Sago starch: An economical substitute for *in vitro* primary screening of starch utilizing microorganisms

The development of industrial biotechnology processing has led to the utilization of microbial enzymes in various applications. One of the industrially important enzymes is amylase, which hydrolyses starch to glucose[1]. Isolation of amylase producers is generally performed on starch agar, which restricts starch as the sole source of carbon with a base of agar-agar[2]. Culture media used are usually gelled with agar, an expensive component. Since its introduction more than hundred years ago by Robert Koch, agar has remained the most frequently used gelling agent for microbial culture media. Its usefulness lies in its stability, high clarity, non-toxic nature and resistance to microbial degradation. Doubts have been raised about the inertness and non-toxic nature of agar, overexploitation of its sources and the exorbitant price of bacteriological grade agar. This has necessitated efforts to look for alternative gelling agents for microbiological culture[3].

Figure 1. Visual comparison of starch agar and sago (sabu) plates.

Figure 2. Sago plate.

Figure 3. Growth of *Aspergillus* spp. (after 48 h of incubation at 27 ± 2°C) on sago plate.

Successful use of isabgol derived from *Plantago ovata* seeds, gum katira exuded from *Cochlospermum religiosum* bark and guar gum from endosperm of *Cyamopsis tetragonoloba* as gelling agent has been reported for microbial culture media[3]. Here we report the successful use of sago starch as an exclusive gelling agent for the isolation of starch-degrading microorganisms. Sabudana/sago is a rich source of carbohydrates and is a popular food in several parts of India. This medium, adjuvated with sago powder – sabudana beads ground to powder (12 g; locally available), arrowroot powder (4 g; locally available), and NaCl (1 g; Qualigens Fine Chemicals, India) was used. These ingredients were added to 100 ml of distilled water. No jellifying agent was required as sago has self-gelling properties. The present study was concerned with the selection of economically available agricultural starchy substrate for the isolation and screening of starch degraders. Sago starch represents an alternative carbon source for fermentation processes that is attractive for both economic and geographical considerations[1]. A comparison of starch agar and sabu media for the isolation and calculation of actual biomass of standard strains of amylase producers, *Bacillus subtilis*, *Aspergillus niger*, *Saccharomyces cerevisiae* was carried out. It has been reported that sago starch can be utilized by *Aspergillus flavus* strain having amylolytic enzymes[4] and *S. cerevisiae*[5].

Sabudana (*Cycas revoluta*) grains powdered in a flour mill and arrowroot (*Marantaceae arundinacea*) powder were purchased at Thane Market, Mumbai, India. All other chemicals used were commercially available and of analytical grade.

For the preparation of sabu solid medium, sabudana powder (12 g) was mixed with arrowroot powder (4 g) and pure NaCl (2 g; Qualigens) in a conical flask (Borosil). Distilled water (100 ml) was added to the above flask and autoclaved at 15 lbs pressure for 15 min. In the treatment, pH of the medium was adjusted to 7–8 before autoclaving. Next the thick, viscous medium was poured in sterile Borosil petri plates. The mixture was maintained left at 25–27°C until the medium solidified. The sabu medium was left at room temperature after solidification and at 37°C to check for any contamination.

Known amylase producing strains of *A. niger* (NCIM strain), *S. cerevisiae* and *B. subtilis* were maintained in potato dextrose agar. They were then individu-

Table 1. Comparison between properties of sabu medium and starch agar

	Starch agar	Sabu medium
Colour	Brown	White
Opacity	Transparent	Opaque
Surface	Very smooth	Smooth
Consistency	Thick	Thick
Possibility of liquid medium	Yes	No
Growth of microorganisms after 24 h of incubation at 27 ± 2°C		
Aspergillus niger	+	+++
Saccharomyces cerevisiae	+	+
Bacillus subtilis	+	+
Weight (in g) of biomass after 24 h of incubation at 27 ± 2°C		
A. niger	6.2	6.9
S. cerevisiae	5.7	5.5
B. subtilis	5.1	4.8

+ indicates growth, + + + indicates good growth.

ally streaked on sabu medium and starch agar and incubated at 37 ± 2°C to observe for growth after every 24 h. The growth of microorganisms was scraped out and actual biomass was compared. All the growth patterns were studied in triplicate.

Using the method described above a thick, firm gel of sabu medium was formed in the plates after solidification. No contamination was observed after 24 h of incubation at both room temperature and at 37°C. The three indicator organisms grew efficiently in the sabu medium, especially *A. niger*, which showed maximum growth. Details of appearance of the medium and comparison of the sabu medium with standard starch agar are given in Table 1. Biomass comparison was also done as shown in Table 2.

Starch agar[2] is the standard medium used for isolating starch-hydrolysing microorganisms for routine microbiological work. Sabu medium is more economical than starch agar. The components used for this formulation are simple and easily available all round the year. Table 3 gives a cost comparison of starch agar and sabu medium.

Sabudana is generally used for making soups, candy, pudding in the food industry, for production of glue in plywood industry, and for surface treatment process in the paper industry[1]. Sabudana can also be used in microbiology as a primary screening medium. Sago medium being about ten times cheaper than its counterpart provides a cost-effective alternative. Besides having a cost advantage, it poses no problem in adjustment of pH and dispensing. Moreover, as it is produced commercially under controlled conditions using renewable sources, its increased demands can be met without any fear of exploitation of its sources. Media gelled with sago is better suited for cultivation of *Aspergillus* species. Biomass comparison showed maximum growth for *Aspergillus*. Like agar, it is biocompatible and degradable and therefore poses no threat to the environment on being disposed after

Table 2. Comparison between components of starch agar and sabu medium

Starch agar[2]		Sabu medium	
Peptone	1.5 g	–	–
Starch	2 g	Sabudana (powder)	12 g
Yeast/beef extract	0.5 g	Arrowroot (powder)	4 g
NaCl	0.5 g	NaCl	2 g
Agar–agar	2.5–3 g	–	–
Distilled water	100 ml	Distilled water	100 ml
pH	7.4	pH	7.14–8.0
Number of plates prepared in 100 ml	5	Number of plates prepared in 100 ml	4
Time taken to solidify	20 min	Time taken to solidify	25–30 min
Concentration of starch	2%	Concentration of starch	90%

Table 3. Cost comparison of components of starch agar and sabu medium

Star agar		Sabu medium	
Components	Price (in rupees) (minimum) per 500 g	Components	Price (in rupees) (minimum) per 500 g
Peptone	500–896	–	–
Starch	370–5300	Sabudana grains (1 kg)	30
Yeast/beef extract	500–1000	Arrowroot powder (100 g)	5
NaCl	75–100	NaCl (500 g)	75–95
Agar-agar	1400–2000	–	

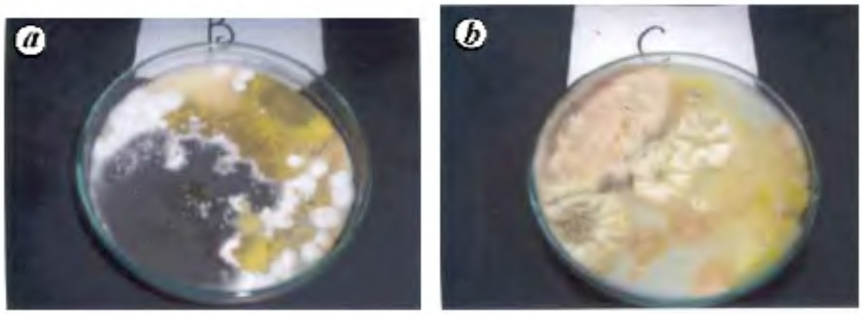

Figure 4. Pigmented (yellow, white and black; ***a***) and (lemon yellow, white and pink; ***b***) growth of fungus on sago plate after 48 h of incubation at room temperature (27 ± 2°C).

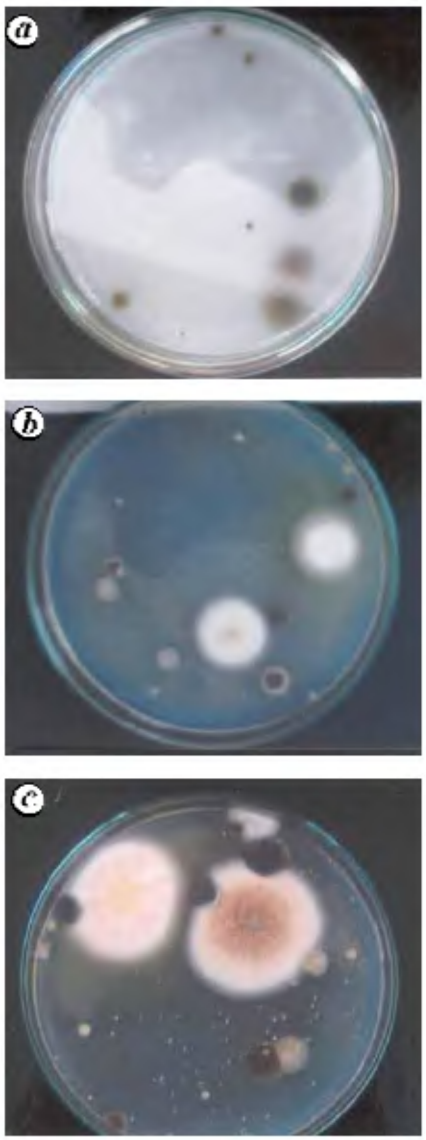

Figure 5*a–c*. Bacterial and fungal growth on sago medium after 48 h of incubation at room temperature (27 ± 2°C).

use. Other advantages of sago medium are: (i) easy availability of components all round the year, and (ii) easy and quick preparation process. The medium can be studied further by improvising upon its quality.

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Uranium–thorium-rich zircon in a granitoid dyke along the Shyok Suture Zone, Nubra–Shyok River Valley, Northern Ladakh, India

In northern India, the Ladakh block lies between the Indian Plate to its south and the Eurasian Plate to its north. To its west, it is largely separated from the Kohistan Complex by the Nanga Parbat–Haramosh syntaxis and to its east, it is cut-off from the Lhasa block by the Karakoram Fault[1]. Most workers have interpreted the Ladakh block and Kohistan Complex as a single accreted island-arc terrane[1–5]. The Ladakh block is delineated by two suture zones, viz. the Indus and the Shyok, which mark the closing of different branches of the Tethys Ocean and finally the collision of India with Asia at 60–50 Ma. The Shyok Suture Zone lies to the north of the Indus Suture Zone and is interpreted as a suture embodying the rocks of a backarc basin[1].

Rocks of the Shyok Suture Zone, trending northwest–southeast across the Nubra–Shyok Valley, occur in deformed tectonic slices between the Ladakh batholith to the southwest and the Karakoram batholith to the northeast. Across the Nubra–Shyok Valley and the adjoining Karakoram block, these tectonic slices comprise a variety of sedimentary, metamorphic and volcano-plutonic rocks, referred to as an accretionary complex[2,6]. The geological structure of the Shyok Suture Zone has been recently discussed elsewhere[1,2,6–8].

Udmaru village is situated on a volcanic rock formation known as the Shyok Volcanics, along the Shyok Suture Zone in the Nubra–Shyok River Valley. These Cretaceous Shyok Volcanics mainly consist of basalts and andesites. North of Udmaru, a ~5–10 m thick granite–pegmatite dyke dissecting across the Cretaceous Shyok Volcanics for a considerable distance has been reported[9]. A preliminary study revealed that a sample of hornblende–biotite-bearing monzogranite contained abundant, small-to-medium grained, euhedral, greenish-coloured zircon. Geochemical analysis of the separated zircon grains showed exceptionally high concentration of both uranium and thorium (0.31–5.36% U and 0.76–1.43% Th; Table 1). The major and trace element geochemical data of granitoid are 71 wt% SiO_2, 14.74 wt% Al_2O_3, 2 wt% Fe_2O_3, 1203 ppm Sr and 2135 ppm Ba. The purpose of this short communication is to report the presence of highly radioactive zircon within the Ladakh block of the India–Asia collision zone. Detailed work is in progress.

Table 1. U and Th contents in zircon from a monzogranite dyke along the Shyok Suture Zone in the Nubra–Shyok River Valley, Ladakh Himalaya. Samples were analysed by TIMS at the Isotope Laboratory of the University of Tuebingen, Germany under the aegis of the Alexander von Humboldt Fellowship

Sample weight (separated zircon; mg)	Uranium	Thorium
0.0168	3847.3 ppm	8736 ppm
0.0103	5.36%	1.43%
0.0132	3102.3 ppm	1.11%
0.0192	3070.8 ppm	7644 ppm

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