides. The polymer from *L. lipoferus* contains mannose and glucuronic acid; the polymer from *L. starkeyi* contains, in addition, galactose and a trisaccharide. This trisaccharide is composed of mannose and glucuronic acid in the molar ratio 1:2. The difference in polysaccharide composition may be the most significant physiological basis yet discovered for separation of species in the genus *Lipomyces*.

INTRODUCTION

During a survey of extracellular yeast polysaccharides, strains designated as *Lipomyces starkeyi* Lodder et Kreger-van Rij and *L. lipoferus* (den Dooren de Jong) Lodder et Kreger-van Rij were found to produce different extracellular polymers. Taxonomic differences based on assimilation tests are not clear-cut in this ascomycete genus. Reinforcement of these data by differences in macromolecular composition and structure, which must in turn reflect genetic distinctions, ought to be of value in species identification. The finding of distinct differences in the extracellular heteropolysaccharides produced by lipomyces strains suggests a basis for separation of the genus into two species.

METHODS

The organisms were grown in yeast extract+malt extract+peptone medium containing 5% glucose hydrate as previously described (Slodki, Wickerham & Bandoni, 1966). Polymers were isolated from cell-free culture liquors by precipitation with 1.2-1.5 volumes of methanol. At these methanol concentrations, precipitation of polymer required addition of a small amount of potassium acetate. The reprecipitated polymers were taken up in water, dialysed and lyophilized.

Methods used for paper chromatographic analysis and determination of component ratios are described elsewhere (Slodki *et al.* 1966).

RESULTS

Table 1 lists the optical rotations and approximate molar ratios of O-acetyl, total hexose and glucuronic acid. Mannose and galactose both occur in the polymers from *Lipomyces starkeyi*. Mannose is the only hexose present in the *L.*

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lipoferus polymers. The difference in hexose composition alone does not account for the optical rotations of the two polymer types. As shown in Fig. 1, hydrolysates of the *L. starkeyi* polysaccharides also contain a characteristic acidic oligosaccharide that gives, on paper chromatograms, a deep red-brown stain with the *o*-aminodiphenyl spray reagent (Timmell, Glaudemans & Currie, 1956). Data in Table 1 and Fig. 1 also serve to characterize culture NRRL Y-6833 as belonging to *L. lipoferus*. Previous taxonomic criteria, based largely on assimilation tests (Wickerham, 1951) failed to place this organism definitively in either *lipomyces* species.

Table 1. *Properties of lipomyces species and polysaccharides*

Species	Source	NRRL no.	Lactose assimilation	Poly-mer yield*	$[\alpha]_D^{25}\dagger$	Hexoses present		Molar ratios		
						Man-nose	Galac-tose	Acetyl:	Hexose:	Glucur-nate
<i>L. starkeyi</i>	Starkey 74	Y-1388	+	1.01	0°	+	+	0.9	2.2	1
<i>L. starkeyi</i>	Starkey 74b	Y-2543	—	0.80	— 4°	+	+	0.6	2.0	1
<i>L. lipoferus</i>	A. C. Thayson	Y-1351	+	0.56	+42°	+	—	1.1	2.5	1
<i>L. lipoferus</i>	Type strain, CBS	Y-2542	+	0.83	+40°	+	—	1.1	3.0	1
<i>L. starkeyi</i> ‡	David Jones	Y-6833	—	0.93	+54°	+	—	1.2	2.0	1

* g. 100 ml. culture.

† c, 0.5, water.

‡ Classification according to Brady & Jones (1964).

A preliminary attempt was made to ascertain the nature of the acidic oligosaccharide in the *Lipomyces starkeyi* Y-2543 polysaccharide. A small portion of the polymer (37 mg.) was hydrolyzed in 2 N-HCl (2 ml., boiling water bath, 1 hr). After neutralization to pH 4.2 with Ag₂CO₃, the mixture was filtered and then decationized by passage through an Amberlite IR-120 (H⁺) column. The acidic solution was allowed to percolate into a Dowex 1-4 X, acetate, 200-400 mesh microcolumn (VanEtten & McGrew, 1957). Elution of the column was done with an acetic acid gradient established by passing 1.5 M-acetic acid into 125 ml. water. The fractions (5-7 ml.) were examined for the presence of carbohydrate by the phenol + sulphuric acid reagent (DuBois *et al.* 1956). After pooling, the various peaks were examined by paper chromatography. The free hexoses, mannose and galactose, were not adsorbed and were present in approximately equal amounts. As shown by hydrolysis and paper chromatography, the oligosaccharides in the two major elution peaks contained only mannose and glucuronic acid. This composition was confirmed by the sulphuric acid + carbazole test (Gregory, 1960) for uronic acid and by the sulphuric acid + cysteine test (Dische, Shettles & Osnos, 1949) for hexoses. The latter test confirmed the presence of galactose in the unadsorbed fraction only and indicated that this hexose must not be involved in relatively stable uronide linkages.

The first peak eluted consisted primarily of an aldobiuronic acid contaminated with a much smaller amount of free glucuronic acid. On paper chromatograms, the aldobiuronic acid had the characteristics of 2-O-(β-D-glucopyranosyluronic acid-D-mannose) (Słodki *et al.* 1966; Sloneker & Jeanes, 1962). These characteristics include an orange-brown spot with the *o*-aminodiphenyl spray, a yellow colour with the periodate Schiff spray (Hardy & Buchanan, 1963) and no reaction with alkaline triphenyltetrazolium (Wallenfels, 1950). The occurrence of the aldobiuronic

acid is not surprising. Previous experience at the Northern Laboratory has been that microbial polysaccharides which contain glucuronic acid and mannose generally possess this disaccharide (J. H. Sloneker, personal communication).

Fig. 1. Chromatogram of lipomyces polysaccharide hydrolyzates. Descending irrigation in ethyl acetate+acetic acid+pyridine+water (5+1+5+3, v/v) for 16 hr; *o*-aminodiphenyl spray reagent (Slodki *et al.* 1966). Strains NRRL Y-1388 and Y-2543 were designated *L. starkeyi*; strains Y-1351 and Y-2542 were classified as *L. lipoferus*.

The second peak contained a single component, the oligosaccharide, giving a red-brown spot on chromatograms sprayed with *o*-aminodiphenyl. The reducing end group was determined by alkaline iodine titration (Slodki, 1963); a mannose:

glucuronic acid:reducing group molar ratio 1.0:2.0:1.1 was obtained. Based on the glucuronic acid analysis, the free acid form gave $[\alpha]_D^{25} + 30^\circ$ (c, 0.23, water). On paper chromatograms, the substance is tetrazolium negative and gives a yellow spot with the periodate Schiff reagent. Hydrolysis and paper chromatography gave spots visualized by the spray reagents which corresponded to mannose, glucuronic acid and the aldobiuronic acid of the first peak. Thus, the trisaccharide is probably glucuronic acid α -linked to 2-O-(β -D-glucopyranosyluronic acid)-D-mannose.

DISCUSSION

Assignment of lipomyces strains to either of the two species comprising the genus has been difficult on the basis of morphological and taxonomic criteria. The principal criterion for separating lipomyces strains has been the ability of *L. lipoferus* and the inability of *L. starkeyi* to assimilate lactose (Lodder & Kreger-van Rij, 1952). Culture NRRL Y-6333 received from D. Jones under the name *L. starkeyi* did not assimilate lactose. In our experience this strain is unusually weak in its ability to assimilate compounds in still medium. Even shaking does not permit assimilation of more than trace amounts of xylose, erythritol, dulcitol, sorbitol, α -methylglucoside, and salicin, although these compounds are strongly assimilated by strains classified by various investigators as either *L. lipoferus* or *L. starkeyi*. Brady & Jones (1964) have classified Jones strain as *L. starkeyi*. Though it does not assimilate lactose, its polymer is like that of the *L. lipoferus* strains, which are recognized as belonging to a separate species (Lodder & Kreger-van Rij, 1952) because they assimilate lactose. Connell, Skinner & Hurd (1954) suggested that the two species should be considered as one, because they believed that no clear means had been found to differentiate strains at the species level. It is perhaps fortunate that lipomyces strains produce one or the other of two distinctive extracellular polysaccharides differing not only in a particular component sugar (galactose), but also in respect to intrinsic polymer structure. This difference represents strong evidence that at least two distinctive species comprise the genus *Lipomyces*.

Among our data from routine taxonomic studies we have found no easily determined characteristic that correlates with the difference in composition of the polymers. In any event, this work casts additional doubt on the validity of separating the two species of the genus *Lipomyces* on the basis of lactose assimilation.

REFERENCES

- BRADY, B. L. & JONES, D. (1964). Entry 70. *Trans. Br. Mycol. Soc. (Brit. Rec.)*, **47**, 298.
CONNELL, G. H., SKINNER, C. E. & HURD, R. C. (1954). *Lipomyces starkeyi* on the skin surface of the human body. *Mycologia*, **46**, 12.
DISCHE, Z., SHETTLES, L. B. & OSNOS, M. (1949). New specific color reactions of hexoses and spectrophotometric micromethods for their determination. *Arch. Biochem.* **22**, 169.
DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. & SMITH, F. (1956). Colorimetric method for determination of sugars and related substances. *Analyt. Chem.* **28**, 350.
GREGORY, J. D. (1960). The effect of borate on the carbazole reaction. *Arch. Biochem. Biophys.* **89**, 157.
HARDY, F. E. & BUCHANAN, J. G. (1963). The identification of carbohydrates giving derivatives of malonaldehyde on oxidation with sodium periodate. *J. Chem. Soc.* 5881.

- LODDER, J. & KREGER-VAN RIJ, N. J. W. (1952). *The Yeasts, a Taxonomic Study*. Amsterdam: North Holland Publishing Co.
- SŁODKI, M. E. (1963). Structure of *Hansenula capsulata* NRRL Y-1842 phosphomannan. *Biochim. biophys. Acta*, **69**, 96.
- SŁODKI, M. E., WICKERHAM, L. J. & BANDONI, R. J. (1966). Extracellular heteropolysaccharides from *Cryptococcus* and *Tremella*: a possible taxonomic relationship. *Can. J. Microbiol.* (in the Press).
- SLONEKER, J. H. & JEANES, A. (1962). Exocellular bacterial polysaccharide from *Xanthomonas campestris* NRRL B-1459. Part 1. Constitution. *Can. J. Chem.* **40**, 2066.
- STARKEY, R. L. (1946). Lipid production by a soil yeast. *J. Bact.* **51**, 33-50.
- TIMMELL, T. E., GLAUDEMANS, C. P. J. & CURRIE, A. L. (1956). Spectrophotometric method for the determination of sugars. *Analyt. Chem.* **28**, 1916.
- VANETTEN, C. H. & MCGREW, C. E. (1957). Ion exchange micromethods for separation of fermentation acids. Determination of fumaric acid in fermentation broth. *Analyt. Chem.* **29**, 1506.
- WALLENFELS, K. (1950). Detection of reducing sugars in paper chromatograms and quantitative evaluation. *Naturwiss.* **37**, 491. Abstracted (1951) in *Chem. Abstr.* **45**, 4604.
- WICKERHAM, L. J. (1951). Taxonomy of yeasts. 1. Techniques of classification. *U.S. Dept. Agr. Tech. Bull.* no. 1029.