Vol. 42, No. 18
Sept. 20, 1973]

CELLULOLY.IC ACTIVITY IN SOME LAND SNAILS OF SOUTH INDIA

R. KASINATHAN, D. CHANDRAMOHAN AND R. NATARAJAN
Centre of Advanced Study in Marine Biology, Porto Novo, Tamil Nadu

INTRODUCTION

OF the thirty or more enzymes reported to be associated with the digestive tract of the land snail, *Helix*, more than twenty are carbohydrases[1,2]. Among the carbohydrases, the cellulase, which forms a major portion, has been reported from many members of the gastropods[1-10]. Florkin and Lozet[11] have demonstrated the presence of cellulose-decomposing bacteria (cellulolytic bacteria) in the digestive tract of *H. pomatia* and suggested that the bacteria were responsible for the breakdown of cellulose in the intestine of snails. Myers and Northcote[12] reported the occurrence of a number of cellulases in the digestive tract of *H. pomatia* and suggested that one or more of these enzymes might have originated from the intestinal microflora while the rest might have been secreted by the animal itself. However, Galli and Giese[7] opined that the cellulolytic bacteria present in the gut of *Tegula* were relatively unimportant. They reported that only four strains of bacteria isolated from the gut of this animal were capable of breaking down structural carbohydrates and these were present in small numbers only. Parnas[9] investigated the source of cellulases in the snail *Levantina hierosolyma* by comparing the cellulolytic activity of normal snails with those whose digestive tract had been sterilized with antibiotics. They conclude that the cellulase of the digestive diverticula of *Levantina* is secreted by the animal whereas the cellulolytic activity of the crop and salivary glands may be from the bacterial source or from the passage of enzymes from the digestive diverticula. Very recently, reporting on cellulolytic micro-organisms in the digestive tract of the snail *Achatina fulica*, Soedigdo *et al.*[10], suggested that these might serve a nutritional function in this snail. There seems to be good evidence therefore that at least some gastropods possess the ability to secrete cellulase. It was therefore considered worthwhile to investigate some of the locally available gastropods for their cellulase activity and to study the origin of such enzymes in the digestive tracts of snails.

MATERIALS AND METHODS

The present investigation was carried out on three species of Mesogastropoda (Cyclophoridae) belonging to three genera, *viz.*, *Cyclophorus jerdoni* (Benson), *Pterocyclus bilabiatus* (Sowerby) and *Theobaldius ravidus* (Benson). All the species were collected at Alagarkoil Hills, 20 kilometers north of Madurai, South India. The first two species were collected at an altitude of 1,000 meters and the last one at 400 meters. All the three species generally occur in crevices crawling on moss-covered rocks, but they are also found among humus and decaying leaves on which they feed. Under laboratory conditions, the animals were maintained in sterile cabinets and fed with sterile filter-paper[9]. In order to obtain different degrees of sterile conditions in the digestive tracts, various antibiotics were employed as given in Table I. The cellulase activity was estimated in different parts of the digestive tract. The humus-soil mixture and the faecal pellets were also analysed for cellulase activity. The cellulase activity of the crude enzyme was determined according to the method of Soedigdo *et al.*[10]. The change in extinction E between 0- and 30– min incubation, divided by the mg of protein/ml was designated as the specific activity (S.A.). Protein was determined according to the method of Lowery *et al.*,[13] using bovine serum albumin as standard. The cellulase activity in the humus-soil mixture was estimated by adopting the method as outlined by Skujins[14]. The cellulolytic bacterial population was estimated by employing Dubos cellulose medium[15]. The potentialities of the various bacterial isolates to produce cellulase in liquid Dubo's medium were also investigated. The various isolates were inoculated individually in different flasks and incubated at room temperature ($28 \pm 2°$) for a maximum period of 21 days and the cell-free filtrates were used as the enzyme source for cellulolytic activity.

RESULTS AND DISCUSSION

The cellulolytic activity could be detected throughout the digestive tract in all the three species investigated (Table I). Generally maximum activities could be recorded in either stomach or intestine only, except in *T. ravidus* where slightly higher activities were observed in the oesophagus and salivary glands. *C. jerdoni* showed the highest specific activity in the stomach region. The freshly collected animals (field animals) always showed higher activities than the filter-paper fed animals under laboratory conditions. The source of cellulase in the alimentary canal in *Cyclophorus*

TABLE I

Cellulase activity in different regions of the alimentary canal of the snails and in faecal pellets (specific activity × 10^3)

Material	*C. jerdoni*			*P. bilabiatus*			*T. ravidus*		
	1	2	3	1	2	3	1	2	3
Oesophagus along with salivary glands and oesophageal pouches	70	95	16	179	20	70	186	41	52
Pre-stomach	64	87	36	243	361	12	42	34	67
Post-stomach	99	174	205	199	43	78	91	73	16
Intestine with rectum	960	42	124	420	42	45	153	67	62
Faecal pellets	190	20	20	116	10	40	108	49	52

1. Field animal. 2. Filter-paper fed animals. 3. Filter-paper + Streptomycin + Achromycin + Greisovin fed animals.

was investigated by comparing the cellulolytic activity of normal snails with those whose digestive tract has been sterilized with antibiotics as reported earlier by Parnas[9]. In antibiotic treated animals, after 48 hrs the different regions of the digestive tract were cultured on nutrient agar medium and cellulolytic activities were estimated only after confirming that the entire tract was sterile. The sterile animals also exhibited cellulolytic activity in various regions. While *C. jerdoni* showed highest activity in the post-stomach region, the other two species had maximum activities in the oesophagous and pre-stomach regions. Persistence of cellulolytic activity in the hepatopancreas of active snails following treatment with antibiotics had already been established[9]. A reduction in cellulolytic activity was recorded in filter-paper fed animals when compared to normal animals though no appreciable differences could be noted in the cellulase activities between the non-sterile and sterile filter-paper fed animals. This may be taken as an indication that microbial populations perhaps do not contribute appreciably to the "cellulase pool" in the animal. Quite possibly the snails are capable of secreting cellulase.

Presently, however, bacteriological studies show that cellulose-decomposing bacteria may also contribute to the cellulase pool in the animal to a limited extent. All the three species used presently were found to harbour cellulolytic bacteria. Totally, twenty bacterial strains were isolated from the three species based on their colony characters but only six strains were found to produce cellulase in synthetic media. These strains exhibited only weak activities (Table II). It is also evident from the results (Table III), that the post-stomach and

TABLE II

Cellulase activity of different cellulolytic bacteria of the alimentary canal of the snails

Sl. No.	Strain	Specific Activity (× 10^3)	*C. jerdoni*	*P. bilabiatus*	*T. ravidus*
1.	C.8	8	+	+	+
2.	C.11	10	+	+	+
3.	C.19	26	+	+	+
4.	C.31	8	−	−	+
5.	C.10	17	+	+	+
6.	C.12	23	+	+	−

+ Present, − Absent.

intestine harboured more cellulolytic bacteria in all the three species. However in relation to total bacteria, these cellulose digesting bacteria were observed to occur at very low concentrations (Table III). The cellulolytic activity in the faecal pellets indicated that obviously some amount of enzyme secreted inside the alimentary canal is excreted through the pellets. The possible entry of cellulase into the alimentary canal through the feed is also ruled out because of the fact that the humus-soil complex, which form the food for the animals in nature, contained only a trace of activity (S.A. 1–3). Considering all these it may be concluded that the snails by themselves are capable of secreting cellulase and perhaps the cellulolytic bacteria also contribute to the cellulase pool to some extent.

TABLE III

Total and cellulolytic bacteria in the alimentary canal of the snails (× 10^6/g contents on oven dry basis)

		Field animals		Filter-paper fed animals		Antibiotic treated animals	
		Total	Cellulolytic	Total	Cellulolytic	Total	Cellulolytic
Oesophagus along with salivary gland and oesophageal pouches	I	6·12	1·93	7·32	1·20	—	—
	II	6·63	2·12	4·00	0·82	—	—
	III	2·70	1·05	2·31	0·42	—	—
Pre stomach	I	11·92	2·34	8·06	1·98	—	—
	II	16·00	2·90	10·50	2·10	—	—
	III	8·20	2·10	2·50	0·96	—	—
Post-stomach	I	130·57	10·00	120·60	6·86	—	—
	II	271·00	26·66	86·60	18·28	—	—
	III	248·50	13·60	20·77	9·28	—	—
Intestine with rectum	I	112·00	3·50	25·00	2·52	—	—
	II	215·60	18·50	39·53	10·55	—	—
	III	105·50	20·52	27·37	10·44	—	—
Faecal pellets	I	345·00	8·35	88·05	3·86	—	—
	II	272·00	25·50	12·72	8·94	—	—
	III	344·00	14·50	4·68	1·62	—	—

I : *C. jerdoni.* II : *P. bilabiatus.* III : *T. ravidus.*

Incidentally it may also be pointed out here, that, the total cellulase activity observed in the stomach of *Cyclophorus* is nearly two times more than in *Helix*[12] which is used commonly as the source for cellulase in western countries. In India *Cyclophorus* can be most successfully employed as the source for the extraction of cellulase for all experimental purposes in the laboratories.

ACKNOWLEDGEMENTS

We are grateful to the University Grants Commission, India, and to Smithsonian Institution, Washington, D.C., U.S.A. (Grant SFC-7–0067) for generous financial support which facilitated the present study.

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