Isolation and Enrichment of Polysaccharide Degraders
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**Title:**
Isolation and Enrichment of polysaccharide degraders  
Begun 1/10/02

**Statement of Purpose:**
To isolate and enrich polysaccharide degraders from using limiting media such as chitin or agar, which are difficult to degrade as the sole carbon and energy source, once the organisms are plated out and forced to use the limited media.

**Methodology and Observations:**

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Add a small amount of agar and chitin suspension to each of two separate room-temperature medium flasks (done by TA). Add a tablespoon of marine kelp environment to each, the chitin because the chitin itself is more turbid than the agar. There was no sand in either flask, however, a small amount of kelp-like green material was added to the loops in the chitin suspension.

Examine macroscopically; is something in there to notice.

**Macro chitin:** continues to be turbid, with the floating suspension being milky white, slightly hazy, and the flake being completely opaque; however, the overall suspension is still translucent. There is lots of sedimentation mainly due to the chitin on the bottom. The floating suspension does not equal more than 10% of the bottom, TA says do wait.

**Macro agar:** slightly more turbid than last week; however, the overall suspension is quite transparent. It has barely any flaking, floating suspension, but has more sedimentation which is slightly yellow; the floating suspension is hazy and milky white just like the chitin flask.

**Macro agar:** turbid when looked at from below, the view is opaque, both due to the white flaky floating suspension and the yellow sedimentation; however, when looking across the flask, there is a very thin layer of milky white suspension. The in-between is very transparent, and very unobstructed, the suspension floats together and bunches up once tipped to one side.
Chitin: The media has a black look when looking from below, not completely opaque, but the black matter, oily suspension beginning to look like bitumen, with its waxy characteristic of floating. It is dense enough not to float at top of water but a few can below the water line, the yellow sedimentation is slightly less than in agar, but is noticeable. The flat-steel-like suspension, does compress when tilted over, however, when it is held level, the suspension expands to fill the plate level, but does not expand up or down.
1/31/02

Observe the nutrient media flasks

A - flasks floating; milky white in color - though still small transparent media

B - growth up top

C - same as above, however more growth, and debris occurring on bottom of flask

C: certainly not opaque (looks eye level of flask; but slightly opaque when looking from below)

2/1/02 - stuck at both on plate. A: blue-green, C: pple

A - flaking, yellow growth instead, floating, no growth along sides, yellow growth but not bright, dull yellow; slightly turbid when shaken, however settling occurs quickly; flakes are slightly transparent, appear to move as one mass, appear to change together with few inside, flakes floating around, when broken, mass finally moves through media.

C - mass of floating colonies are much more compact than agar; obvious changing and restricted turbidity due to colony formation. Yellow (dull) like color, slightly transparent like color, has hardened nodules at bottom (most likely due to the media), mass breaks up quickly when shaken, not quickly like the agar based phyto-see. In both, there is considerable clearance around the direct bottom, most of the growth occurs along side bottom.

- bottom view
2/19/02

Chitin / Agar

detailed macro/micro check plate for growth. If growth streak again for colonies, if not restreak using E. coli growth.

Agar

dense heavy growth.

yellow colonies, not smooth, jagged edges to the colonies.

colonies, cut into the media, making a pitted appearance; colony appears as pitted.

Chitin

Definite heavy growth; also yellow colonies, seem to be plastic-like and plaque having granular, jagged edges, crinkled into the pits of agar. Not isolated, growth along the streak procedure; no growth is pretty much restricted to the lines of the streaking process — definite structures and not usual exponential growth.
Cuboid micro: small, red, shaped; solid color; too small to see differentiation between internal structures. Smooth, edged, aggregates into directional clumps. Seemingly moving, though all in some way so may likely due to the micro under the cone slide. No evidence of filamentous organisms like in the agar, all rods one with one length of being the same size.

Ragi: Small and decolor; solid color; smooth, aggregate into small lattice structures. Mobile: Filamentous some of them. Filamentous ones are aggregating into lattice structures, possibly as long as oval ones.
colonies eating into agar, pitted colony appearance, no real isolation of colonies seems to have large clumping, some areas appear darker as because more isolated, which usually means larger plagues.

spores present; not completely one mass at tip; more isolated than thought at first.

micro: motile rods, straight not bended, dark throughout, small (small enough to show up as only dark) circular cells. Found pinpoint dark cells, slow migration seem to be congoating larger circles (non-motile)

interposed of circular pinpoint colonies causing lattice type structure, seems to have bonding problems within lattice formation
Chitin:

solid colony growth at 40°C streaked into chitin medium did not cling and form plaques on agar 4A. Much easier to get isolated colonies; however, they are faint so it is hard to see in comparison to the colonies. Colors are opaque yellow, not as dark as agar.

Micro:
- Dark central shape, lacks vacuole inside, smoothed edges
- Also some have lighter clearing than a dented center; also small.
- Some are flagellated but remain same size.

Rod types are two times as long as dark center types most prevalent in all types so far not solid colored

Non-motile.
- Also have thin rod shape, almost like spermatid with a thin head and long tails; there is no clearance with; also these are non-motile, sometimes rods are slightly motile.

Motile: there are smaller central motiled hektrin 20; moving about seem to aggregate together and 47 long rod types.
Chitin / Acid - Streak again for isolation
while spots (like bubbles, spherical, maybe settling of gas)

3/20

Agar

Pitted colonies; possible isolation of colonies; the yellow color is not as
dark as before; spotty pattern, the streak isn't contiguous, it is breaking
into lines.

Micro, rods; congregation around certain food points; only 4 of them are
regular, a few may be conical but not

more than 3 per slide.

A few are more spherical than the others

coming with 0; but definitely not spheres

very hard not singular, outside these large clusters

that appear; cells on smooth edge;
colonies continue to eat into chitin, distinguishing the small white dots is difficult due to the chitin media; pitted colony appearance, seems to have slight clumping as evidenced by lack of stratification in colony isolation. One long continuous band for initial streak. Some areas are cluster yellow; not a uniform color throughout.

misc: long string of rods; in a similar position much in the same direction; only appear in large clusters or long chains in cells not connected; cells are also not found singularly on this slide mount; all are associated with the large clusters; smooth edges; non-motile; there are no spherical cells; all are rods.
solid yellow
solid culture
white dots permeate all colonies

white dots, colonies sometimes coart inside yellow colonies

agora

claymore says plate is contaminated
solid yellow, stippled appearance, smooth, ends into
white dots, colonies do not exhibit white
det contaminants
uses these for plating

micro.

slightly more rods, segmented, too small to see individual vacuoles;
do not cluster; range from 2 to 5 segments long

some segments are longer than others, end segments

seem to be longest in volume

contaminant, seemingly small-edges
all yellow not isolated colonies at all continues to eat into media yellow as before dorsal regions are more yellow (darker yellow)

Micro: slightly mobile, high colony growth, each cell is small
round shaped, clump near colony center, less organized than before which were all aligned in same direction, these are in all directions

and some aren't organized

very few not tinged red and are in morphological change not associated with large colonies
micro: yellowish, the rods are floating, perhaps due to the amount of power because no flagella is immediately present and identifiable. The rods are not segmented, as they close together as opposed to last week; the size continues to be variable, the shape is definitely rod shaped, the colonies are clumped together; last weeks isolates must have been contaminated or sparse, they continue to have small, rounded edges and are too small to identify interior structures.
hard to see because of the chitin plate, taking into the
chitin, some streaks are very faint, the outer lines are
not smooth at all, have outgrowth beyond the streak here

small red shaped cells congregating around a single, the cells
are rarely not in pairs at the very least, lightly mobile,
for wiggling under the light, the cell
groupings aren't as large as before
with group of 5-6 being
usual common. The cells are
smooth and all pretty the
same size, very little derivation
in size.
Summary:

The polysaccharide degraders took quite some time to evolve into recognizable, extractable organisms in the liquid media. The macroscopic observations of the two media flasks was hindered by an inability to adequately describe and later draw the activity, a digital camera would have provided far better detail. Once the level of suspended feathery white flotsam-like growth had incubated for 3 weeks, the TA decided it was time to plate. The liquid suspension was shaken, and a loopful of the media was transferred to respective chitin and agar plates. Upon streaking onto plates, the characterization and description of the colonies were far easier, however, the isolation of the polysaccharide degraders was difficult. Purification was thought to have been established 2 weeks later with all of the colonies being yellow (a member of Flavobacterium possibly), however, isolation was nearly impossible. It was noticed that there were small white colonies interspersed within the yellow colonies and upon the TA’s request, the yellow colonies were plucked, purified, and isolated in the following week (4/11). Regardless of the contamination in both agar and chitin until noticed, the microscopic observations always contained rod shaped organisms, and in all but one observation, those organisms grouped together.

Upon the first streak (2/14), the colonies for both agar and developed into yellow, non-smooth, almost plastic looking plaques. The colonies for both appeared to eat into the media, with the colonies inhabiting inside the pits. Microscopically, these cells began to display the morphologies consistent with *flavobacterium*, exhibiting rod-shaped cells as well as not having sporylation. There were no lattice structures, however, the rod-shapes formed groupings and clumps fixed around a certain point.

Finally in the last purification step, the colonies did not change the characteristics from the previous week. The plaques were still eating into the plates; forming solid bands of yellow deposits, however, finally a bit of isolation took place, with noticeable small clusters and isolated colonies later on in the second portion of the final streak. As less of the colonies appeared due to isolation, the thick solid bands at the initial streak site became more and more striated, with few deviations on either agar or chitin from the pattern of streaks. The colonies followed the inoculating loop track almost perfectly, with little colony spreading. Microscopically, the cells appeared dark, with hardly any visibility inside the cells. Both agar and chitin appeared nearly identical under both macroscopic and microscopic observation. For both, groupings of cells did not reach numbers above 20 except on rare occasions, however, groupings of 5 or 6 were in close proximity, but always appeared oriented in different directions.

The experiment was a success because the water did contain a polysaccharide degrader. The experiment was successful because the continuous streaking allowed for isolation, making the identification of the isolate much easier.
Isolate Description:

This lab was designed to purify and isolate polysaccharide degraders, which commonly appeared in two forms in this lab, as either yellow colonies (flavobacterium) or white colonies (cytophaga). Because the colonies were consistently yellow for the entire isolation portion on the media plates, the isolate was considered to be of the Flavobacterium group.

*Flavobacterium* are ubiquitous organisms, much like bacillus. They are found in soil, but more commonly in water environments. *Flavobacterium* can exist in almost every water systems including distilled water lines and dental chair spray units. They can also survive in intravenous anesthetics, eyes, urine, and stool samples. Most species are yellow pigmented rods, which was the main feature in hypothesizing that what was isolated was indeed *Flavobacterium*.

*Flavobacterium* are fastidious microbes, are facultative aerobes, and are nonfermentative gram-negative bacilli. The percent G+C ranges from 31-42%. All but one species produces indole, which is a unique characteristics among non fermenters. They are chemoorganotrophs, which have respiratory metabolism. Some species are acid producing, while other strains are holotolerant, while others still fix nitrogen. *Flavobacterium* has found numerous niches in the environment. Reproduction within these environments, however, does not include a resting stage or spore formation. There are three hundred and twelve strains of *Flavobacterium*, which account for the number of strains that exhibit different characteristics. In testing for *Flavobacterium*, it tests positive for phosphatase, oxidase, and catalase. Of the three hundred and twelve strains, two hundred and fifty of these strains are acid producing. The acid producing strains include the species meningosepticum, the strain that causes the deadly disease meningitis.