

septate at the base, 2 to 3 times coiled at the apex and branched. Branches 2 to 6 times coiled; finely roughened, brown, broader at the base and tapering towards the apex. Asci cylindrical, hyaline, stipitate, 8-spored, $35.5-50.5 \times 4.5-6.5 \mu$. Ascospores uniseriate, lemon-shaped, lenticular, sub-apiculate, dark brown.

Subculture deposited at C.M.I., Kew, England under $5.6-7.7 \times 4.3-5.6 \mu$.

the accession number IMI 160294.

3. *Achaetomiella virescens* v. Arx, 1970, The Genera of Fungi Sporulating in Pure Culture, J. Cramer, Lebre, p. 247.

Perithedia partly superficial, partly immersed in culture, subglobose to ellipsoidal, ostiolate, dark olivaceous brown, $152.2-214.6 \times 127.6-171.0 \mu$. Hairs few, mostly present near the apex around the ostiole, simple, septate, brown, finely verrucose. Asci clavate, stipitate, 8-spored, evanescent, $28.3-44.0 \times 10.5-15.0 \mu$. Ascospores irregularly arranged, oval ovate to broadly ellipsoidal, brown, with terminal germ pores, $8.9-11.4 \times 5.6-6.8 \mu$.

Subculture deposited at C.M.I., Kew, England under the accession number IMI 161617.

The authors are grateful to Mr. A. Johnston, Director and Drs. Booth and Hawksworth of C.M.I., Kew, England for their help in the identification of these fungi.

Department of Botany,
Govt. Science College,
Jabalpur 482 001,
December 5, 1977.

D. P. TIWARI.
P. D. AGRAWAL.
A. R. LODH.

PHYLLACHORA CROTONIS (COOKE) SACC. A NEW RECORD FROM INDIA

Phyllachora crotonis (Cooke) Sacc., was collected by the authors from the leaf spot of *Croton oblongifolius* Roxb. Mahson, a wild host growing in Rajmahal hill, Bihar. It develops characteristic tar-spot symptoms on the leaves. It has not been reported from India¹⁻⁴ and the host is also a new record. It was reported earlier⁵ from the leaves of *Crotonis sylvatici*. The morphological details of the fungus are as follows:

Stroma black, rounded, erumpent, amphigenous, scattered, shining, about 0.20 mm in diameter, convex. Pseudoperithecia in dense group, paraphysate, asci clavate, octosporous $100-140 \mu \times 4-6 \mu$; ascospores hyaline uniseriate, oval, $12-16 \mu \times 3-4.5 \mu$.

The specimen is deposited at C.M.I., Kew, England, under accession no. IMI 215181.

The authors are grateful to Professor K. S. Bilgrami for proper laboratory facilities and also to Dr. A.

Johnston and Dr. A. Sivanesan of C.M.I., Kew, England, for their help in identification of the fungus.

Microbiology Laboratory, A. K. SHRIVASTAVA.
P.G. Department of Botany, P. L. SINGH.
Bhagalpur University,
Bhagalpur 812 007,
January 16, 1978.

1. Butler, E. J. and Bisby, G. R., "Fungi of India" (revised of R. S. Vasudevan); I.C.A.R., New Delhi, 1954.
2. Vasudeva, R. S., "Fungi of India. Supplement-1", I.C.A.R., New Delhi, 1962.
3. Tandon, R. N. and Chandra, S. "Supplement to the list of Indian Fungi", University of Allahabad Studies, Allahabad, 1964-69.
4. Mukerji, K. G. and Juneja, R. C., "Supplement to the list of Indian Fungi", Emkay Publication, New Delhi, 1974.
5. Saccardo, P., *Syll. Fungo.* 1883, 2, 599.

NEUTRAL SALT SOLUBLE COLLAGEN FROM THE ADDUCTOR MUSCLE OF AMUSIUM (BIVALVIA)

VIRCHOW⁸ pointed out that the fibroblast secreted a soluble substance which became fibrillated outside the cell. Wolbach⁹ found that the collagen formation was preceded by the formation of amorphous matrix which was considered to be mucopolysaccharide. Cooper and Davidson² studied the effect of ultraviolet irradiation on soluble collagen. Collagen extracted with dilute acetic acid was shown to form a fibrous precipitate having all the tinctorial properties of collagen. Neutral salt soluble fraction can be treated as the precursor of insoluble collagen. So far, collagen studies have not been made on tissues other than the conventional skin, tendon and granulation tissue. The present study deals with the extraction of neutral salt soluble fraction from the adductor muscle of a marine bivalve *Amusium pleuronectes* (Linn.).

Material and Methods

Procedure of Cooper and Davidson² was followed. The adductor muscles from young growing animals were extracted with five times their weight of 10% (W/W) NaCl solution at 0-4° for 96 hours. The extraction was repeated thrice. The extracts were centrifuged (14000 g and -2°). The clear extracts were saturated with solid (NH₄)₂ SO₄ and the precipitated proteins were collected by centrifugation. The precipitate was dissolved in M NaCl and dialysed against a large volume of this solvent to remove excess of salts. The protein solution was then filtered and brought to pH 3.4 with acetic acid. After

standing overnight the precipitated protein was collected and redissolved in 0.5 N acetic acid and centrifuged for 1 hr (32000 g at -2°) to remove insoluble material. This is now 0.5 N acetic acid soluble collagen and it was precipitated by the addition of solid NaCl to give a final concentration of 5% (W/V). The collagen was dissolved in 0.5 N acetic acid and the solution centrifuged at 3200 g at -2° for one hour. The solution was dialysed against three lots of 0.02 M Na_2HPO_4 until precipitation was complete. The precipitated collagen was redissolved in 0.15 N acetic acid and dialysed against several changes of this solvent and then freeze-dried. The amino acid analysis was done on Technicon auto analyser.

TABLE I

Amino acid composition of the neutral salt soluble collagen

Amino acids	Residues/1,000 total residues
Hydroxyproline	80.0
Aspartic acid	37.96
Threonine	7.7
Serine	12.18
Glutamic acid	52.78
Proline	93.4
Glycine	280.5
Alanine	25.3
Valine	8.12
Methionine	0.812
Isoleucine	13.2
Leucine	18.9
Tyrosine	4.12
Phenylalanine	6.5
Hydroxylysine	2.4
Histidine	6.7
Lysine	30.45
Arginine	62.73

The amino acid composition of the neutral salt soluble fraction is not identical with that of insoluble collagen. The concentrations of the following amino acids in the soluble fraction are lower than those of insoluble fraction—hydroxyproline, aspartic acid, proline, glycine, glutamic acid, alanine, methionine, valine, hydroxylysine and arginine. The following amino acids occur in higher concentrations in soluble fractions tyrosine, threonine, serine, isoleucine, leucine, phenylalanine, histidine and lysine. Harkness⁴ pointed out that all fractions of collagen type have high contents of both hydroxyproline and glycine but contain small amounts of tyrosine. On the other hand water soluble

and citrate insoluble proteins show a reverse relationship in high contents of tyrosine and small amounts of hydroxyproline and glycine.

Neutral salt soluble fraction is not derived from ordinary fibrils. This fraction seems to be the precursor of native collagen that will be polymerized. Within this precursor the peptide chains are in the process of synthesis. The differences observed in the amino acid composition of the insoluble collagen and soluble collagen suggest that any conversion of this soluble protein to collagen fibres must involve the addition of protein fraction relatively rich in tyrosine, histidine, lysine and proline and relatively low in hydroxyproline, alanine and serine. But in the present study it was found that proline content is not higher than the native collagen. It is tentatively suggested by Bowes *et al.*¹ that fibre formation may involve the association of soluble protein with mucopolysaccharide fraction. It may be that some enzymatic modification of this molecule is required before polymerization can occur as in the case of fibrinogen fibrin transformation. The physiological role of this collagen seems to impart high viscosity to its solutions.

I thank Prof. R. Natarajan, Director, Centre of Advanced Study in Marine Biology, Porto Novo for suggesting the problem and Prof. D. S. Jackson, Head of the Department of Medical Biochemistry, School of Medicine, University of Manchester for giving me the facilities to carry out the analyses.

Centre of Advanced Study
 in Marine Biology,
 Parangipettai 608 502,
 Tamil Nadu, India,
 March 18, 1977.

M. KALYANI.

1. Bowes, J. H., Elliot, R. J., and Moss, J. A. "Nature and Structure of Collagen". Ed. by Randall, J. T. London. Butterworths scientific publications., 1953, p. 199.
2. Cooper, D. R., and Davidson, R. J., *Biochem. J.*, 1965, 97, 139.
3. Harkness, R. D., Marks, A. M., Muir, H. M., and Neuberger, A. "Nature and Structure of Collagen". Ed. by Randall, J. T. London, Butterworths scientific publications., 1953, p. 208.
4. —, "Recent advances in Gelatin and Glue Research". Pergamon press, London, 1958, p. 58.
5. Jackson, D. S. *Ibid.*, 1958, 50.
6. Maser, M. D. and Rice, R. V. *Biochem. Biophys. Acta*, 1962, 63, 255.
7. Piez, K. A. and Gross, J. *Ibid.*, 1959, 34, 24.
8. Virchow, R. *Die Cellular Pathologie*, Berlin A. Hirschwald., 1871, p. 40.
9. Walbach, S. B., *Proc. Nutrit. Soc.*, 1953, 12, 247.